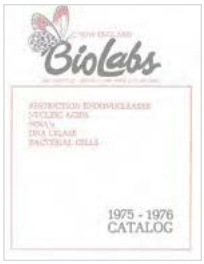
NEW ENGLAND Biolabs®

CATALOG & TECHNICAL REFERENCE

2025~26



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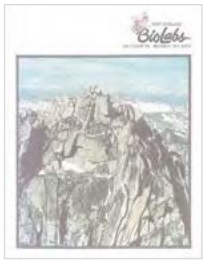

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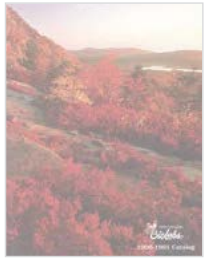

1990

1992

1993

1995

SHAPING the *science* of TOMORROW

1996

1998

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2017

2019

2021

2023

50 YEARS of *passion* for SCIENCE

Over 50 years ago, Donald Comb had an idea — an experiment really — to start a company that would be unlike any other. A company that put people over profit and substance over story. That experiment, New England Biolabs, would go on to become a mainstay of the modern molecular biology laboratory, and our brand would become synonymous with science, quality and technical support.

At NEB, we continue to be guided by the foundational principles that Don envisioned: the belief that science should be used to expand our understanding of the world around us; the belief that scientists can also act as humanitarians; the believe that all species, great and small, benefit when we care for the environment; and the belief that great science is fundamentally creative and artistic, and changes the way that we see and experience the world. While our company has grown, our values have not wavered.

As I reflect upon the past 50 years, I am thankful for and humbled by our loyal customers who share these similar values. We were thrilled to celebrate you and your achievements as part of our 50th anniversary celebration.

As we look toward the next 50 years, I am excited to continue to build a sustainable business that is focused on enabling the scientific community, fostering curiosity, and giving back to those around us. We have much work to do, and I am excited that we are able to do it together.

As always, if there is anything you believe we should be doing differently, please share your thoughts with us.

SALVATORE RUSSELLO
CEO, NEW ENGLAND BIOLABS, INC.

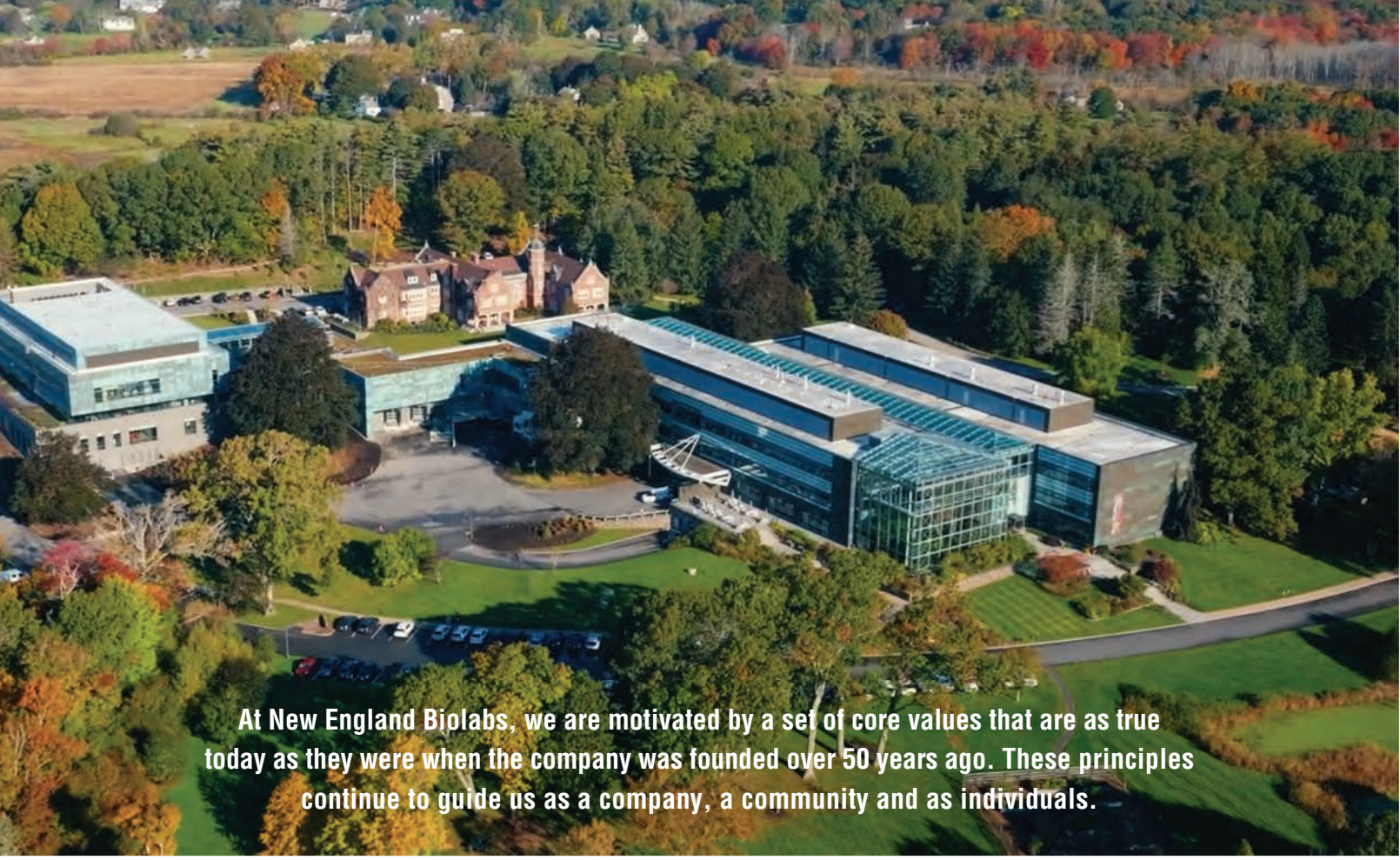
GET TO KNOW US!

NEB scientists and staff regularly attend large society shows, as well as regional tradeshows, conferences, workshops and other local events.

NEB events
Visit our events page to find out where we will be each week.

nebtv webinar series
Find webinars hosted by NEB scientists.

LESSONS from Lab & Life
Listen to NEB scientists share helpful tips in our podcast series.



At New England Biolabs, we are motivated by a set of core values that are as true today as they were when the company was founded over 50 years ago. These principles continue to guide us as a company, a community and as individuals.

Advancement of Science

We believe that basic research and the cultivation of scientific knowledge is critical for us to stay connected with our customers and to drive scientific breakthroughs. At NEB, over 35 labs participate in research projects, which are aided by post-doctoral fellows and students in Masters and Ph.D. programs. NEB researchers have authored or co-authored over 1,500 publications to date, the vast majority of which are in peer-reviewed journals. To learn more, visit **www.neb.com/research**.

Sustainability Commitment

NEB was founded on the idea that we can advance science while simultaneously protecting the environment, preserving biodiversity, and being a good corporate citizen.

We continuously strive to advance ecologically sound and ethical practices in all that we do. From pioneering the first EPS shipping box takeback program in the 1970s to becoming one of the first certified B Corp™ companies in the life sciences in 2021, we are constantly seeking new ways to minimize our environmental impact, support our employees, and advance community health and equity, both locally and globally.

While we are proud of what we've accomplished over the last 50 years, and we know we can do more. Our sustainability plan outlines our targets for the future and provides insight into some of the unique ways that NEB employees across the company are advancing sustainability in life science. To learn more, visit **www.neb.com/environmentalphilosophy**.

Certified B Corporation

Social Responsibility

We see opportunities where science can be used to improve lives, and we continue to be guided by our responsibility to each other and our community to work towards a kinder and more just world. This philosophy lies behind NEB's longstanding commitment to its parasitology research program, which contributes to the understanding and treatment of poorly-funded and understudied tropical diseases. NEB also supports several organizations devoted to humanitarian efforts. Further, we recognize that we must work together to build a more equitable society and improve diversity, equity and inclusion in our workplace. To learn more, visit **www.neb.com/corporateresponsibility**.

Delivering the Highest Quality Product

It is our goal to deliver best-in-class product quality and technical support. NEB holds ISO 13485:2016 and ISO 9001:2015 certifications at its manufacturing facilities in Ipswich, Rowley, and Beverly, MA, USA. Our manufacturing facility in Rowley, MA produces GMP-grade* materials for customers requiring an enhanced level of quality documentation and support. NEB Lyophilization Sciences®, located in Oxfordshire, UK, is equipped to develop and manufacture lyophilized molecular biology reagents for the life sciences, and is also ISO 13485:2016 and ISO 9001:2015 certified. We are constantly improving the stringency and range of our quality controls to ensure that our products will perform to your expectations, every time.

nqa ISO 13485 Registered Medical Devices

nqa ISO 9001 Registered Quality Management

nqa ISO 14001 Registered Environmental Management

*See page 6 for more details.

NEB Facilities in the U.S.

NEB headquarters is located in Ipswich, MA, and features a LEED® certified, state-of-the-art research and production facility. Approximately 15 minutes away, our production facility in Rowley, MA, is designed to serve the needs of customers in regulated markets and is used for manufacture of GMP-grade materials (see page 6). Also in Rowley, our packaging facility is responsible for kitting and packaging of a selection of NEB products. We have two locations in Beverly, MA, which is approximately 20 minutes from our main campus. Our Beverly Organic Synthesis Facility is responsible for synthesis and manufacture of oligonucleotides, modified nucleotides, and affinity beads/resins. Our R&D facility at Dunham Ridge houses many of our Research and Application & Product Development groups. To learn more, visit **www.neb.com/AboutNEB**.

Discover NEB and what makes it unique.

Photo credit: © Robert Benson Photography

Photo credit: © Robert Benson Photography

PARTNERING WITH NEB

NEB has over 50 years of experience in the discovery, development and manufacture of molecular biology reagents. These are essential components in a vast array of genomic and proteomic technologies that continue to transform our understanding of the world we live in, and ultimately the diagnosis and treatment of disease. With experience in fields as diverse as next generation sequencing, RNA biology, qPCR, and protein engineering, NEB is ready to work with you to help bring your technologies to market. Further, our global distribution network can help to ensure that your products have worldwide reach.

Customized Solutions

Creating the right partnership is essential when pioneering a new life science product. Every aspect of development — technical expertise, reagent optimization, manufacturing scale, turnaround time, reagent quality, and comprehensive logistical support — is vital for achieving your objectives. NEB's Customized Solutions Team is here to help, and serves as a bridge to the support and resources you need to navigate these complexities and ensure your success.
Learn more: **www.neb.com/customized-solutions**.

International Business

Our ability to successfully operate as both a research institute and a commercial enterprise in service of our customers is amplified by the extent of our global reach. The International Business Team at NEB operates worldwide to generate sustainable growth through an exceptional network of commercial operations that includes wholly-owned subsidiaries located in Australia, Canada, China, France, Germany, Japan, Singapore, South Korea and the United Kingdom. Additionally, NEB works with over 60 international distribution partners. Together, our subsidiary and distribution network enables us to support customers in more than 90 countries. By leveraging the talents and assets of NEB, including scientific and commercial resources, we ensure that our customers are serviced by a stable, ethical and engaged global network. More information can be found on the inside back cover or by contacting **globaldev@neb.com**.

NEBnow Freezer Program Network

With NEBnow® on-site freezers, enjoy convenient and affordable access to NEB's high quality reagents, anytime. Our NEBnow Freezer Program Team works closely with your institution to customize inventory best suited to your research program. Save time and avoid shipping fees with consolidated shipments. For more information, visit **www.neb.com/NEBnow** or contact **freezers@neb.com**.

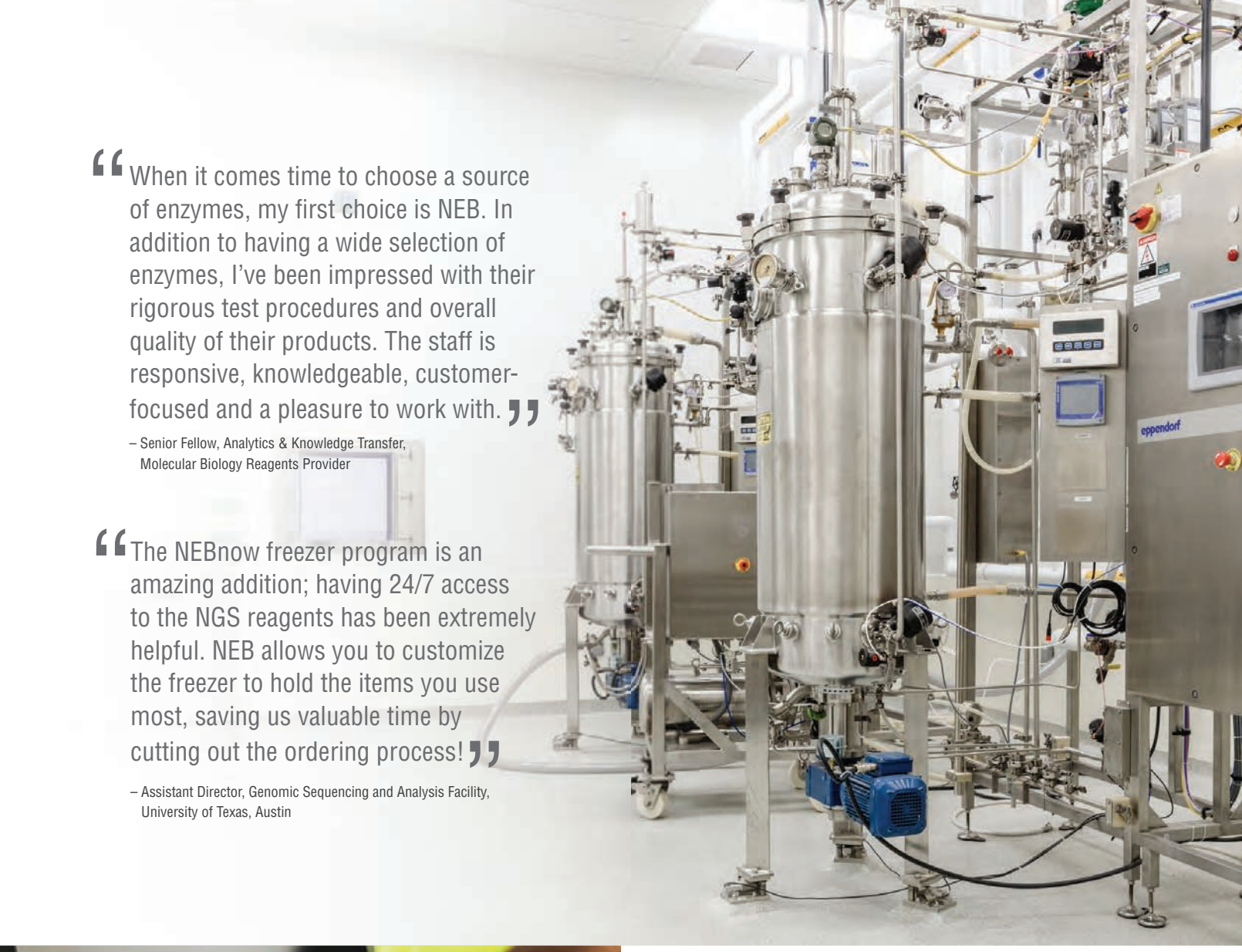
E-business/B2B Integration

NEB offers a wide range of B2B integrations to streamline your eProcurement initiatives. We support industry-standard platforms, and catalog sourcing solutions, such as punchout, which allows us to partner with you to make purchasing efficient, transparent, less resource-intensive, and cost-effective. Our experienced eBusiness Team will tailor a solution to your unique needs.
To find out more visit **www.neb.com/B2B**.

Enzymes for Innovation

The NEB catalog highlights a variety of enzyme functionalities found in nature or engineered for specific purposes. However, in molecular biology, new tools can often lead to new discoveries. Taking advantage of our enzymology expertise, we offer a growing selection of novel enzymes with interesting and unique activities for manipulating DNA, RNA, proteins and glycans, even if specific applications for them have yet to be discovered. If you are looking for an enzyme functionality that it is not currently available, visit **www.enzymesforinnovation.com** or contact **enzymesforinnovation@neb.com**.

See page 6 for more details.



> When it comes time to choose a source of enzymes, my first choice is NEB. In addition to having a wide selection of enzymes, I've been impressed with their rigorous test procedures and overall quality of their products. The staff is responsive, knowledgeable, customer-focused and a pleasure to work with.

– Senior Fellow, Analytics & Knowledge Transfer, Molecular Biology Reagents Provider

> The NEBnow freezer program is an amazing addition; having 24/7 access to the NGS reagents has been extremely helpful. NEB allows you to customize the freezer to hold the items you use most, saving us valuable time by cutting out the ordering process!

– Assistant Director, Genomic Sequencing and Analysis Facility, University of Texas, Austin

Practicing Ethical Science

NEB is committed to practicing ethical science – we believe it is our job as researchers to ask the important questions that, when answered, help preserve our quality of life and the world that we live in. However, this research should always be done in a safe and ethical manner. Learn more at **www.neb.com/neb-ethics**.

SUPPORTING REGULATED MARKETS

At NEB, we view every challenge as an opportunity. We know that your teams are pushing the boundaries of what is known today to develop innovative solutions for diagnosing and treating disease. Whether you are performing your first build or one of many, accessing innovative and critical materials at the scale you need is an important first step in bringing your assay or treatment to market.

With over 50 years of experience, we can draw upon our expertise in enzymology and reagent manufacturing to find solutions that best fit your needs. As an extension of your team, we will equip you with high-quality enzymes and reagents, whether you are at the stage of validation and verification, or preparing to scale-up production for a commercial launch. Our focus on understanding and supporting your needs means that we can offer you flexibility and customization, from development through to commercial scale. **We are committed to your success.**

Understanding your Needs and Minimizing Risks with GMP-grade* product manufacturing

NEB's expertise in enzyme manufacturing positions us to best anticipate your needs and minimize risk when transitioning from research use only (RUO) to GMP-grade reagents. The product attributes that differentiate NEB's GMP-grade products include:

- Bioburden and/or endotoxin specifications on products
- Certified animal-free origin and manufacturing process
- Qualified equipment, utilities, QC test methods and controlled manufacturing processes to deliver the highest levels of lot-to-lot consistency
- Validated QC methods designed to provide customers with quantitative values, where appropriate

NEB's GMP-grade products are produced in compliance with the ISO 13485:2016 Quality Management Standard, and are also in line with many of the principles defined in U.S. 21 CFR 820 "Quality System Regulation" – Medical Devices.

Our dedicated facility in Rowley, MA includes Quality Control and Production functions ranging from a shipping/receiving area and dedicated warehouse, to separate inoculation preparation, fermentation, purification and fill suites. To learn more, visit **www.neb.com/GMP**.

Learn about our manufacturing capabilities.

GMP* for more information about GMP-grade reagents for nucleic acid therapeutic manufacturing, visit the DNA Modifying Enzymes and RNA Analysis Chapters

*"GMP-grade" is a branding term NEB uses to describe reagents manufactured or finished at NEB's Rowley facility. The Rowley facility was designed to manufacture reagents under more rigorous infrastructure and process controls to achieve more stringent product specifications and customer requirements. Reagents manufactured at NEB's Rowley facility are manufactured in compliance with ISO 9001 and ISO 13485 quality management system standards. However, at this time, NEB does not manufacture or sell products known as Active Pharmaceutical Ingredients (APIs), nor does NEB manufacture its products in compliance with all of the Current Good Manufacturing Practice regulations.

Supporting Scientists Developing Molecular Diagnostics

The COVID-19 pandemic elevated many of the challenges faced by clinical labs, requiring new and innovative solutions to address them. Technology development is happening faster than ever before, increasing the need for innovation and thinking differently about how diagnostics should be developed, manufactured and deployed.

Many scientists know NEB as a trusted reagent provider to the life science community. What many do not know is that we also offer products and support for scientists who are working to develop diagnostics. Extensive molecular biology and enzymology experience enable us to help customers solve the challenges inherent in technology development and ultimately in scale-up and commercialization. Visit **www.neb.com/MDx** to learn more.

New England Biolabs Lyophilization Sciences

New England Biolabs Lyophilization Sciences Ltd. (NEB Lyo Sciences) is equipped to develop and manufacture lyophilized molecular biology reagents for the life sciences, including research, applied and the molecular diagnostics sectors. The NEB Lyophilization Sciences Team are experts in the design, development and manufacture of innovative solutions for ambient stored products. Visit **www.neb.com/lyosciences** to learn more.

NEW ENGLAND Biolabs®

The employee photo has been a tradition in the catalog for over 30 years.

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Deutschland

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Fax: +49/(0)69/305-23149

Email: info.de@neb.com
Online-Shop und technische Information:
www.neb-online.de

Kostenfreie Servicenummern

Telefon: 0800/246-5227 (in DE)
Fax: 0800/246-5229 (in DE)
Telefon: 00800/246-52277 (in AT)

Öffnungszeiten

Wir stehen Ihnen montags–freitags von 8:30 bis 17:30 Uhr persönlich zur Verfügung.

Kostenfreier Technischer Support

Nutzen Sie bei technischen Fragen gerne kostenfrei die Expertise unseres wissenschaftlichen Beratungsteams. Telefon: 0800/246-5227 (in DE) bzw. 00800/246-52277 (in AT) oder Email: techsupport.de@neb.com

24 Stunden Lieferservice

Bei Bestelleingang werktags (Mo-Do) bis 16:00 Uhr erhalten Sie Ihre Ware am nächsten Tag! Bestellungen, die vor einem Wochenende/Feiertag eingehen, werden am kommenden Montag/Werktag versendet.

Kühlversand ohne Styropor

Wir versenden in Kreislaufverpackungen aus nachwachsenden Rohstoffen – ganz ohne Styropor. Diese bestehen aus recyclingfähiger Kartonage aus Altpapier und nutzen die natürlichen isolierenden und dämmenden Eigenschaften von Hanf.

Verpackungs-/Transportpauschale

Deutschland
Kurier-/Kühlsendungen 17,50 €;
frachtkostenfrei ab einem Nettowert von 300 €.

Österreich
Kurier-/Kühlsendungen 25 €;
frachtkostenfrei ab einem Nettowert von 350 €.

Bestellungen über unseren Online-Shop, versenden wir ab einem Nettowert von 150,- Euro versandkostenfrei.

NEB erhebt keine zusätzlichen Mindermengen- oder Trockeneiszuschläge.

Garantie und Gewährleistung

NEB GmbH garantiert die Qualität aller Produkte im Rahmen der jeweiligen Produktspezifikation. Bei Nichterfüllung liefern wir kostenfreien Ersatz. Die Reklamation von fehlerhaften Produkten muss innerhalb von 60 Tagen nach Erhalt der Ware erfolgen.

Zahlung

Die Rechnungsbeträge sind innerhalb von 30 Tagen nach Erhalt der Ware rein netto zur Zahlung fällig.

Erstausstattungsrabatt

Wir sind Ihnen bei der Erstausstattung Ihres neuen Labors gerne behilflich und entwickeln mit Ihnen gemeinsam eine optimale Start-Strategie.

Konsignationslager/ Freezer Programm

Wir stellen Ihnen gerne ein individuell zugeschnittenes Depot der von Ihnen häufig benötigten Produkte vor Ort zur Verfügung. Unmittelbarer Zugriff auf diese Produkte und minimaler Verwaltungsaufwand sind die Leitgedanken des NEB Freezer Programms. Bitte fordern Sie unsere detaillierten Unterlagen an!

Großmengen, Sonderformulierungen und -abfüllungen, GMP-grade*

Wir bieten Ihnen unsere Katalogprodukte auch in kundenspezifischen Mengen, Formulierungen oder Verpackungen an. Viele Produkte bieten wir außerdem in "GMP Qualität" an.
Bitte richten Sie Ihre Anfragen an unsere kostenfreie Servicenummer oder direkt an **custom.de@neb.com.**
*Weitere Informationen auf S.6.

Beschränkungen und Haftung

Die Produkte und Angebote von NEB richten sich ausschließlich an gewerbliche Nutzer, Universitäten, Forschungs- und Bildungseinrichtungen etc., nicht jedoch an Verbraucher/Privatpersonen. NEB Produkte sind Forschungsreagenzien und ausschließlich für *in vitro* Anwendungen bestimmt. Die Verwendung durch ausgebildetes Laborpersonal wird vorausgesetzt. Weder NEB GmbH noch NEB Inc. haften für Schäden, die sich aus dem Gebrauch von NEB Produkten, direkt oder indirekt, ergeben oder ergeben könnten.

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Für weitere Informationen kontaktieren Sie bitte NEBs Global Business Development Team unter gbd@neb.com.

Unsere vollständigen Allgemeinen Geschäftsbedingungen finden Sie im Internet unter www.neb-online.de.

Für Irrtümer oder Druckfehler übernehmen wir keine Haftung.

Preise

Eine Preisliste aller Katalogprodukte finden Sie unter
www.neb-online.de/Katalogpreise

Supporting Non-Profits and Foundations

New England Biolabs has played a role in the establishment of several organizations that are advancing social responsibility and environmental stewardship worldwide.

New England Biolabs Foundation

The New England Biolabs Foundation is a private independent foundation whose mission is to foster community-based conservation of landscapes and seascapes, and the bio-cultural diversity found in these places. The foundation supports projects in selected countries of Central America, Andean South America, West Africa, and in coastal communities on the North Shore of Massachusetts. Learn more at **NEBF.org**.

creative action INSTITUTE
home of ArtCorps

Creative Action Institute catalyzes community-driven solutions that advance gender equality and build a sustainable planet. Through our experiential trainings, convenings and coaching, we develop leadership, build networks and support grassroots advocacy. Visit **CreativeActionInstitute.org** to learn more.

Northeastern University
The Ocean Genome Legacy Center of New England Biolabs
dedicated to preserving the biological diversity of the sea
www.northeastern.edu/ogl

The Ocean Genome Legacy Center of New England Biolabs is a non-profit research center dedicated to the conservation of marine genome diversity and maintaining a repository of genomic DNA from marine organisms around the world. Learn more at **northeastern.edu/ogl**.

Restriction Endonucleases

AatII
AccI
Acc65I
AciI
AclI
AcuI
AfeI
AflII
AflIII
AgeI-HF
AhdI
AleI-v2
AluI
AlwI
AlwNI
ApaI
ApaLI
ApeKI
ApoI-HF
AscI
AseI
AsiSI
AvaI
AvaII
AvrII
BaeI
BaeGI
BamHI
BamHI-HF
BanI
BanII
BbsI
BbsI-HF
BbvI
BbvCI
BccI
BceAI
BcgI
BciVI
BclI
BclI-HF
BcoDI
BfaI
BfuAI
BglI
BglII
BlpI
BmgBI
BmrI
BmtI-HF
BpmI
Bpu10I
BpuEI
BsaI-HFv2
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BsaWI
BsaXI
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BseYI
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BsiEI
BsiHKAI
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BsiWI-HF
BsmI
BsmAI
BsmBI-v2
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Bsp1286I
BspCNI
BspDI
BspEI
BspHI
BspMI
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BspQI-HF
BsrI
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BsrDI
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BssSI-v2
BstAPI
BstBI
BstEII-HF
BstNI
BstUI
BstXI
BstYI
BstZ17I-HF
Bsu36I
BtgI
BtgZI
BtsI-v2
BtsIMutI
BtsCI
Cac8I
ClaI
CspCI
CviKI-1
CviQI
DdeI
DpnI
DpnII
DraI
DraIII-HF
DrdI
EaeI
EagI-HF
EarI
EciI
Eco53kI
EcoNI
EcoO109I
EcoP15I
EcoRI
EcoRI-HF
EcoRV
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Esp3I
FatI
FauI
Fnu4HI
FokI
FseI
FspI
HaeII
HaeIII
HgaI
HhaI
HincII
HindIII
HindIII-HF
HinfI
HinP1I
HpaI
HpaII
HphI
Hpy99I
Hpy166II
Hpy188I
Hpy188III
HpyAV
HpyCH4III
HpyCH4IV
HpyCH4V
KasI
KpnI-HF
MboI
MboII
MfeI-HF
MluI-HF
MluCI
MlyI
MmeI
MnlI
MscI
MseI
MslI
MspI
MspA1I
MspJI
MwoI
NaeI
NarI
NciI
NcoI
NcoI-HF
NdeI
NgoMIV
NheI-HF
NlaIII
NlaIV
NmeAIII
NotI
NotI-HF
NruI-HF
NsiI
NsiI-HF
NspI
PacI
PaeR7I
PaqCI
PciI
PflFI
PflMI
PleI
PluTI
PmeI
PmlI
PpuMI
PshAI
PsiI-v2
PspGI
PspOMI
PspXI
PstI
PstI-HF
PvuI-HF
PvuII
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SacII
SalI
SalI-HF
SapI
Sau3AI
Sau96I
SbfI-HF
ScaI-HF
ScrFI
SexAI
SfaNI
SfcI
SfiI
SfoI
SgrAI
SmaI
SmlI
SnaBI
SpeI-HF
SphI
SphI-HF
SrfI
SspI-HF
StuI
StyI-HF
StyD4I
SwaI
TaqI-v2
TfiI
TseI
Tsp45I
TspMI
TspRI
Tth111I
XbaI
XcmI
XhoI
XmaI
XmnI
ZraI

DNA Polymerases & Amplification Technologies

DNA Modifying Enzymes & Cloning Technologies

Nucleic Acid Purification

NEBNext Reagents for Next Generation Sequencing

Markers & Ladders (DNA, RNA & Protein)

Genome Editing

RNA Reagents

Protein Expression & Purification

Competent Cells

Glycobiology & Protein Analysis Tools

Epigenetics

Cellular Analysis

Reference Appendix

PASSION FOR SCIENCE

As you explore this year's catalog, you will find minireviews highlighting the remarkable achievements of our 2024 Passion in Science® awardees. Their stories remind us of the extraordinary potential within the scientific community to lead with compassion, creativity and courage. We are honored to celebrate their contributions, and we hope their journeys inspire you as much as they have inspired us.

Minireviews were authored by Joanne Gibson, Joanne is a Marketing Communications Writer at NEB. She received her Ph.D. in Molecular Biology from the University of Sydney, Australia.

View this sculpture in 3D.

EXPLORE 3D

Sculpture on the NEB campus in Ipswich, MA, USA

Celebrating Passion for Science

Science has the power to illuminate, inspire and transform — not only our understanding of the natural world but also the communities we live in. At New England Biolabs (NEB), this belief is at the heart of everything we do. For over 50 years, NEB has been driven by foundational values: a commitment to advancing scientific discovery, fostering environmental stewardship, embracing creativity, and recognizing the humanity in science. It is with these values in mind that we established the Passion in Science Awards®, a celebration of scientists who go beyond the bench to impact the world in profound and lasting ways.

The Passion in Science Awards was first introduced in 2014 to honor those unsung heroes of the scientific community who devote not only their research, but their lives to causes that align with the broader ideals of a compassionate, equitable and sustainable world.

The inception of the Passion in Science Awards marked a natural extension of NEB's mission. From our earliest days, we have believed that scientific research thrives in an ecosystem where curiosity, collaboration and a sense of shared responsibility flourish. As we were celebrating our 40th anniversary in 2014, we thought it fitting to celebrate customers who share similar values as NEB — those individuals whose efforts exceed their scientific accomplishments to include meaningful contributions to society and the planet. These awards were envisioned as an event to share their stories and amplify their impact.

Since then, the Passion in Science Awards have grown into a signature initiative for NEB, held again in 2016, 2019, and most recently in 2024 during our 50th anniversary celebration. Each event has celebrated an inspiring array of innovators, mentors, artists and advocates whose work enriches their fields and uplifts their communities. These individuals remind us that science is not an isolated endeavor but deeply intertwined with culture, ethics and our collective future.

The four award categories highlight a diverse range of achievements that we believe reflect NEB's vision for science as a tool for positive change:

- **Scientific Mentorship** recognizes individuals who nurture the next generation of scientists, fostering environments of inclusion, support and intellectual growth.
- **Environmental Stewardship** celebrates those working to protect and restore our natural world, addressing urgent challenges such as climate change and biodiversity loss.
- **Art and Creativity** honors the intersection of science and the arts, where innovation flourishes and new perspectives develop.
- **Humanitarian Duty** shines a light on scientists who dedicate themselves to addressing social inequalities, improving global health or advancing education.

In an era shaped by global challenges — from environmental crises to social inequities — the Passion in Science Awards highlight actionable efforts and inspiring achievements, showcasing how cross-disciplinary approaches can lead to impactful solutions. It celebrates those individuals who truly demonstrate a passion for science.

Restriction Endonucleases

The leader in the discovery & production of restriction enzymes.

Having supplied restriction enzymes to the research community for almost 50 years, NEB has earned the reputation of being a leader in enzyme technologies. Working continuously to be worthy of that distinction, NEB strives to develop enzymes of the highest purity and unparalleled quality. NEB scientists continue to improve our existing portfolio, as well as explore the utility of NEB reagents in new technologies. As a result, NEB scientists continue to publish scientific papers and be awarded grants in this area. NEB has an extensive research and development group. We are proud to have been there first: the first to commercialize a recombinant enzyme, the first to introduce a nicking enzyme, and the first to supply rAlbumin formulations. In addition, NEB has a continuing history of innovation by engineering restriction enzymes with altered specificities and improved performance. Through ongoing research in these areas, we are committed to driving the innovations that allow us to offer maximum performance and convenience.

Featured Tools and Resources

Visit **NEBrestrictionenzymes.com** to find additional online tools, video tech tips and tutorials to help you in setting up restriction enzyme reactions.

Icon Descriptions

The gene encoding this enzyme was cloned at NEB.

This enzyme is purified from a recombinant source.

This enzyme has been engineered for maximum performance.

Time-Saver qualified enzymes will digest 1 µg of substrate DNA in 5–15 minutes using 1 µl of enzyme under recommended reaction conditions. These enzymes can also be used overnight with no loss of sample.

2*site Indicates that the restriction enzyme requires two or more sites for cleavage.

NEB r1.1 NEB r2.1 NEB r3.1 rCutSmart NEB U
Indicates which reaction buffer is supplied with the enzyme for optimal activity. Enzymes with buffer requirements not met by one of the four standard NEBuffers are supplied with their own unique NEBuffer (NEB U). NEBuffers are color-coded (NEB r1.1 – yellow, NEB r2.1 – blue, NEB r3.1 – red, rCutSmart – green) and supplied as 10X stocks with each enzyme. For more information, consult the Performance Chart found in the Technical Reference section.

Epi This enzyme is EpiMark validated for epigenetics studies.

rAlbumin This enzyme is supplied with a separate tube of Recombinant Albumin (rAlbumin). To obtain 100% activity, rAlbumin should be added to the 1X reaction mix to a final concentration as indicated.

dam dcm CpG This restriction enzyme is sensitive to dam, dcm, or CpG methylation (note that CpG methylation is applicable to eukaryotic genomic DNA only). For more information, see the methylation sensitivity section of the Technical Reference.

65° 80° No Indicates whether or not the enzyme can be heat inactivated. Enzymes are first tested by incubation at 65°C for 20 minutes; any enzyme not inactivated at 65°C is then tested by incubation at 80°C for 20 minutes. If an enzyme can be heat inactivated, the temperature is indicated in the icon.

25° 37° 50° 55° 60° 65° 75°
Indicates the enzyme's optimal incubation temperature.

dilA dilB dilC Indicates which diluent buffer (A, B or C) is recommended for making dilutions of restriction enzymes. More information can be found in the Technical Reference section.

What are restriction enzymes?

- AatII
- AccI
- Acc65I
- AciI
- AclI
- AcuI
- AfeI
- AflII
- AflIII
- AgeI-HF
- AhdI
- AleI-v2
- AluI
- AlwI
- AlwNI
- ApaI
- ApaLI
- ApeKI
- ApoI-HF
- AscI
- AseI
- AsiSI
- AvaI
- AvaII
- AvrII
- BaeI
- BaeGI
- BamHI
- BamHI-HF
- BanI
- BanII
- BbsI
- BbsI-HF
- BbvI
- BbvCI
- BccI
- BceAI
- BcgI
- BciVI
- BclI
- BclI-HF
- BcoDI
- BfaI
- BfuAI
- BglI
- BglII
- BlpI
- BmgBI
- BmrI
- BmtI-HF
- BpmI
- Bpu10I
- BpuEI
- BsaI-HFv2
- BsaAI
- BsaBI
- BsaHI
- BsaJI
- BsaWI
- BsaXI
- BseRI
- BseYI
- BsgI
- BsiEI
- BsiHKAI
- BsiWI
- BsiWI-HF
- BslI
- BsmI
- BsmAI
- BsmBI-v2
- BsmFI
- BsoBI
- Bsp1286I
- BspCNI
- BspDI
- BspEI
- BspHI
- BspMI
- BspQI
- BspQI-HF
- BsrI
- BsrBI
- BsrDI
- BsrFI-v2
- BsrGI-HF
- BssHII
- BssSI-v2
- BstAPI
- BstBI
- BstEII-HF
- BstNI
- BstUI
- BstXI
- BstYI
- BstZ17I-HF
- Bsu36I
- BtgI
- BtgZI
- BtsI-v2
- BtsIMutI
- BtsCI
- Cac8I
- ClaI
- CspCI
- CviKI-1
- CviQI
- DdeI
- DpnI
- DpnII
- DraI
- DraIII-HF
- DrdI
- EaeI
- EagI-HF
- EarI
- EciI
- Eco53kI
- EcoNI
- EcoO109I
- EcoP15I
- EcoRI
- EcoRI-HF
- EcoRV
- EcoRV-HF
- Esp3I
- FatI
- FauI
- Fnu4HI
- FokI
- FseI
- FspI
- HaeII
- HaeIII
- HgaI
- HhaI
- HincII
- HindIII
- HindIII-HF
- HinfI
- HinP1I
- HpaI
- HpaII
- HphI
- Hpy99I
- Hpy166II
- Hpy188I
- Hpy188III
- HpyAV
- HpyCH4III
- HpyCH4IV
- HpyCH4V
- KasI
- KpnI-HF
- MboI
- MboII
- MfeI-HF
- MluI-HF
- MluCI
- MlyI
- MmeI
- MnlI
- MscI
- MseI
- MslI
- MspI
- MspA1I
- MspJI
- MwoI
- NaeI
- NarI
- NciI
- NcoI
- NcoI-HF
- NdeI
- NgoMIV
- NheI-HF
- NlaIII
- NlaIV
- NmeAIII
- NotI
- NotI-HF
- NruI-HF
- NsiI
- NsiI-HF
- NspI
- PacI
- PaeR7I
- PaqCI
- PciI
- PflFI
- PflMI
- PleI
- PluTI
- PmeI
- PmlI
- PpuMI
- PshAI
- PsiI-v2
- PspGI
- PspOMI
- PspXI
- PstI
- PstI-HF
- PvuI-HF
- PvuII
- PvuII-HF
- RsaI
- RsrII
- SacI-HF
- SacII
- SalI
- SalI-HF
- SapI
- Sau3AI
- Sau96I
- SbfI-HF
- ScaI-HF
- ScrFI
- SexAI
- SfaNI
- SfcI
- SfiI
- SfoI
- SgrAI
- SmaI
- SmlI
- SnaBI
- SpeI-HF
- SphI
- SphI-HF
- SrfI
- SspI-HF
- StuI
- StyI-HF
- StyD4I
- SwaI
- TaqI-v2
- TfiI
- TseI
- Tsp45I
- TspMI
- TspRI
- Tth111I
- XbaI
- XcmI
- XhoI
- XmaI
- XmnI
- ZraI

Recombinant Enzyme

Nicking Endonucleases 54

- Nb.BbvCI
- Nb.BsmI
- Nb.BsrDI
- Nb.BssSI
- Nb.BtsI
- Nt.AlwI
- Nt.BbvCI
- Nt.BsmAI
- Nt.BspQI
- Nt.BstNBI
- WarmStart Nt.BstNBI
- Nt.CviPII

Homing Endonucleases 56

- I-CeuI
- I-SceI
- PI-PspI
- PI-SceI

Reaction Buffers 57

NEBuffer 1
NEBuffer 2
NEBuffer 3
NEBuffer 4
rCutSmart Buffer
NEBuffer Set (r1.1, r2.1, r3.1 and rCutSmart)
NEBuffer Set (EcoRI/SspI, DpnII)
S-adenosylmethionine (SAM)
Nuclease-free Water
NEBuffer r2.1
NEBuffer r3.1

Diluent Buffers 57

Diluent A (with rAlbumin)
Diluent B (with rAlbumin)
Diluent C (with rAlbumin)

Gel Loading Dyes 57

Gel Loading Dye, Blue (6X)
Gel Loading Dye, Orange (6X)
Gel Loading Dye, Purple (6X)
Gel Loading Dye, Purple (6X), no SDS

Other 57

Recombinant Albumin, Molecular Biology Grade
NEB Tube Opener

Learn about dam, dcm and CpG methylation.

Enzymes in green are supplied with rCutSmart Buffer.

Looking to bring **convenience** to your **workflow**?

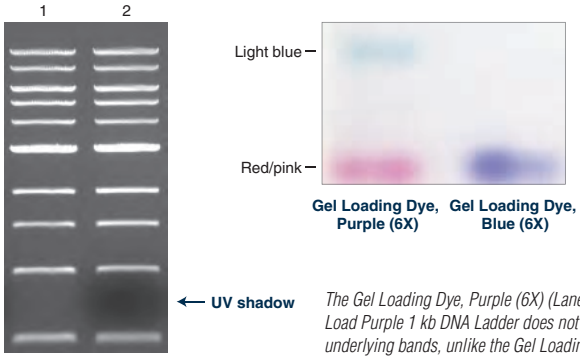
Speed up digestions with Time-Saver™ Qualified Restriction Enzymes

There are > 180 NEB restriction enzymes that can digest DNA in 5–15 minutes; many of which are supplied with rCutSmart Buffer or are High-Fidelity (HF®) Restriction Enzymes. If you prefer, you can also digest overnight with no unwanted star activity. All of our enzymes are rigorously tested for nuclease contamination. Only NEB can offer enzymes with the power to digest in 5–15 minutes, and the flexibility to withstand overnight digestions with no loss of sample.

www.neb.com/timesaver

Improve your analysis with our Purple Gel Loading Dye

Our Gel Loading Dye, Purple (6X), which is supplied with most restriction enzymes and all HF enzymes, sharpens bands and eliminates the UV shadow seen with other dyes. This solution contains SDS, which often results in sharper bands, as some restriction enzymes are known to remain bound to DNA following cleavage.



The Gel Loading Dye, Purple (6X) (Lane 1) included in the Quick-Load Purple 1 kb DNA Ladder does not cast a UV shadow over the underlying bands, unlike the Gel Loading Dye, Blue (6X) (Lane 2).

Simplify reaction setup and double digestion with rCutSmart™ Buffer

Over 210 enzymes are 100% active in a single buffer, rCutSmart Buffer, making it significantly easier to set up double digest reactions. Since rCutSmart Buffer includes Recombinant Albumin, there are fewer tubes and pipetting steps to worry about. Additionally, many DNA modifying enzymes are 100% functionally active in rCutSmart Buffer, eliminating the need for subsequent purification.

www.NEBCutSmart.com

Same high performance, now with BSA-free reaction buffer

To address the increased need for BSA-free reagents, NEB has switched our BSA-containing reaction buffers to Recombinant Albumin (rAlbumin)-containing buffers. We are also in the process of transitioning our enzyme formulations to contain rAlbumin. NEB has rigorously tested these changes and has not seen a difference in performance with these changes.

www.neb.com/BSA-free

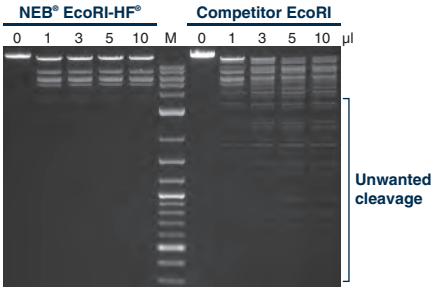
Learn about our switch to Recombinant Albumin.

Looking to **optimize performance** in your **reaction**?

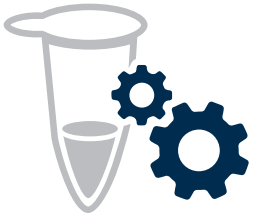
Choose High-Fidelity (HF®) Restriction Enzymes

NEB High-Fidelity (HF) restriction enzymes have the same specificity as native enzymes, with the added benefits of reduced star activity, rapid digestion (5–15 minutes), and 100% activity in rCutSmart Buffer. Enjoy the improved performance of our engineered enzymes at the same price as the native enzymes!

www.neb.com/HF



EcoRI-HF (NEB #R3101) shows no star activity in overnight digests, even when used at higher concentrations. *50 µl rxns were set up using 1 µg of Lambda DNA, the indicated amount of enzyme and the recommended reaction buffer. Rxns were incubated overnight at 37°C. Marker M is the 1 kb DNA Ladder (NEB #N3232).*

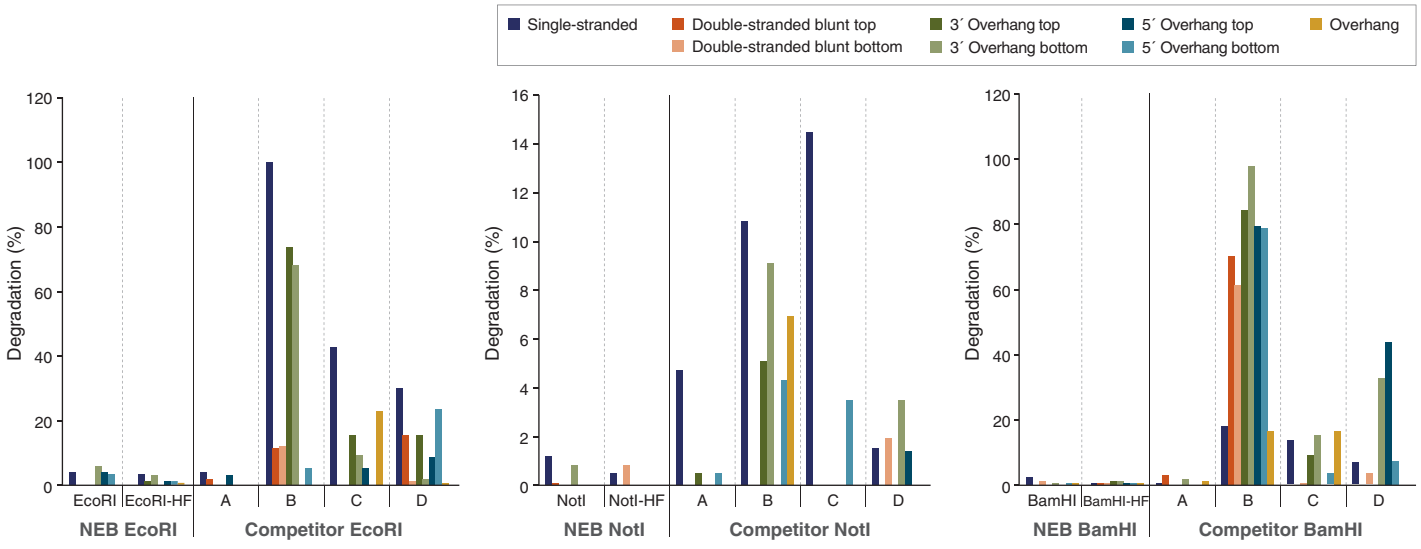


Benefit from industry-leading quality controls

NEB's reputation as a leader in enzyme technologies stems from the quality and reliability of our restriction enzymes. All of our restriction enzymes undergo stringent quality control testing, ensuring the highest levels of purity and lot-to-lot consistency.

www.neb.com/quality

Restriction Enzyme Competitor Study: Nuclease Contamination



EcoRI, NotI, and BamHI from multiple suppliers were tested in reactions containing a fluorescent labeled single stranded, double stranded blunt, 3´ overhang or 5´ overhang containing oligonucleotides. *The percent degradation is determined by capillary electrophoresis and peak analysis. The resolution is at the single nucleotide level.*

Learn about the benefits of HF enzymes.

AatII

rCutSmart · RR · dilB · 37° · 80° · CpG

#R0117S 500 units
#R0117L 2,500 units

5′... G A C G T ▼C ...3′
3′... C ▲T G C A G ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: May exhibit star activity in NEBuffer r2.1.

AclI

rCutSmart · RR · dilB · 37° · 80° · CpG

#R0598S 300 units
#R0598L 1,500 units

5′... A A ▼C G T T ... 3′
3′... T T G C ▲A A ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

AccI

rCutSmart · RR · dilA · 37° · 80° · CpG

#R0161S 1,000 units
#R0161L 5,000 units

5′... G T ▼M K A C ...3′
3′... C A K M ▲T G ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

AcuI

rCutSmart · RR · dilB · 37° · 65°

#R0641S 300 units
#R0641L 1,500 units

5′..CTGA AG $(N)_{16}$ ▼... 3′
3′..GACT TC $(N)_{14}$ ▲... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

Acc65I

NEB r3.1 · RR · dilA · 37° · 65° · CpG · dcm

#R0599S 2,000 units
#R0599L 10,000 units

5′... G ▼G T A C C ...3′
3′... C C A T ▲G G ...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	75	100	25

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Some Combinations of Overlapping
CpG: Blocked by Some Combinations of Overlapping

Note: May exhibit star activity in NEBuffer 2.1.

AfeI

rCutSmart · RR · dilB · 37° · 65° · CpG

#R0652S 200 units
#R0652L 1,000 units

5′... A G ▼C G C T ... 3′
3′... T C G ▲C G A ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	25	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

AciI

rCutSmart · RR · dilA · 37° · 65° · CpG

#R0551S 200 units
#R0551L 1,000 units

5′... C ▼C G C ...3′
3′... G G C ▲G ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	25	100	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

AflII

rCutSmart · RR · dilA · 37° · 65°

#R0520S 2,000 units
#R0520L 10,000 units

5′... C ▼T T A A G ... 3′
3′... G A A T ▲T C ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

AflIII

#R0541S 250 units
#R0541L 1,250 units

5′...A▼CRYGT...3′
3′...TGYRC▲A...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	100	50

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

AgeI-HF®

#R3552S 300 units
#R3552L 1,500 units

5′...A▼CCGGT...3′
3′...TGGCC▲A...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

AhdI

#R0584S 1,000 units
#R0584L 5,000 units

5′...GACNNN▼NNGTC...3′
3′...CTGNN▲NNNCAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	25	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Some Combinations of Overlapping

AleI-v2

#R0685S 500 units
#R0685L 2,500 units

5′...CACNN▼NNGTG...3′
3′...GTGNNN▲NCAC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Overlapping

AluI

#R0137S 1,000 units
#R0137L 5,000 units

5′...AG▼CT...3′
3′...TC▲GA...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	50	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

AlwI

#R0513S 500 units
#R0513L 2,500 units

5′...GGATC(N)$_4$▼...3′
3′...CCTAG(N)$_5$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	10	100

Methylation Sensitivity:
dam: Blocked
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

AlwNI

#R0514S 500 units
#R0514L 2,500 units

5′...CAGNNN▼CTG...3′
3′...GTC▲NNNGAC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	50	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Not Sensitive

ApaI

#R0114S 5,000 units
#R0114L 25,000 units

5′...GGGCC▼C...3′
3′...C▲CCGGG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 50,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	25	<10	100

Activity at 25°C: 100%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Blocked by Overlapping

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ApaLI

#R0507S 2,500 units
#R0507L 12,500 units
for high (5X) concentration
#R0507M 12,500 units

5′. . . G▼TGCAC . . . 3′
3′. . . CACGT▲G . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	10	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

ApeKI

#R0643S 250 units
#R0643L 1,250 units

5′. . . G▼CWGC . . . 3′
3′. . . CGWC▲G . . . 5′

Reaction Conditions: NEBuffer r3.1, 75°C

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	100	10

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

ApoI-HF®

#R3566S 1,000 units
#R3566L 5,000 units

5′. . . R▼AATTY . . . 3′
3′. . . YTTAA▲R . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

AscI

#R0558S 500 units
#R0558L 2,500 units

5′. . . GG▼CGCGCC . . . 3′
3′. . . CCGCGC▲GG . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	10	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

AseI

#R0526S 2,000 units
#R0526L 10,000 units
for high (5X) concentration
#R0526M 10,000 units

5′. . . AT▼TA AT . . . 3′
3′. . . TA AT▲TA . . . 5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 and 50,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	100	10

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in NEBuffer r2.1. Star activity may result from a glycerol concentration of >5%.

AsiSI

#R0630S 500 units
#R0630L 2,500 units

5′. . .GCGAT▼CGC . . . 3′
3′. . .CGC▲TAGCG . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: Star activity may result from extended digestion.

AvaI

#R0152S 2,000 units
#R0152L 10,000 units
for high (5X) concentration
#R0152T 2,000 units
#R0152M 10,000 units

5′. . .C▼YCGRG . . . 3′
3′. . .GRGCY▲C . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	100	25	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

AvaII

#R0153S 2,000 units
#R0153L 10,000 units
for high (5X) concentration
#R0153M 10,000 units

5′. . . G▼GWCC . . . 3′
3′. . . CCWG▲G . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	75	10	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Blocked by Overlapping

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

AvrII

#R0174S 100 units
#R0174L 500 units

5′. . . C▼CTAGG . . . 3′
3′. . . GGATC▲C . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BamHI-HF®

#R3136S 10,000 units
#R3136L 50,000 units
for high (5X) concentration
#R3136T 10,000 units
#R3136M 50,000 units

5′. . . G▼GATCC . . . 3′
3′. . . CCTAG▲G . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BaeI

#R0613S 250 units

5′. .▼$_{10}$(N) AC (N)$_4$GTAYC (N)$_{12}$▼. .3′
3′. .▲$_{15}$(N) TG (N)$_4$CATRG (N)$_7$▲. .5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Activity at 25°C: 100%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

BanI

#R0118S 5,000 units

5′. . . G▼GYRCC . . . 3′
3′. . . CCRYG▲G . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	25	<10	100

dcm: Blocked by Some Combinations of Overlapping
CpG: Blocked by Some Combinations of Overlapping

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

BaeGI

#R0708S 500 units

5′. . . GKGCM▼C . . . 3′
3′. . . C▲MCGKG . . . 5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	75	100	25

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BanII

#R0119S 2,000 units

5′. . . GRGCY▼C . . . 3′
3′. . . C▲YCGRG . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion.

BamHI

#R0136S 10,000 units
#R0136L 50,000 units
for high (5X) concentration
#R0136T 10,000 units
#R0136M 50,000 units

5′. . . G▼GATCC . . . 3′
3′. . . CCTAG▲G . . . 5′

Reaction Conditions: NEBuffer r3.1, 37°C

Concentration: 20,000 and 100,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in NEBuffer r1.1, r2.1 and rCutSmart Buffers. Star activity may result from a glycerol concentration of >5%.

BbsI

#R0539S 300 units
#R0539L 1,500 units

5′. . . GAAGAC (N)$_2$▼. . . 3′
3′. . . CTTCTG (N)$_6$▲. . . 5′

Reaction Conditions: NEBuffer r2.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	25	75

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

BbsI-HF®

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · Diluent Buffer B · 37° Incubation Temperature · 65° Heat Inactivation

#R3539S 300 units
#R3539L 1,500 units
for high (2X) concentration
#R3539M 1,000 units

5′... G A A G A C $(N)_2^{\blacktriangledown}$... 3′
3′... C T T C T G $(N)_{6\blacktriangle}$... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	10	100

Concentration: 20,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BbvI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · 2*site Multiple Recognition Site · Diluent Buffer B · 37° Incubation Temperature · 65° Heat Inactivation

#R0173S 300 units

5′... G C A G C $(N)_8^{\blacktriangledown}$ ... 3′
3′... C G T C G $(N)_{12\blacktriangle}$... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

BbvCI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Diluent Buffer B · 37° Incubation Temperature · No Heat Inactivation · CpG Methylation Sensitivity

#R0601S 100 units
#R0601L 500 units

5′... C C$^{\blacktriangledown}$T C A G C ... 3′
3′... G G A G T$_{\blacktriangle}$C G ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 2,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	50	100

dcm: Not Sensitive
CpG: Impaired by Overlapping

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

BccI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Diluent Buffer A · 37° Incubation Temperature · 65° Heat Inactivation

#R0704S 1,000 units
#R0704L 5,000 units

5′... C C A T C $(N)_4^{\blacktriangledown}$... 3′
3′... G G T A G $(N)_{5\blacktriangle}$... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with 2 mM DTT. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

BceAI

NEB r3.1 · Cloned at NEB · Recombinant Enzyme · Diluent Buffer A · 37° Incubation Temperature · 65° Heat Inactivation · CpG Methylation Sensitivity

#R0623S 50 units
#R0623L 250 units

5′... A C G G C $(N)_{12}^{\blacktriangledown}$... 3′
3′... T G C C G $(N)_{14\blacktriangle}$... 5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

dcm: Not Sensitive
CpG: Blocked

Note: May exhibit star activity in NEBuffer r1.1, r2.1 and rCutSmart Buffers. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

BcgI

NEB r3.1 · Cloned at NEB · Recombinant Enzyme · 2*site Multiple Recognition Site · Diluent Buffer A · 37° Incubation Temperature · 65° Heat Inactivation · CpG Methylation Sensitivity · dam Methylation Sensitivity

#R0545S 250 units

5′... $^{\blacktriangledown}_{10}$(N) C G A $(N)_6$ T G C $(N)_{12}^{\blacktriangledown}$... 3′
3′... $_{\blacktriangle 12}$(N) G C T $(N)_6$ A C G $(N)_{10\blacktriangle}$... 5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	75	100	50

Methylation Sensitivity:
dam: Impaired by Overlapping
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

Note: May exhibit Star Activity in NEBuffer r2.1 and rCutSmart Buffer.

BciVI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Diluent Buffer C · 37° Incubation Temperature · 80° Heat Inactivation

#R0596S 200 units
#R0596L 1,000 units

5′... G T A T C C $(N)_6^{\blacktriangledown}$... 3′
3′... C A T A G G $(N)_{5\blacktriangle}$... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	25	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BclI

NEB r3.1 · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Diluent Buffer A · 37° Incubation Temperature · No Heat Inactivation · dam Methylation Sensitivity

#R0160S 3,000 units

5′... T$^{\blacktriangledown}$G A T C A ... 3′
3′... A C T A G$_{\blacktriangle}$T ... 5′

Reaction Conditions: NEBuffer r3.1, 37°C.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	75

Methylation Sensitivity:
dam: Blocked
dcm: Not Sensitive
CpG: Not Sensitive

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

BclI-HF

rCutSmart | dil B | 37° | 65° | dam

#R3160S 3,000 units
#R3160L 15,000 units

5′... T▼G A T C A ...3′
3′... A C T A G▲T ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Blocked
dcm: Not Sensitive
CpG: Not Sensitive

BglI

NEB r3.1 | dil B | 37° | 65° | CpG

#R0143S 2,000 units
#R0143L 10,000 units

5′... G C C N N N N▼N G G C ...3′
3′... C G G N▲N N N N C C G ...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	25	100	10

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

BcoDI

rCutSmart | dil B | 37° | No | CpG

#R0542S 1,000 units

5′... G T C T C (N)$_1$▼... 3′
3′... C A G A G (N)$_5$▲... 5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	75	75	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Some Combinations of Overlapping

BglII

NEB r3.1 | dil A | 37° | No

#R0144S 2,000 units
#R0144L 10,000 units
for high (5X) concentration
#R0144M 10,000 units

5′... A▼G A T C T ...3′
3′... T C T A G▲A ...5′

Reaction Conditions: NEBuffer r3.1, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	100	<10

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BfaI

rCutSmart | dil B | 37° | 80°

#R0568S 500 units
#R0568L 2,500 units

5′... C▼T A G ...3′
3′... G A T▲C ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	10	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion.

BlpI

rCutSmart | dil A | 37° | No

#R0585S 500 units
#R0585L 2,500 units

5′... G C▼T N A G C ...3′
3′... C G A N▲T C G ...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BfuAI

NEB r3.1 | 2*site | dil B | 50° | 65° | CpG

#R0701S 250 units
#R0701L 1,250 units

5′... A C C T G C (N)$_4$▼... 3′
3′... T G G A C G (N)$_8$▲... 5′

Reaction Conditions: NEBuffer r3.1, 50°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Activity at 37°C: 25%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	25	100	10

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

BmgBI

NEB r3.1 | dil B | 37° | 65° | CpG

#R0628S 500 units
#R0628L 2,500 units

5′... C A C▼G T C ...3′
3′... G T G▲C A G ...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	10	100	10

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: Star activity may result from a glycerol concentration of >5%.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

BmrI

#R0600S 100 units

5′... A C T G G G (N)$_5$▼... 3′
3′... T G A C C C (N)$_4$▲... 5′

Reaction Conditions: NEBuffer r2.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	75	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in rCutSmart Buffer.

BpuEI

#R0633S 500 units

5′... C T T G A G (N)$_{16}$▼... 3′
3′... G A A C T C (N)$_{14}$▲... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in NEBuffer r1.1, and NEBuffer r3.1.

BmtI-HF®

#R3658S 300 units
#R3658L 1,500 units

5′... G C T A G▼C ... 3′
3′... C▲G A T C G ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BsaI-HF®v2

#R3733S 1,000 units
#R3733L 5,000 units

5′... G G T C T C (N)$_1$▼... 3′
3′... C C A G A G (N)$_5$▲... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Impaired by Some Combinations of Overlapping
CpG: Blocked by Some Combinations of Overlapping

Note: Available as a GMP-grade reagent. See page 6 for details.

BpmI

#R0565S 100 units
#R0565L 500 units

5′... C T G G A G (N)$_{16}$▼... 3′
3′... G A C C T C (N)$_{14}$▲... 5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion.

BsaAI

#R0531S 500 units

5′... Y A C▼G T R ... 3′
3′... R T G▲C A Y ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Bpu10I

#R0649S 200 units

5′... C C▼T N A G C ... 3′
3′... G G A N T▲C G ... 5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	25	100	25

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

BsaBI

#R0537S 2,000 units

5′... G A T N N▼N N A T C ... 3′
3′... C T A N N▲N N T A G ... 5′

Reaction Conditions: rCutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 37°C: 25%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	75	100

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

Note: Star activity may result from extended digestion.

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

BsaHI

rCutSmart | RR | dilC | 37° | 80° | CpG | dcm

#R0556S 2,000 units

5′...GR▼CGYC...3′
3′...CYGC▲RG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Some Combinations of Overlapping
CpG: Blocked

BseRI

rCutSmart | RR | dilA | 37° | 80°

#R0581S 200 units
#R0581L 1,000 units

5′...GAGGAG(N)$_{10}$▼...3′
3′...CTCCTC(N)$_{8}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	75	100

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BsaJI

rCutSmart | RR | dilA | 60° | 80°

#R0536S 1,000 units

5′...C▼CNNGG...3′
3′...GGNNC▲C...5′

Reaction Conditions: rCutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Activity at 37°C: 25%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BseYI

NEB r3.1 | RR | dilB | 37° | 80° | CpG

#R0635S 100 units
#R0635L 500 units

5′...C▼CAGC...3′
3′...GGGTC▲G...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	100	50

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

BsaWI

rCutSmart | RR | dilA | 60° | 80°

#R0567S 250 units

5′...W▼CCGGW...3′
3′...WGGCC▲W...5′

Reaction Conditions: rCutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	50	100

Activity at 37°C: 50%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BsgI

rCutSmart | RR | 2*site | dilB | 37° | 65°

#R0559S 50 units
#R0559L 250 units

5′...GTGCAG(N)$_{16}$▼...3′
3′...CACGTC(N)$_{14}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	25	100

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BsaXI

rCutSmart | dilC | 37° | 70°

#R0609S 100 units
#R0609L 500 units

5′...▼$_{9}$(N)AC(N)$_{5}$CTCC(N)$_{10}$▼...3′
3′...▲$_{12}$(N)TG(N)$_{5}$GAGG(N)$_{7}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in NEBuffer r1.1, and NEBuffer r2.1.

BsiEI

rCutSmart | RR | dilA | 60° | 70° | CpG

#R0554S 1,000 units

5′...CGRY▼CG...3′
3′...GC▲YRGC...5′

Reaction Conditions: rCutSmart Buffer, 60°C.

Concentration: 10,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

BsiHKAI

#R0570S 1,000 units

5′...GWGCW▼C...3′
3′...C▲WCGWG...5′

Reaction Conditions: rCutSmart Buffer, 65°C

Concentration: 10,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BsmI

#R0134S 500 units
#R0134L 2,500 units

5′...GAATGCN▼...3′
3′...CTTAC▲GN...5′

Reaction Conditions: rCutSmart Buffer, 65°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	<10	100

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BsiWI

#R0553S 300 units
#R0553L 1,500 units

5′...C▼GTACG...3′
3′...GCATG▲C...5′

Reaction Conditions: NEBuffer r3.1, 55°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 37°C: 25%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	100	25

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: May exhibit star activity in NEBuffer r2.1.

BsmAI

#R0529S 1,000 units
#R0529L 5,000 units

5′...GTCTC(N)$_1$▼...3′
3′...CAGAG(N)$_5$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

BsiWI-HF®

#R3553S 300 units
#R3553L 1,500 units

5′...C▼GTACG...3′
3′...GCATG▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

BsmBI-v2

#R0739S 200 units
#R0739L 1,000 units

5′...CGTCTC(N)$_1$▼...3′
3′...GCAGAG(N)$_5$▲...5′

Reaction Conditions: NEBuffer r3.1, 55°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	100	25

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

BslI

#R0555S 1,000 units
#R0555L 5,000 units

5′...CCNNNNN▼NNGG...3′
3′...GGNN▲NNNNNCC...5′

Reaction Conditions: rCutSmart Buffer, 55°C

Concentration: 10,000 units/ml

Activity at 37°C: 50%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	75	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Some Combinations of Overlapping
CpG: Blocked by Some Combinations of Overlapping

BsmFI

#R0572S 100 units
#R0572L 500 units

5′...GGGAC(N)$_{10}$▼...3′
3′...CCCTG(N)$_{14}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Blocked by Overlapping

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

BsoBI

rCutSmart · dilA · 37°C · 80°C

#R0586S 10,000 units

5′...C▼Y C G R G...3′
3′...G R G C Y▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BspEI

NEB r3.1 · dilB · 37°C · 80°C · CpG · dam

#R0540S 1,000 units
#R0540L 5,000 units

5′...T▼CCGGA...3′
3′...AGGCC▲T...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	10	100	<10

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Impaired

Bsp1286I

rCutSmart · dilA · 37°C · 65°C

#R0120S 500 units

5′...G D G C H▼C...3′
3′...C▲H C G D G...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	25	25	100

Methylation Sensitivity:
dam methylation: Not Sensitive
dcm methylation: Not Sensitive
CpG methylation: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

BspHI

rCutSmart · dilA · 37°C · 80°C · dam

#R0517S 500 units
#R0517L 2,500 units

5′...T▼C A T G A...3′
3′...A G T A C▲T...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	25	100

Methylation Sensitivity:
dam: Impaired by Overlapping
dcm: Not Sensitive
CpG: Not Sensitive

BspCNI

rCutSmart · dilA · 37°C · 80°C

#R0624S 100 units

5′...C T C A G $(N)_{10}$▼...3′ and 5′...C T C A G $(N)_9$▼...3′
3′...G A G T C $(N)_{5}$▲...5′ 3′...G A G T C $(N)_7$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	75	10	100

Activity at 25°C: 100%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BspMI

NEB r3.1 · 2*site · dilB · 37°C · 65°C

#R0502S 100 units

5′...A C C T G C $(N)_4$▼...3′
3′...T G G A C G $(N)_8$▲...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	100	10

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in NEBuffer r2.1.

BspDI

rCutSmart · dilA · 37°C · 80°C · CpG · dam

#R0557S 2,000 units

5′...A T▼C G A T...3′
3′...T A G C▲T A...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	75	50	100

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Blocked

BspQI

NEB r3.1 · dilB · 50°C · 80°C

#R0712S 500 units
#R0712L 2,500 units

5′...G C T C T T C $(N)_1$▼...3′
3′...C G A G A A G $(N)_4$▲...5′

Reaction Conditions: NEBuffer r3.1, 50°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 37°C: 50%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Now available as a GMP-grade reagent. Star activity may result from a glycerol concentration of >5%.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

BspQI-HF®

#R3712L 2,500 units
#R3712S 500 units

5′...GCTCTTC(N)$_1$▼...3′
3′...CGAGAAG(N)$_4$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BsrFI-v2

#R0682S 1,000 units

5′...R▼CCGGY...3′
3′...YGGCC▲R...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	25	0	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

BsrI

#R0527S 1,000 units
#R0527L 5,000 units

5′...ACTGGN▼...3′
3′...TGAC▲CN...5′

Reaction Conditions: NEBuffer r3.1, 65°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	100	10

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BsrGI-HF®

#R3575S 1,000 units
#R3575L 5,000 units

5′...T▼GTACA...3′
3′...ACATG▲T...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BsrBI

#R0102S 1,000 units
#R0102L 5,000 units

5′...CCG▼CTC...3′
3′...GGC▲GAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

BssHII

#R0199S 500 units
#R0199L 2,500 units
for high (5X) concentration
#R0199M 2,500 units

5′...G▼CGCGC...3′
3′...CGCGC▲G...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 and 25,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

BsrDI

#R0574S 200 units
#R0574L 1,000 units

5′...GCAATGNN▼...3′
3′...CGTTAC▲NN...5′

Reaction Conditions: NEBuffer r2.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

Activity at 65°C: 100%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	75	25

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

BssSI-v2

#R0680S 200 units
#R0680L 1,000 units

5′...C▼ACGAG...3′
3′...GTGCT▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	25	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

BstAPI

rCutSmart · RR · dilA · 60° · 80° · CpG

#R0654S 200 units
#R0654L 1,000 units

5′...GCANNNNNTGC...3′
3′...CGTNNNNNNACG...5′

Reaction Conditions: rCutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	25	100

Activity at 37°C: 25%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

BstBI

rCutSmart · RR · dilA · 65° · No · CpG

#R0519S 2,500 units
#R0519L 12,500 units

5′...TTCGAA...3′
3′...AAGCTT...5′

Reaction Conditions: rCutSmart Buffer, 65°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	10	100

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

BstEII-HF®

rCutSmart · RR · e · dilA · 37° · No

#R3162S 2,000 units
#R3162L 10,000 units
for high (5X) concentration
#R3162M 10,000 units

5′...GGTNACC...3′
3′...CCANTGG...5′

Reaction Conditions: rCutSmart Buffer, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	10	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BstNI

NEB r3.1 · RR · dilA · 60° · No

#R0168S 3,000 units
#R0168L 15,000 units

5′...CCWGG...3′
3′...GGWCC...5′

Reaction Conditions: NEBuffer r3.1, 60°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	100	75

Activity at 37°C: 25%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BstUI

rCutSmart · RR · dilA · 60° · No · CpG

#R0518S 1,000 units
#R0518L 5,000 units

5′...CGCG...3′
3′...GCGC...5′

Reaction Conditions: rCutSmart Buffer, 60°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	25	100

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

BstXI

NEB r3.1 · RR · dilB · 37° · 80° · dcm

#R0113S 1,000 units
#R0113L 5,000 units

5′...CCANNNNNNTGG...3′
3′...GGTNNNNNNACC...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	100	25

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Some Combinations of Overlapping
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

BstYI

rCutSmart · RR · dilA · 60° · No

#R0523S 2,000 units

5′...RGATCY...3′
3′...YCTAGR...5′

Reaction Conditions: rCutSmart Buffer, 60°C

Concentration: 10,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	75	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BstZ17I-HF®

rCutSmart · RR · e · dilA · 37° · No · CpG

#R3594S 1,000 units
#R3594L 5,000 units

5′...GTATAC...3′
3′...CATATG...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

Bsu36I

#R0524S 1,000 units
#R0524L 5,000 units

5′...CC▼TNAGG...3′
3′...GGANT▲CC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BsIMutI

#R0664S 100 units

5′...CAGTGNN▼...3′
3′...GTCACNN▲...5′

Reaction Conditions: rCutSmart Buffer, 55°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 1,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Activity at 37°C: 50%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BtgI

#R0608S 1,000 units

5′...C▼CRYGG...3′
3′...GGYRC▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BtsCI

#R0647S 2,000 units

5′...GGATGNN▼...3′
3′...CCTAC▲NN...5′

Reaction Conditions: rCutSmart Buffer, 50°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	25	100

Activity at 37°C: 25%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BtgZI

#R0703S 100 units
#R0703L 500 units

5′...GCGATG(N)$_{10}$▼...3′
3′...CGCTAC(N)$_{14}$▲...5′

Reaction Conditions: rCutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

Activity at 37°C: 50%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	25	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired

Note: Star activity may result from a glycerol concentration of >5%.

Cac8I

#R0579L 500 units

5′...GCNN▼GC...3′
3′...CGN▲NCG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	75	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

BtsI-v2

#R0667S 500 units
#R0667L 2,500 units

5′...GCAGTGNN▼...3′
3′...CGTCAC▲NN...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	25	100

dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

ClaI

#R0197S 1,000 units
#R0197L 5,000 units

5′...AT▼CGAT...3′
3′...TAGC▲TA...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	50	100

Concentration: 10,000 units/ml

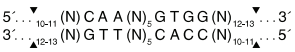
Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Blocked

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

CspCI

rCutSmart | RR | 2*site | dilA | 37° | 65°

#R0645S 500 units



Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	10	100

dcm: Not Sensitive
CpG: Not Sensitive

Note: The exact positions of cleavage can vary ± one base depending on the sequence of the DNA flanking the recognition site and the digestion conditions

CviKI-1

rCutSmart | RR | dilA | 37° | No

#R0710S 250 units

Reaction Conditions: rCutSmart Buffer, 37°C.

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

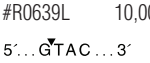
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

CviQI

NEB r3.1 | RR | dilC | 25° | No

#R0639S 2,000 units
#R0639L 10,000 units



Reaction Conditions: NEBuffer r3.1, 25°C

Concentration: 10,000 units/ml

Activity at 37°C: 25%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	75

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in NEBuffer r2.1, and rCutSmart Buffer.

DdeI

rCutSmart | RR | dilB | 37° | 65°

#R0175S 1,000 units
#R0175L 5,000 units

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

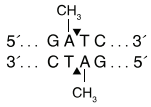
Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

DpnI

rCutSmart | RR | dilB | 37° | 80° | CpG | Epi

#R0176S 1,000 units
#R0176L 5,000 units



Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	75	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

Note: DpnI cleaves only when its recognition site is methylated. DNA purified from a dam^+ strain will be a substrate for DpnI.

DpnII

NEBU | RR | dilB | 37° | 65° | dam | Epi

#R0543S 1,000 units
#R0543L 5,000 units
for high (5X) concentration
#R0543T 1,000 units
#R0543M 5,000 units

Reaction Conditions:
Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 and 50,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	25	100	25

Methylation Sensitivity:
dam: Blocked
dcm: Not Sensitive
CpG: Not Sensitive

Note: Will exhibit star activity in NEBuffer 3.1. We recommend the use of NEB DpnII Unique Buffer.

DraI

rCutSmart | RR | dilA | 37° | 65°

#R0129S 2,000 units
#R0129L 10,000 units

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	75	50	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

DraIII-HF®

rCutSmart | RR | e | dilB | 37° | No | CpG

#R3510S 1,000 units
#R3510L 5,000 units

Reaction Conditions: rCutSmart Buffer, 37°C.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Overlapping

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

DrdI

rCutSmart · Cloned at NEB · Recombinant Enzyme · diluent A · 37° · Heat Inactivation 65° · CpG

#R0530S 300 units
#R0530L 1,500 units

5′...GACNNNN▼NNGTC...3′
3′...CTGNN▲NNNNCAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	10	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked by Some Combinations of Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

EciI

rCutSmart · Cloned at NEB · Recombinant Enzyme · diluent A · 37° · Heat Inactivation 65° · CpG

#R0590S 100 units
#R0590L 500 units

5′...GGCGGA(N)$_{11}$▼...3′
3′...CCGCCT(N)$_{9}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	50	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked by Some Combinations of Overlapping

Note: Star activity may result from extended digestion.

EaeI

rCutSmart · Cloned at NEB · Recombinant Enzyme · diluent A · 37° · Heat Inactivation 65° · CpG · dcm

#R0508S 200 units
#R0508L 1,000 units

5′...Y▼GGCCR...3′
3′...RCCGG▲Y...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	<10	100

Concentration: 5,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Blocked by Overlapping

CpG: Blocked by Overlapping

Eco53kI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · diluent A · 37° · Heat Inactivation 65° · CpG

#R0116S 1,000 units

5′...GAG▼CTC...3′
3′...CTC▲GAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 25°C: 50%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	<10	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked by Some Combinations of Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

EagI-HF®

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · diluent B · 37° · Heat Inactivation 65° · CpG

#R3505S 500 units
#R3505L 2,500 units

for high (5X) concentration

#R3505M 2,500 units

5′...C▼GGCCG...3′
3′...GCCGG▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked

EcoNI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · diluent A · 37° · Heat Inactivation 65°

#R0521S 1,000 units
#R0521L 5,000 units

5′...CCTNN▼NNNAGG...3′
3′...GGANNN▲NNTCC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	75	100

Concentration: 10,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

EarI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · diluent B · 37° · Heat Inactivation 65° · CpG

#R0528S 500 units
#R0528L 2,500 units

5′...CTCTTC(N)$_{1}$▼...3′
3′...GAGAAG(N)$_{4}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	10	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Impaired by Overlapping

EcoO109I

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · diluent A · 37° · Heat Inactivation 65° · dcm

#R0503S 2,000 units

5′...RG▼GNCCY...3′
3′...YCCNG▲GR...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Blocked by Overlapping

CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · Recombinant Enzyme · Epi EpiMark Validated · Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · diluent A Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

EcoP15I

#R0646S 500 units

5′...CAGCAG(N)$_{25}$▼...3′
3′...GTCGTC(N)$_{27}$▲...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Supplement with 1X ATP. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

EcoRI

#R0101S 10,000 units
#R0101L 50,000 units

for high (5X) concentration
#R0101T 10,000 units
#R0101M 50,000 units

5′...G▼AATTC...3′
3′...CTTAA▲G...5′

Reaction Conditions: NEBuffer EcoRI/SspI, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	50	50

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

Note: May exhibit star activity in NEBuffer r2.1 or rCutSmart Buffer.

EcoRI-HF®

#R3101S 10,000 units
#R3101L 50,000 units

for high (5X) concentration
#R3101T 10,000 units
#R3101M 50,000 units

5′...G▼AATTC...3′
3′...CTTAA▲G...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

EcoRV

#R0195S 4,000 units
#R0195L 20,000 units

for high (5X) concentration
#R0195T 4,000 units
#R0195M 20,000 units

5′...GAT▼ATC...3′
3′...CTA▲TAG...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	100	10

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Some Combinations of Overlapping

EcoRV-HF®

#R3195S 4,000 units
#R3195L 20,000 units

for high (5X) concentration
#R3195T 4,000 units
#R3195M 20,000 units

5′...GAT▼ATC...3′
3′...CTA▲TAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Some Combinations of Overlapping

Esp3I

#R0734S 300 units
#R0734L 1,500 units

5′...CGTCTC(N)$_1$▼...3′
3′...GCAGAG(N)$_5$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

FatI

#R0650S 50 units
#R0650L 250 units

5′...▼CATG...3′
3′...GTAC▲...5′

Reaction Conditions: NEBuffer r2.1, 55°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	50	50

Activity at 37°C: 100%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

FauI

#R0651S 200 units

5′...CCCGC(N)$_4$▼...3′
3′...GGGCG(N)$_6$▲...5′

Reaction Conditions: rCutSmart Buffer, 55°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Activity at 37°C: 50%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: Star activity may result from a glycerol concentration of >5%.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

Fnu4HI

rCutSmart | RR | Time-Saver | dilA | 37° | No heat inactivation | CpG

#R0178S 200 units
#R0178L 1,000 units

5´. . . G C^N G C . . . 3´
3´. . . C G N_C G . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

HaeII

rCutSmart | RR | Time-Saver | dilA | 37° | 80° | CpG

#R0107S 2,000 units
#R0107L 10,000 units

5´. . . R G C G C^Y . . . 3´
3´. . . Y_C G C G R . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

FokI

rCutSmart | RR | 2+site | dilA | 37° | 65° | CpG | dcm

#R0109S 1,000 units
#R0109L 5,000 units

5´. . . G G A T G (N)$_9$^ . . . 3´
3´. . . C C T A C (N)$_{13}$_ . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	75	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Impaired by Overlapping
CpG: Impaired by Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

HaeIII

rCutSmart | RR | Time-Saver | dilA | 37° | 80°

#R0108S 3,000 units
#R0108L 15,000 units
for high (5X) concentration
#R0108T 3,000 units
#R0108M 15,000 units

5´. . . G G^C C . . . 3´
3´. . . C C_G G . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	25	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

FseI

rCutSmart | RR | Time-Saver | dilB | 37° | 65° | CpG | dcm

#R0588S 100 units
#R0588L 500 units

5´. . . G G C C G G^C C . . . 3´
3´. . . C C_G G C C G G . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	75	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Impaired by Some Combinations of Overlapping
CpG: Blocked

HgaI

NEB r1.1 | RR | dilA | 37° | 65° | CpG

#R0154S 100 units

5´. . . G A C G C (N)$_5$^ . . . 3´
3´. . . C T G C G (N)$_{10}$_ . . . 5´

Reaction Conditions: NEBuffer r1.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

FspI

rCutSmart | RR | Time-Saver | dilC | 37° | No heat inactivation | CpG

#R0135S 500 units
#R0135L 2,500 units

5´. . . T G C^G C A . . . 3´
3´. . . A C G_C G T . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

HhaI

rCutSmart | RR | Time-Saver | dilA | 37° | 65° | CpG

#R0139S 2,000 units
#R0139L 10,000 units

5´. . . G C G^C . . . 3´
3´. . . C_G C G . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

rCutSmart or NEB r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | Epi EpiMark Validated | e Engineered for Performance | 2+site Multiple Recognition Site | Time-Saver Qualified | dilA Diluent Buffer | 37° Incubation Temperature | 65° Heat Inactivation | dam Methylation Sensitivity | rAlbumin Recombinant Albumin

HincII

rCutSmart | RR | dilB | 37° | 65° | CpG

#R0103S 1,000 units
#R0103L 5,000 units

5′...GTY▼RAC...3′
3′...CAR▲YTG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked by Some Combinations of Overlapping

HinP1I

rCutSmart | RR | dilA | 37° | 65° | CpG

#R0124S 2,000 units

5′...G▼CGC...3′
3′...CGC▲G...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked

HindIII

NEB r2.1 | RR | dilB | 37° | 80°

#R0104S 10,000 units
#R0104L 50,000 units

for high (5X) concentration
#R0104T 10,000 units
#R0104M 50,000 units

5′...A▼AGCTT...3′
3′...TTCGA▲A...5′

Reaction Conditions: NEBuffer r2.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	50	50

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

Note: Star activity may result from extended digestion.

HpaI

rCutSmart | RR | dilA | 37° | No | CpG

#R0105S 500 units
#R0105L 2,500 units

5′...GTT▼AAC...3′
3′...CAA▲TTG...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 5,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	75	25	100

CpG: Blocked by Some Combinations of Overlapping

Note: May exhibit star activity in NEBuffer r2.1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

HindIII-HF®

rCutSmart | RR | e | dilB | 37° | 80°

#R3104S 10,000 units
#R3104L 50,000 units

for high (5X) concentration
#R3104T 10,000 units
#R3104M 50,000 units

5′...A▼AGCTT...3′
3′...TTCGA▲A...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

HpaII

rCutSmart | RR | dilA | 37° | 80° | CpG | Epi

#R0171S 2,000 units
#R0171L 10,000 units

for high (5X) concentration
#R0171M 10,000 units

5′...C▼CGG...3′
3′...GGC▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	<10	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked

HinfI

rCutSmart | RR | dilA | 37° | 80° | CpG

#R0155S 5,000 units
#R0155L 25,000 units

for high (5X) concentration
#R0155T 5,000 units
#R0155M 25,000 units

5′...G▼ANTC...3′
3′...CTNA▲G...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked by Some Combinations of Overlapping

HphI

rCutSmart | RR | dilB | 37° | 65° | dam | dcm

#R0158S 1,000 units
#R0158L 5,000 units

5′...GGTGA(N)$_8$▼...3′
3′...CCACT(N)$_7$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	<10	100

Methylation Sensitivity:

dam: Blocked

dcm: Blocked

CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

Hpy99I

rCutSmart · RR · dilA · 37° · 65° · CpG

#R0615S 100 units
#R0615L 500 units

5′... CGWCG ...3′
3′... GCWGC ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	10	<10	100

Concentration: 2,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

HpyAV

rCutSmart · RR · 37° · 65° · CpG

#R0621S 100 units
#R0621L 500 units

5′... CCTTC $(N)_6$ ...3′
3′... GGAAG $(N)_5$ ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

Hpy166II

rCutSmart · RR · dilC · 37° · 65° · CpG

#R0616S 1,000 units

5′... GTNNAC ...3′
3′... CANNTG ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

HpyCH4III

rCutSmart · RR · dilA · 37° · 65°

#R0618S 250 units
#R0618L 1,250 units

5′... ACNGT ...3′
3′... TGNCA ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	25	<10	100

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Hpy188I

rCutSmart · RR · dilA · 37° · 65° · dam

#R0617S 1,000 units
#R0617L 5,000 units

5′... TCNGA ...3′
3′... AGNCT ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	50	100

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

HpyCH4IV

rCutSmart · RR · dilA · 37° · 65° · CpG

#R0619S 500 units
#R0619L 2,500 units

5′... ACGT ...3′
3′... TGCA ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	25	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Hpy188III

rCutSmart · RR · dilB · 37° · 65° · CpG · dam

#R0622S 500 units
#R0622L 2,500 units

5′... TCNNGA ...3′
3′... AGNNCT ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	10	100

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Blocked by Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

HpyCH4V

rCutSmart · RR · dilA · 37° · 65°

#R0620S 100 units
#R0620L 500 units

5′... TGCA ...3′
3′... ACGT ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	25	100

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

KasI

rCutSmart | dilB | 37° | 65° | CpG

#R0544S 250 units
#R0544L 1,250 units

5′... G▼GCGCC ...3′
3′... CCGCG▲G ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: Star activity may result from a glycerol concentration of >5%.

KpnI-HF®

rCutSmart | e | dilA | 37° | No

#R3142S 4,000 units
#R3142L 20,000 units
for high (5X) concentration
#R3142M 20,000 units

5′... GGTAC▼C ...3′
3′... C▲CATGG ...5′

Reaction Conditions: rCutSmart Buffer, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	25	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

MboI

rCutSmart | dilA | 37° | 65° | dam

#R0147S 500 units
#R0147L 2,500 units
for high (5X) concentration
#R0147M 2,500 units

5′...▼GATC ...3′
3′... CTAG▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 and 25,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Methylation Sensitivity:
dam: Blocked
dcm: Not Sensitive
CpG: Not Sensitive

Note: MboI is blocked by *dam* methylation, however Sau3AI is not sensitive to *dam* methylation.

MboII

rCutSmart | 2*site | dilC | 37° | 65° | dam

#R0148S 300 units
#R0148L 1,500 units

5′... GAAGA $(N)_8$▼... 3′
3′... CTTCT $(N)_7$▲... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	50	100

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit Star Activity in NEBuffer r1.1.

MfeI-HF®

rCutSmart | e | dilA | 37° | No

#R3589S 500 units
#R3589L 2,500 units

5′... C▼AATTG ...3′
3′... GTTAA▲C ...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	25	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

MluI-HF®

rCutSmart | e | dilA | 37° | No | CpG

#R3198S 1,000 units
#R3198L 5,000 units

5′... A▼CGCGT ...3′
3′... TGCGC▲A ...5′

Reaction Conditions: rCutSmart Buffer, 37°C.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

MluCI

rCutSmart | dilA | 37° | No

#R0538S 1,000 units
#R0538L 5,000 units

5′...▼AATT ...3′
3′... TTAA▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	10	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

MlyI

rCutSmart | dilA | 37° | 65°

#R0610S 1,000 units
#R0610L 5,000 units

5′... GAGTC $(N)_5$▼... 3′
3′... CTCAG $(N)_5$▲... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

MmeI

rCutSmart | RR | 2*site | dilB | 37° | 65° | CpG

#R0637S 100 units
#R0637L 500 units

5′...TCCRAC(N)$_{20}$▼...3′
3′...AGGYTG(N)$_{18}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Concentration: 2,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

MnlI

rCutSmart | RR | dilB | 37° | 65°

#R0163S 500 units
#R0163L 2,500 units

5′...CCTC(N)$_{7}$▼...3′
3′...GGAG(N)$_{6}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	50	100

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

MscI

rCutSmart | RR | dilC | 37° | 80° | dcm

#R0534S 250 units
#R0534L 1,250 units
for high (5X) concentration
#R0534M 1,250 units

5′...TGG▼CCA...3′
3′...ACC▲GGT...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 and 25,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Not Sensitive

Note: The single MscI site in pBR322 overlaps a dcm methylation site; consequently, pBR322 which has been grown in a dcm– host should be used for cloning.

MseI

rCutSmart | RR | dilA | 37° | 65°

#R0525S 500 units
#R0525L 2,500 units
for high (5X) concentration
#R0525M 2,500 units

5′...T▼TAA...3′
3′...AAT▲T...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	75	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

MslI

rCutSmart | RR | dilA | 37° | 80°

#R0571S 500 units
#R0571L 2,500 units

5′...CAYNN▼NNRTG...3′
3′...GTRNN▲NNYAC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

MspI

rCutSmart | RR | dilA | 37° | No | Epi

#R0106S 5,000 units
#R0106L 25,000 units
for high (5X) concentration
#R0106T 5,000 units
#R0106M 25,000 units

5′...C▼CGG...3′
3′...GGC▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	50	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

MspA1I

rCutSmart | RR | dilB | 37° | 65° | CpG

#R0577S 500 units
#R0577L 2,500 units

5′...CMG▼CKG...3′
3′...GKC▲GMC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

MspJI

rCutSmart | RR | dilB | 37° | 65° | Epi

#R0661S 200 units
#R0661L 1,000 units

5′...^{m}CNNR(N)$_{9}$▼...3′
3′... GNNY(N)$_{13}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with 1X Enzyme Activator Solution. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

rCutSmart or NEB r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | Epi EpiMark Validated | Engineered for Performance | 2*site Multiple Recognition Site | Time-Saver Qualified | dilA Diluent Buffer | 37° Incubation Temperature | 65° Heat Inactivation | dam Methylation Sensitivity | rAlbumin Recombinant Albumin

MwoI

#R0573S 500 units
#R0573L 2,500 units

5′. . . GCNNNNN▼NNGC . . . 3′
3′. . . CGNN▲NNNNNCG . . . 5′

Reaction Conditions: rCutSmart Buffer, 60°C

Concentration: 5,000 units/ml

Activity at 37°C: 25%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

NcoI

#R0193S 1,000 units
#R0193L 5,000 units

for high (5X) concentration
#R0193T 1,000 units
#R0193M 5,000 units

5′. . . C▼CATGG . . . 3′
3′. . . GGTAC▲C . . . 5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

NaeI

#R0190S 500 units
#R0190L 2,500 units

5′. . . GCC▼GGC . . . 3′
3′. . . CGG▲CCG . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	25	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

NcoI-HF®

#R3193S 1,000 units
#R3193L 5,000 units

for high (5X) concentration
#R3193M 5,000 units

5′. . . C▼CATGG . . . 3′
3′. . . GGTAC▲C . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

NarI

#R0191S 500 units
#R0191L 2,500 units

5′. . . GG▼CGCC . . . 3′
3′. . . CCGC▲GG . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	10	100

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

NdeI

#R0111S 4,000 units
#R0111L 20,000 units

5′. . . CA▼TATG . . . 3′
3′. . . GTAT▲AC . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

NciI

#R0196S 2,000 units
#R0196L 10,000 units

5′. . . CC▼SGG . . . 3′
3′. . . GGS▲CC . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	25	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Overlapping

NgoMIV

#R0564S 1,000 units
#R0564L 5,000 units

5′. . . G▼CCGGC . . . 3′
3′. . . CGGCC▲G . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C.

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

dcm: Not Sensitive
CpG: Blocked

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

NheI-HF®

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · Diluent Buffer C · 37° Incubation Temperature · 80° Heat Inactivation · CpG Methylation Sensitivity

#R3131S 1,000 units
#R3131L 5,000 units
for high (5X) concentration
#R3131M 5,000 units

5′... G▼CTAGC ...3′
3′... CGATC▲G ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	25	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

NlaIII

rCutSmart · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · Diluent Buffer B · 37° Incubation Temperature · 65° Heat Inactivation

#R0125S 500 units
#R0125L 2,500 units

5′...CATG▼...3′
3′...▲GTAC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

NlaIV

rCutSmart · Cloned at NEB · Recombinant Enzyme · Diluent Buffer B · 37° Incubation Temperature · 65° Heat Inactivation · CpG Methylation Sensitivity · dcm Methylation Sensitivity

#R0126S 200 units

5′...GGN▼NCC...3′
3′...CCN▲NGG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Blocked by Overlapping

NmeAIII

rCutSmart · Cloned at NEB · Recombinant Enzyme · 2*site Multiple Recognition Site · Diluent Buffer B · 37° Incubation Temperature · 65° Heat Inactivation

#R0711S 250 units

5′...GCCGAG(N)$_{20\text{-}21}$▼...3′
3′...CGGCTC(N)$_{18\text{-}19}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	<10	100

CpG: Not Sensitive

Note: The cleavage point may shift one base pair depending on the DNA sequence context between the recognition site and the position of cleavage. For a given sequence, generally one cut site will predominate.

NotI

NEB r3.1 · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · Diluent Buffer C · 37° Incubation Temperature · 65° Heat Inactivation · CpG Methylation Sensitivity

#R0189S 500 units
#R0189L 2,500 units
for high (5X) concentration
#R0189M 2,500 units

5′... GC▼GGCCGC ...3′
3′... CGCCGG▲CG ...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	100	25

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

NotI-HF®

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · Diluent Buffer A · 37° Incubation Temperature · 65° Heat Inactivation · CpG Methylation Sensitivity

#R3189S 500 units
#R3189L 2,500 units
for high (5X) concentration
#R3189M 2,500 units

5′... GC▼GGCCGC ...3′
3′... CGCCGG▲CG ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	25	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

NruI-HF®

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · Diluent Buffer A · 37° Incubation Temperature · No Heat Inactivation · CpG Methylation Sensitivity · dam Methylation Sensitivity

#R3192S 1,000 units
#R3192L 5,000 units

5′...TCG▼CGA...3′
3′...AGC▲GCT...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	0	25	50	100

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Blocked

NsiI

NEB r3.1 · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · Diluent Buffer B · 37° Incubation Temperature · 65° Heat Inactivation

#R0127S 1,000 units
#R0127L 5,000 units

5′...ATGCA▼T...3′
3′...T▲ACGTA...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	75	100	25

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · Recombinant Enzyme · Epi EpiMark Validated · Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

NsiI-HF®

rCutSmart · RR · e · dilB · 37° · 80°

#R3127S 1,000 units
#R3127L 5,000 units

5′...ATGCA▼T...3′
3′...T▲ACGTA...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	20	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

NspI

rCutSmart · RR · e · dilA · 37° · 65°

#R0602S 250 units
#R0602L 1,250 units

5′...RCATG▼Y...3′
3′...Y▲GTACR...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

PacI

rCutSmart · RR · e · dilA · 37° · 65°

#R0547S 250 units
#R0547L 1,250 units

5′...TTAAT▼TAA...3′
3′...AAT▲TAATT...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	75	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

PaeR7I

rCutSmart · RR · e · dilA · 37° · No · CpG

#R0177S 2,000 units

5′...C▼TCGAG...3′
3′...GAGCT▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

PaqCI®

rCutSmart · RR · 2*site · dilB · 37° · 65° · CpG

#R0745S 200 units
#R0745L 1,000 units

5′...CACCTGC(N)$_4$▼...3′
3′...GTGGACG(N)$_8$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with PaqCI Activator. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Overlapping

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

PciI

r3.1 · RR · dilB · 37° · 80°

#R0655S 200 units
#R0655L 1,000 units

5′...A▼CATGT...3′
3′...TGTAC▲A...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	75	100	50

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit Star Activity in rCutSmart Buffer.

PflFI

rCutSmart · RR · e · dilA · 37° · 65°

#R0595S 2,000 units

5′...GACNN▼NGTC...3′
3′...CTGNN▲NCAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

PflMI

r3.1 · RR · e · dilA · 37° · 65° · dcm

#R0509S 1,000 units
#R0509L 5,000 units

5′...CCANNNN▼NTGG...3′
3′...GGTNNNN▲NACC...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	0	100	100	50

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

PleI

rCutSmart | RR | 2*site | dilA | 37° | 65° | CpG

#R0515S 1,000 units

5′...GAGTC(N)$_4$▼...3′
3′...CTCAG(N)$_5$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	25	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked by Some Combinations of Overlapping

PluTI

rCutSmart | RR | 2*site | dilA | 37° | 65° | CpG

#R0713S 500 units

5′...GGCGC▼C...3′
3′...C▲CGCGG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	25	<10	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked

PmeI

rCutSmart | RR | e | dilA | 37° | 65° | CpG

#R0560S 500 units
#R0560L 2,500 units

5′...GTTT▼AAAC...3′
3′...CAAA▲TTTG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	10	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked by Some Combinations of Overlapping

PmlI

rCutSmart | RR | e | dilA | 37° | 65° | CpG

#R0532S 2,000 units
#R0532L 10,000 units

5′...CAC▼GTG...3′
3′...GTG▲CAC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked

PpuMI

rCutSmart | RR | e | dilB | 37° | 80° | dcm

#R0506S 500 units
#R0506L 2,500 units

5′...RG▼GWCCY...3′
3′...YCCWG▲GR...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Blocked by Overlapping

CpG: Not Sensitive

PshAI

rCutSmart | RR | e | dilA | 37° | 65° | CpG

#R0593S 1,000 units
#R0593L 5,000 units

5′...GACNNN▼NGTC...3′
3′...CTGNNN▲NCAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	10	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked by Some Combinations of Overlapping

PsiI-v2

rCutSmart | RR | e | e | dilB | 37° | 65°

#R0744S 400 units
#R0744L 2,000 units

5′...TTA▼TAA...3′
3′...AAT▲ATT...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	10	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

PspGI

rCutSmart | RR | dilA | 75° | 80° | dcm

#R0611S 1,000 units

5′...▼CCWGG...3′
3′...GGWCC▲...5′

Reaction Conditions: rCutSmart Buffer, 75°C

Concentration: 10,000 units/ml

Activity at 37°C: 25%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	50	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Blocked

CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

rCutSmart or NEB r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | Epi EpiMark Validated | e Engineered for Performance | 2*site Multiple Recognition Site | Time-Saver Qualified | dilA Diluent Buffer | 37° Incubation Temperature | 65° Heat Inactivation | dam Methylation Sensitivity | rAlbumin Recombinant Albumin

PspOMI

rCutSmart | RR | dilB | 37° | 65° | CpG | dcm

#R0653S 1,500 units
#R0653L 7,500 units

5′...G▼GGCCC...3′
3′...CCCGG▲G...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	<10	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Impaired by Some Combinations of Overlapping

CpG: Blocked by Overlapping

PspXI

rCutSmart | RR | dilB | 37° | No | CpG

#R0656S 200 units
#R0656L 1,000 units

5′...VC▼TCGAGB...3′
3′...BGAGCT▲CV...5′

Reaction Conditions: rCutSmart Buffer, 37°C.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	100	25	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Impaired

PstI

NEB r3.1 | RR | dilC | 37° | 80°

#R0140S 10,000 units
#R0140L 50,000 units

for high (5X) concentration
#R0140T 10,000 units
#R0140M 50,000 units

5′... CTGCA▼G...3′
3′... G▲ACGTC...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	75	100	50

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

Note: May exhibit star activity in rCutSmart Buffer.

PstI-HF®

rCutSmart | RR | e | dilC | 37° | No

#R3140S 10,000 units
#R3140L 50,000 units

for high (5X) concentration
#R3140T 10,000 units
#R3140M 50,000 units

5′... CTGCA▼G...3′
3′... G▲ACGTC...5′

Reaction Conditions: rCutSmart Buffer, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	75	50	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

PvuI-HF®

rCutSmart | RR | e | dilB | 37° | No | CpG

#R3150S 500 units
#R3150L 2,500 units

5′...CGAT▼CG...3′
3′...GC▲TAGC...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked

PvuII

NEB r3.1 | RR | dilB | 37° | No

#R0151S 5,000 units
#R0151L 25,000 units

for high (5X) concentration
#R0151T 5,000 units
#R0151M 25,000 units

5′... CAG▼CTG...3′
3′... GTC▲GAC...5′

Reaction Conditions: NEBuffer r3.1, 37°C.

Concentration: 10,000 and 50,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

Note: May exhibit star activity in rCutSmart Buffer.

PvuII-HF®

rCutSmart | RR | e | dilB | 37° | No

#R3151S 5,000 units
#R3151L 25,000 units

for high (5X) concentration
#R3151M 25,000 units

5′... CAG▼CTG...3′
3′... GTC▲GAC...5′

Reaction Conditions: rCutSmart Buffer, 37°C.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

RsaI

rCutSmart | RR | dilA | 37° | No | CpG

#R0167S 1,000 units
#R0167L 5,000 units

5′...GT▼AC...3′
3′...CA▲TG...5′

Reaction Conditions: rCutSmart Buffer, 37°C.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	<10	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked by Some Combinations of Overlapping

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

RsrII

rCutSmart · RR · 2°site · diiC · 37° · 65° · CpG

#R0501S 500 units
#R0501L 2,500 units

5′...CG▼GWCCG...3′
3′...GCCWG▲GC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	75	10	100

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

SalI-HF®

rCutSmart · RR · e · diiA · 37° · 65° · CpG

#R3138S 2,000 units
#R3138L 10,000 units
for high (5X) concentration
#R3138T 2,000 units
#R3138M 10,000 units

5′... G▼TCGAC...3′
3′... CAGCT▲G...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	100	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

SacI-HF®

rCutSmart · RR · e · diiA · 37° · 65° · CpG

#R3156S 2,000 units
#R3156L 10,000 units
for high (5X) concentration
#R3156M 10,000 units

5′... GAGCT▼C...3′
3′... C▲TCGAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

SapI

rCutSmart · RR · diiB · 37° · 65°

#R0569S 250 units
#R0569L 1,250 units

5′...GCTCTTC(N)$_1$▼...3′
3′...CGAGAAG(N)$_4$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	50	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

SacII

rCutSmart · RR · 2°site · diiA · 37° · 65° · CpG

#R0157S 2,000 units
#R0157L 10,000 units

5′...CCGC▼GG...3′
3′...GG▲CGCC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Sau3AI

NEB r1.1 · RR · diiA · 37° · 65° · CpG

#R0169S 200 units
#R0169L 1,000 units

5′...▼GATC...3′
3′...CTAG▲...5′

Reaction Conditions: NEBuffer r1.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

SalI

NEB r3.1 · RR · diiA · 37° · 65° · CpG

#R0138S 2,000 units
#R0138L 10,000 units
for high (5X) concentration
#R0138T 2,000 units
#R0138M 10,000 units

5′... G▼TCGAC...3′
3′... CAGCT▲G...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	100	<10

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Sau96I

rCutSmart · RR · diiA · 37° · 65° · CpG · dcm

#R0165S 1,000 units

5′...G▼GNCC...3′
3′...CCNG▲G...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Blocked by Overlapping

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2°site Multiple Recognition Site · Time-Saver Qualified · diiA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

SbfI-HF®

rCutSmart · 37° · 80°

#R3642S 500 units
#R3642L 2,500 units

5′... CCTGCAGG...3′
3′... GGACGTCC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	25	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

ScaI-HF®

rCutSmart · dilB · 37° · 80°

#R3122S 1,000 units
#R3122L 5,000 units
for high (5X) concentration
#R3122M 5,000 units

5′... AGTACT...3′
3′... TCATGA...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

ScrFI

rCutSmart · dilC · 37° · 65° · CpG · dcm

#R0110S 1,000 units

5′...CCNGG...3′
3′...GGNCC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Blocked by Overlapping

Note: Star activity may result from extended digestion.

SexAI

rCutSmart · dilA · 37° · 65° · dcm

#R0605S 200 units
#R0605L 1,000 units

5′...ACCWGGT...3′
3′...TGGWCCA...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	75	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

SfaNI

r3.1 · dilB · 37° · 65° · CpG

#R0172S 300 units

5′...GCATC(N)$_5$▼...3′
3′...CGTAG(N)$_9$▲...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	75	100	25

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Some Combinations of Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

SfcI

rCutSmart · dilB · 37° · 65°

#R0561S 200 units
#R0561L 1,000 units

5′...CTRYAG...3′
3′...GAYRTC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	50	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

SfiI

rCutSmart · 2°site · dilC · 50° · No · CpG · dcm

#R0123S 3,000 units
#R0123L 15,000 units

5′...GGCCNNNNNGGCC...3′
3′...CCGGNNNNNCCGG...5′

Reaction Conditions: rCutSmart Buffer, 50°C.

Concentration: 20,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Impaired by Overlapping
CpG: Blocked by Some Combinations of Overlapping

SfoI

rCutSmart · dilB · 37° · No · CpG · dcm

#R0606S 500 units
#R0606L 2,500 units

5′... GGCGCC...3′
3′... CCGCGG...5′

Reaction Conditions: rCutSmart Buffer, 37°C.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Some Combinations of Overlapping
CpG: Blocked

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

SgrAI

rCutSmart | Cloned at NEB | RR | 2°site | dilA | 37° | 65° | CpG

#R0603S 1,000 units
#R0603L 5,000 units

5′...CR▼CCGGYG...3′
3′...GY▲GGCCRC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

SmaI

rCutSmart | Cloned at NEB | RR | Engineered for Performance | dilB | 37° | 65° | CpG

#R0141S 2,000 units
#R0141L 10,000 units

5′...CCC▼GGG...3′
3′...GGG▲CCC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Activity at 25°C: 100%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

SmlI

rCutSmart | Cloned at NEB | RR | dilA | 55° | NO

#R0597S 500 units
#R0597L 2,500 units

5′...C▼TYRAG...3′
3′...GARY▲TC...5′

Reaction Conditions: rCutSmart Buffer, 55°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	75	25	100

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

SnaBI

rCutSmart | Cloned at NEB | RR | dilA | 37° | 80° | CpG

#R0130S 500 units
#R0130L 2,500 units
for high (5X) concentration
#R0130M 2,500 units

5′...TAC▼GTA...3′
3′...ATG▲CAT...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 and 25,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: May exhibit star activity in NEBuffer r1.1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

SpeI-HF®

rCutSmart | Cloned at NEB | RR | Engineered for Performance | Time-Saver Qualified | dilC | 37° | 80°

#R3133S 500 units
#R3133L 2,500 units
for high (5X) concentration
#R3133M 2,500 units

5′...A▼CTAGT...3′
3′...TGATC▲A...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

SphI

NEB r2.1 | Cloned at NEB | RR | dilB | 37° | 65°

#R0182S 500 units
#R0182L 2,500 units
for high (8X) concentration
#R0182M 2,500 units

5′...GCATG▼C...3′
3′...C▲GTACG...5′

Reaction Conditions: NEBuffer r2.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 and 80,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion.

SphI-HF®

rCutSmart | Cloned at NEB | RR | Engineered for Performance | Time-Saver Qualified | dilB | 37° | 65°

#R3182S 500 units
#R3182L 2,500 units
for high (5X) concentration
#R3182M 2,500 units

5′...GCATG▼C...3′
3′...C▲GTACG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	25	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

SrfI

rCutSmart | Cloned at NEB | RR | Time-Saver Qualified | dilB | 37° | 65° | CpG

#R0629S 500 units
#R0629L 2,500 units

5′...GCCC▼GGGC...3′
3′...CGGG▲CCCG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	0	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

rCutSmart or NEB r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | Epi EpiMark Validated | Engineered for Performance | 2°site Multiple Recognition Site | Time-Saver Qualified | dilA Diluent Buffer | 37° Incubation Temperature | 65° Heat Inactivation | dam Methylation Sensitivity | rAlbumin Recombinant Albumin

SspI-HF®

rCutSmart 37° 65°

#R3132S 1,000 units
#R3132L 5,000 units
for high (5X) concentration
#R3132M 5,000 units

5′... A A T ▼ A T T ...3′
3′... T T A ▲ T A A ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

StuI

rCutSmart dilA 37° No dcm

#R0187S 1,000 units
#R0187L 5,000 units
for high (10X) concentration
#R0187M 5,000 units

5′... A G G ▼ C C T ...3′
3′... T C C ▲ G G A ...5′

Reaction Conditions: rCutSmart Buffer, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Concentration: 10,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Not Sensitive

StyI-HF®

rCutSmart dilA 37° 65°

#R3500S 3,000 units
#R3500L 15,000 units

5′... C ▼ C W W G G ...3′
3′... G G W W C ▲ C ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	25	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

StyD4I

rCutSmart dilB 37° 65° CpG dcm

#R0638S 200 units

5′... ▼ C C N G G ...3′
3′... G G N C C ▲ ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Impaired by Overlapping

SwaI

NEB r3.1 dilB 25° 65°

#R0604S 2,000 units
#R0604L 10,000 units

5′... A T T T ▼ A A A T ...3′
3′... T A A A ▲ T T T A ...5′

Reaction Conditions: NEBuffer r3.1, 25°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	100	10

Activity at 37°C: 25%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

TaqI-v2

rCutSmart dilB 65° No dam

#R0149S 4,000 units
#R0149L 20,000 units
for high (5X) concentration
#R0149T 4,000 units
#R0149M 20,000 units

5′... T ▼ C G A ...3′
3′... A G C ▲ T ...5′

Reaction Conditions: rCutSmart Buffer, 65°C.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Concentration: 20,000 and 100,000 units/ml

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Not Sensitive

TfiI

rCutSmart dilC 65° No CpG

#R0546S 500 units

5′... G ▼ A W T C ...3′
3′... C T W A ▲ G ...5′

Reaction Conditions: rCutSmart Buffer, 65°C.

Concentration: 10,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

TseI

rCutSmart dilB 65° No CpG

#R0591L 500 units

5′... G C W G C ...3′
3′... C G W C ▲ G ...5′

Reaction Conditions: rCutSmart Buffer, 65°C.

Concentration: 10,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

Tsp45I

rCutSmart RR dilA 65° No

#R0583S 400 units
#R0583L 2,000 units

5′...▼GTSAC...3′
3′...CASTG▲...5′

Reaction Conditions: rCutSmart Buffer, 65°C.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	<10	100

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Tth111I

rCutSmart RR dilB 65° No

#R0185S 400 units

5′...GACN▼NNGTC...3′
3′...CTGNNN▲NCAG...5′

ßReaction Conditions: rCutSmart Buffer, 65°C.

Concentration: 10,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

TspMI

rCutSmart dilB 75° No CpG

#R0709S 200 units

5′...C▼CCGGG...3′
3′...GGGCC▲C...5′

Reaction Conditions: rCutSmart Buffer, 75°C

Concentration: 5,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	75	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: May exhibit star activity in NEBuffer r1.1, NEBuffer r2.1 or NEBuffer r 3.1.

XbaI

rCutSmart RR dilA 37° 65° dam

#R0145S 3,000 units
#R0145L 15,000 units
for high (5X) concentration
#R0145T 3,000 units
#R0145M 15,000 units

5′...T▼CTAGA...3′
3′...AGATC▲T...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	100	75	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Not Sensitive

TspRI

rCutSmart RR dilB 65° No

#R0582S 1,000 units

5′...NNCASTGNN▼...3′
3′...▲NNGTSACNN...5′

Reaction Conditions: rCutSmart Buffer, 65°C

Concentration: 10,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

XcmI

NEB r2.1 RR dilC 37° 65°

#R0533S 1,000 units
#R0533L 5,000 units

5′...CCANNNNNN▼NNNNTGG...3′
3′...GGTNNNN▲NNNNNACC...5′

Reaction Conditions: NEBuffer r2.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion.

rCutSmart or NEB r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | Epi EpiMark Validated | Engineered for Performance | 2*site Multiple Recognition Site | Time-Saver Qualified | dilA Diluent Buffer | 37° Incubation Temperature | 65° Heat Inactivation | dam Methylation Sensitivity | rAlbumin Recombinant Albumin

XhoI

rCutSmart | RR | dilA | 37° | 65° | CpG

#R0146S 5,000 units
#R0146L 25,000 units
for high (5X) concentration
#R0146M 25,000 units

5′...C▼TCGAG...3′
3′...GAGCT▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired

XmnI

rCutSmart | RR | dilA | 37° | 65°

#R0194S 1,000 units
#R0194L 5,000 units

5′...GAANN▼NNTTC...3′
3′...CTTNNNNAAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	75	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

XmaI

rCutSmart | RR | dilA | 37° | 65° | CpG

#R0180S 500 units
#R0180L 2,500 units
for high (5X) concentration
#R0180M 2,500 units

5′...C▼CCGGG...3′
3′...GGGCC▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 and 50,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired

Note: Star activity may result from a glycerol concentration of >5%.

ZraI

rCutSmart | RR | dilB | 37° | 80° | CpG

#R0659S 200 units
#R0659L 1,000 units

5′...GAC▼GTC...3′
3′...CTG▲CAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	25	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Nancy (left) and Beth Ann (right) are members of our Quality Control Team. Together they have over 45 years of experience at NEB. Currently, Nancy is the Senior QC Operations Manager, while Beth Ann is a Senior Quality Control Scientist. Beth Ann also organizes the NEB Craft Fair.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

Nicking Endonucleases

As a rule, when restriction endonucleases bind to their recognition sequences in DNA, they cleave both strands of the duplex at the same time. Two independent hydrolytic reactions proceed in parallel, most often driven by the presence of two catalytic sites within each enzyme, one for hydrolyzing each strand. One of the focuses of our research program is to engineer restriction enzymes so that they hydrolyze only one strand of the duplex, generating DNA molecules that are "nicked", rather than cleaved. These conventional nicks (3´-hydroxyl, 5´-phosphate) can serve as initiation points for a variety of further enzymatic reactions such as replacement DNA synthesis, strand-displacement amplification (1), exonucleolytic degradation or the creation of small gaps (2).

Nicking endonucleases (NEases) are as simple to use as restriction endonucleases. Since the nicks generated by 6- or 7-base nicking endonucleases do not fragment DNA, their activities are monitored by conversion of supercoiled plasmids to open circles. Alternatively, substrates with nicking sites close enough on opposite strands to create a double-stranded cut can be used instead.

The uses of nicking endonucleases are still being explored. NEases can generate nicked or gapped duplex DNA for DNA mismatch repair studies and for diagnostic applications. The long overhangs that nicking enzymes make can be used in DNA fragment assembly. Nt.BbvCI has been used to generate long and non-complementary overhangs when used with XbaI in the USER® cloning protocol from NEB. Nicking endonucleases are also useful for isothermal DNA amplification, which relies on the production of site-specific nicks. For example, isothermal DNA amplification using Nt.BstNBI in concert with Vent® (exo⁻) DNA Polymerase (NEB #M0257) (EXPAR) has been reported for detection of a specific DNA sequence in a sample (3). Another isothermal DNA amplification technique [Nicking Endonuclease Mediated- DNA Amplification (NEMDA)] (4) has been described using the 3-base cutter Nt.CviPII and *Bst* DNA Polymerase I. Frequent cutting NEases can generate short partial duplex DNA fragments from genomic DNA. These fragments can be used for cloning or used as probes for hybridization-based applications. Nicking enzymes have also been used for genome mapping.

NEB continues to engineer more nicking enzymes, particularly in response to specific customer needs and applications.

References:

(1) Walker, G.T. et al. (1992) *Proc. Natl. Acad. Sci. USA*, 89, 392–396.

(2) Wang, H. and Hays, J.B. (2000) *Mol. Biotechnol.*, 15, 97–104.

(3) Van Ness, J. et al. (2003) *Proc. Natl. Acad. Sci. USA*, 89, 4504–4509.

(4) Chan, S.H. et al. (2004) *Nucl. Acids Res.*, 32, 6187–6199.

Nb.BbvCI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Diluent Buffer A · 37° Incubation Temperature · 80° Heat Inactivation

#R0631S 1,000 units
#R0631L 5,000 units

5´... C C T C A G C ... 3´
3´... G G A G T C G ... 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

Nb.BsrDI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Diluent Buffer A · 37° Incubation Temperature · 80° Heat Inactivation

#R0648S 1,000 units
#R0648L 5,000 units

5´... G C A A T G N N ... 3´
3´... C G T T A C N N ... 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

Nb.BsmI

NEB r3.1 · Cloned at NEB · Recombinant Enzyme · Diluent Buffer A · 65° Incubation Temperature · 80° Heat Inactivation

#R0706S 1,000 units

5´... G A A T G C N ... 3´
3´... C T T A C G N ... 5´

Reaction Conditions: NEBuffer r3.1, 65°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 37°C: 100%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	100	10

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

Nb.BssSI

NEB r3.1 · Cloned at NEB · Recombinant Enzyme · Diluent Buffer B · 37° Incubation Temperature · No Heat Inactivation

#R0681S 1,000 units
#R0681T 5,000 units

5´... C A C G A G ... 3´
3´... G T G C T C ... 5´

Reaction Conditions: NEBuffer r3.1, 37°C

Concentration: 20,000 and 100,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	100	25

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

Learn about nicking enzymes, including WarmStart Nt.BstNBI.

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · Recombinant Enzyme · Epi EpiMark Validated · Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

Nb.BtsI

rCutSmart · dilA · 37° · 80°

#R0707S 1,000 units

5′...GCAGTGNN...3′
3′...CGTCACNN...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	75	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Nt.BspQI

NEB r3.1 · dilB · 50° · 80°

#R0644S 1,000 units

5′...GCTCTTCN...3′
3′...CGAGAAGN...5′

Reaction Conditions: NEBuffer r3.1, 50°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 37°C: 50%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	25	100	10

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Nt.AlwI

rCutSmart · dilA · 37° · 80° · dam

#R0627S 500 units

5′...GGATCNNNNN...3′
3′...CCTAGNNNNN...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	100	100

Methylation Sensitivity:
dam: Blocked
dcm: Not Sensitive
CpG: Not Sensitive

Nt.BstNBI

NEB r3.1 · dilA · 55° · 80°

#R0607S 1,000 units
#R0607L 5,000 units

5′...GAGTCNNNNN...3′
3′...CTCAGNNNNN...5′

Reaction Conditions: NEBuffer r3.1, 55°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	0	10	100	10

Activity at 37°C: 50%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Nt.BbvCI

rCutSmart · dilA · 37° · 80° · CpG

#R0632S 1,000 units
#R0632L 5,000 units

5′...CCTCAGC...3′
3′...GGAGTCG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

WarmStart® Nt.BstNBI

NEB r3.1 · dilA · 55° · 80°

#R0725S 1,000 units

5′...GAGTCNNNNN...3′
3′...CTCAGNNNNN...5′

Reaction Conditions: NEBuffer r3.1, 55°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	0	10	100	25

Activity at 37°C: 0%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Nt.BsmAI

rCutSmart · dilA · 37° · 65° · CpG

#R0121S 500 units

5′...GTCTCNN...3′
3′...CAGAGNN...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Nt.CviPII

rCutSmart · dilA · 37° · 65° · CpG

#R0626S 40 units

5′...CCD...3′
3′...GGH...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

Homing Endonucleases

Homing endonucleases are double stranded DNases that have large, asymmetric recognition sites (12–40 base pairs) and coding sequences that are usually embedded in either introns or inteins (1). Introns are spliced out of precursor RNAs, while inteins are spliced out of precursor proteins (2,3). Homing endonucleases are named using conventions similar to those of restriction endonucleases with intron-encoded endonucleases containing the prefix, "I-" and intein endonucleases containing the prefix, "PI-"(1,7).

Homing endonuclease recognition sites are extremely rare. For example, an 18 base pair recognition sequence will occur only once in every 7 x 10^{10} base pairs of random sequence. This is equivalent to only one site in 20 mammalian-sized genomes (4). However, unlike standard restriction endonucleases, homing endonucleases tolerate some sequence degeneracy within their recognition sequence (5,6). As a result, their observed sequence specificity is typically in the range of 10–12 base pairs.

Homing endonucleases do not have stringently-defined recognition sequences in the way that restriction enzymes do. That is, single base changes do not abolish cleavage but reduce its efficiency to variable extents. The precise boundary of required bases is generally not known. The recognition sequence listed is one site that is known to be recognized and cleaved.

References:
(1) Belfort, M. and Roberts, R.J. (1997) *Nucleic Acids Res.*, 25, 3379–3388.
(2) Dujon, B. et al. (1989) *Gene*, 82, 115–118.
(3) Perler, F.B. et al. (1994) *Nucleic Acids Res.*, 22, 1125–1127.
(4) Jasin, M. (1996) *Trends in Genetics*, 12, 224–228.
(5) Gimble, F.S. and Wang, J. (1996) *J. Mol. Biol.*, 263, 163–180.
(6) Argast, M.G. et al. (1998) *J. Mol. Biol.*, 280, 345–353.
(7) Roberts, R.J. et al. (2003) *Nucleic Acids Res.*, 31, 1805–1812.

I-CeuI

rCutSmart · RR · dilB · 37° · 65°

#R0699S 500 units
#R0699L 2,500 units
(Supplied with 5 µg of plasmid DNA)

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	10	100

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Specificity: The homing or recognition site for this endonuclease is shown below:

5′...TAACTATAACGGTCCTAAGGTAGCGAA...3′
3′...ATTGATATTGCCAGGATTCCATCGCTT...5′

Concentration: 5,000 units/ml

Note: Homing endonucleases do not have stringently-defined recognition sequences in the way that restriction enzymes do. That is, single base changes do not abolish cleavage but reduce its efficiency to variable extents. The precise boundary of required bases is generally not known. The recognition sequence listed is one site that is known to be recognized and cleaved. Note that this enzyme requires a 3-hour incubation time.

I-SceI

rCutSmart · RR · dilB · 37° · 65°

#R0694S 500 units
#R0694L 2,500 units
(Supplied with 5 µg of plasmid DNA)

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	25	100

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Specificity: The homing or recognition site for this endonuclease is shown below:

5′...TAGGGATAACAGGGTAAT...3′
3′...ATCCCTATTGTCCCATTA...5′

Concentration: 5,000 units/ml

Note: Homing endonucleases do not have stringently-defined recognition sequences in the way that restriction enzymes do. That is, single base changes do not abolish cleavage but reduce its efficiency to variable extents. The precise boundary of required bases is generally not known. The recognition sequence listed is one site that is known to be recognized and cleaved.

PI-PspI

NEBU · Cloned at NEB · RR · dilB · 65° · No dam · rAlbumin

#R0695S 500 units
(Supplied with 5 µg of plasmid DNA)

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	10	10

Reaction Conditions: NEBuffer PI-PspI, 65°C. Supplement with Recombinant Albumin, Molecular Biology Grade.

Specificity: The homing or recognition site for this endonuclease is shown below:

5′...TGGCAAACAGCTATTATGGGTATTATGGGT...3′
3′...ACCGTTTGTCGATAATACCCATAATACCCA...5′

Concentration: 5,000 units/ml

Activity at 37°C: 10%

Note: Homing endonucleases do not have stringently-defined recognition sequences in the way that restriction enzymes do. That is, single base changes do not abolish cleavage but reduce its efficiency to variable extents. The precise boundary of required bases is generally not known. The recognition sequence listed is one site that is known to be recognized and cleaved. Note that this enzyme requires a 3-hour incubation time.

PI-SceI

NEBU · RR · dilB · 37° · 65° · rAlbumin

#R0696S 250 units
(Supplied with 5 µg of plasmid DNA)

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	10	10

Reaction Conditions: NEBuffer PI-SceI, 37°C. Supplement with Recombinant Albumin, Molecular Biology Grade. Heat inactivation: 65°C for 20 minutes.

Specificity: The homing or recognition site for this endonuclease is shown below:

5′...ATCTATGTCGGGTGCGGAGAAAGAGGTAATGAAATGG...3′
3′...TAGATACAGCCCACGCCTCTTTCTCCATTACTTTACC...5′

Concentration: 5,000 units/ml

Note: Homing endonucleases do not have stringently-defined recognition sequences in the way that restriction enzymes do. That is, single base changes do not abolish cleavage but reduce its efficiency to variable extents. The precise boundary of required bases is generally not known. The recognition sequence listed is one site that is known to be recognized and cleaved.

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

Recombinant Albumin, Molecular Biology Grade

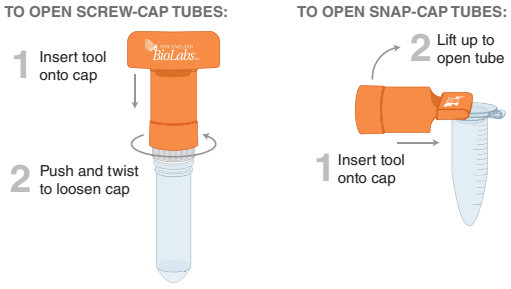
#B9200S 12 mg

Recombinant Albumin, Molecular Biology Grade, is a non-bovine derived albumin that can serve as an alternative to Bovine Serum Albumin (BSA). Like BSA, it has been shown to prevent adhesion of enzymes to reaction tubes and pipette surfaces. It also stabilizes some proteins during incubation. Choose Recombinant Albumin, when there is a need to avoid BSA.

NEB Tube Opener

#C1008S 2 Each

Description: Use to open a variety of microcentrifuge tubes. Can be used for snap-on caps or screw-on caps.



Reaction Buffers

NEBuffer 1
#B7001S 5 ml

NEBuffer 2
#B7002S 5 ml

NEBuffer 3
#B7003S 5 ml

NEBuffer 4
#B7004S 5 ml

rCutSmart Buffer
#B6004S 5 ml

NEBuffer Set (r1.1, r2.1, r3.1 and rCutSmart)
#B7030S 1.25 ml each

NEBuffer Set (EcoRI/SspI, DpnII)
#B7006S 1.25 ml each

S-adenosylmethionine (SAM)
#B9003 0.5 ml

Nuclease-free Water
#B1500S 25 ml
#B1500L 100 ml

NEBuffer r2.1
#B6002S 5 ml

NEBuffer r3.1
#B6003S 5 ml

Description: New England Biolabs provides a color-coded 10X NEBuffer with each restriction endonuclease to ensure optimal (100%) activity. Most of our enzymes are supplied with one of four standard NEBuffers. Occasionally, an enzyme has specific buffer requirements not met by one of the four standard NEBuffers, in which case the enzyme is supplied with its own unique NEBuffer.

The NEBuffer Sets contain multiple vials of buffers, which are indicated in the product name. NEBuffer Set (r1.1, r2.1, r3.1 and rCutSmart) is formulated with Recombinant Albumin.

Nuclease-free Water is ideal for the preparation of reagents and for use in enzymatic reactions. No toxic agents, such as DEPC, are used in the production of this water, so as to avoid inhibition in enzymatic reactions.

Reaction Buffer Compositions: See Performance Chart for Restriction Enzymes or visit www.neb.com for details.

Diluent Buffers

NEW
Diluent A (with rAlbumin)
#B8532S 5 ml

NEW
Diluent B (with rAlbumin)
#B8533S 5 ml

NEW
Diluent C (with rAlbumin)
#B8534S 5 ml

Description: Diluent Buffers are recommended for making dilutions of restriction endonucleases. When necessary, we recommend diluting enzymes just prior to use and suggest that the final concentration of diluted enzymes be at least 1,000 units/ml. Diluent preference for each restriction endonuclease is listed with its catalog entry.

Storage Conditions: Store at –20°C.

Diluent Buffer Compositions: See Performance Chart for Restriction Enzymes or visit www.neb.com for details.

Gel Loading Dyes

Gel Loading Dye, Blue (6X)
#B7021S 4 ml

Gel Loading Dye, Orange (6X)
#B7022S 4 ml

Gel Loading Dye, Purple (6X)
#B7024S 4 ml

Gel Loading Dye, Purple (6X), no SDS
#B7025S 4 ml

Description: Pre-mixed loading dye solutions are available with a choice of tracking dyes, for agarose and non-denaturing polyacrylamide gel electrophoresis. Three of the solutions contain SDS, which often results in sharper bands, as some restriction enzymes remain bound to their DNA substrates following cleavage. Some interference may be observed when using SYBR® or GelRed® as precast dyes in the presence of increased concentrations of SDS. When using these dyes as precast dyes, NEB recommends using our Gel Loading Dye, Purple (6X), no SDS (NEB #B7025). Gel Loading Dyes contain EDTA to chelate up to 10 mM magnesium, thereby stopping the reaction. Bromophenol blue is the standard tracking dye for electrophoresis. It migrates at approximately 300 bp on a standard 1% TBE agarose gel. Orange G will not appear in gel photographs, and runs ahead of all but the smallest restriction fragments, migrating at approximately 50 bp on a standard 1% TBE agarose gel. NEB also offers a unique purple dye which migrates similarly to Bromophenol blue. Specifically chosen, this dye does not leave a shadow under UV light.

Gel Loading Dye Compositions: Visit www.neb.com for details.

Note: Use 5 µl of Gel Loading Dye per 25 µl reaction, or 10 µl per 50 µl reaction. Mix well before loading gel. Store at room temperature.

View video discussing Don's work.

WATCH

Don Spratt teaching students
Credit: Clark University

Building Bridges Between Classrooms and Research Labs

Don Spratt's passion for science education and mentorship didn't begin with a grand plan but through a fortunate encounter and a desire to make a difference. It started when his neighbor, a high school co-op coordinator, mentioned they were looking for a biotech placement for a student. Spratt, a structural biologist at Clark University, immediately offered to host the student for a short-term experience in his lab.

A simple offer to host one student quickly grew into something much larger. Soon, students from several high schools visited Clark University to engage in hands-on science, including purifying proteins and exploring biotechnology. These visits became the core of Spratt's outreach, with over 1500 students participating, creating a powerful word-of-mouth initiative connecting his lab with schools across Worcester, MA, USA.

Despite the challenges of the COVID-19 pandemic, Spratt adapted by shifting his outreach online. Over 18 months, he continued to engage students virtually through interactive sessions, expanding his reach to new schools and maintaining strong ties with Worcester's education community. The shift to virtual learning allowed Spratt to reach more students than ever before.

As in-person programs resumed, Spratt reinstituted his hands-on lab sessions at Clark University, where students participate in protein purification, liquid nitrogen ice cream-making and discussions on STEM careers. He also emphasizes that in addition to technical knowledge, skills such as teamwork, communication and resilience are vital for success in STEM. He aims to help students develop a well-rounded skill set, giving them the confidence to navigate their future pathways and approach challenges with a positive mindset. Many students have gone on to pursue advanced degrees and careers in STEM, including positions at biotech companies like Moderna and Ph.D. programs at prestigious institutions such as UCLA.

"Busy people are happy people, my grandfather used to say," Spratt recalled regarding his packed schedule of teaching, research and mentorship. *"It's a lot of fun, and I wake up every day excited about what I'll do that day,"* he said, emphasizing how fulfilling he finds the balance of his many responsibilities, particularly his outreach work.

As the program continues to expand, Clark University is exploring ways to formalize it by creating lasting infrastructure, including a Science Center for Excellence and Inclusivity, to serve more schools and ensure that the opportunities Spratt initiated continue to benefit students for years to come.

What started as a neighborly conversation has become a far-reaching initiative with the potential to change lives. Through his work, Spratt has shown the immense impact one person's passion can have. His journey from hosting a single student to mentoring over 1,500 highlights the power of mentorship and the importance of creating opportunities for students to explore careers in STEM. As this initiative continues to grow, Spratt's influence will inspire new generations of students to see themselves as future scientists, researchers and innovators.

Associate Professor Don Spratt
Clark University, Worcester, MA, USA
2024 Passion in Science
Science Mentorship and Advocacy Award

DNA Polymerases & Amplification Technologies

NEB has pursued the discovery & development of DNA polymerases for over 35 years.

As the first company to sell *Taq* DNA polymerase and the first to discover a PCR-stable high-fidelity DNA polymerase, NEB established a foundation in developing innovative, high quality tools for the research and diagnostic community.

PCR & qPCR

NEB's product portfolio features a large selection of polymerases for PCR. Q5® High-Fidelity DNA Polymerase offers fidelity 280 times higher than *Taq*, along with superior performance with minimal optimization. One*Taq* is ideal for robust amplification in routine PCR applications. Both are available in hot start formulations, utilizing a novel aptamer-based approach that does not require an activation step.

Fluorescence-based quantitative real-time PCR (qPCR) is the gold standard for detection and quantitation of nucleic acids due to its specificity and sensitivity. Luna qPCR & RT-qPCR products feature an inert blue tracking dye for easy reaction setup and are available for intercalating dye or fluorescent probe-based detection methods.

Isothermal Amplification

Sequence specific isothermal amplification approaches eliminate the need for temperature cycling, providing advantages for certain point-of-care diagnostic needs. NEB's broad suite of reagents continue to enable advancement in isothermal amplification. Nicking enzymes, WarmStart enzymes, strand-displacing DNA polymerases, and RNA polymerases offer flexibility to assemble and design an isothermal amplification platform.

Introducing LyoPrime™ Lyophilized Products

NEB Lyophilization Sciences® has created shelf-stable, lyophilized products that do not sacrifice the high-performance qualities of their liquid counterparts. LyoPrime products include optimized formulations containing enzymes and inhibitors enabling robust detection of RNA via hydrolysis-probe-based RT-qPCR and Detection of DNA or RNA via LAMP/RT-LAMP.

Featured Products

Featured Tools & Resources

Visit **www.neb.com/PCR** to find additional online tools, video tech tips and tutorials to help you in setting up your PCR experiments.

Find an overview of PCR.

RM Recombinant Enzyme

Amplification-based Molecular Diagnostic Applications

NEB has a long history in the development of reliable and convenient tools for amplification, and offers a large selection of products for PCR, qPCR, RT-qPCR and isothermal amplification. Our extensive expertise in this area has allowed us to develop optimized enzymes for a variety of applications, including incorporation into customers' diagnostics. Large volume and/or custom formats are available for all products, with more specific customization details included below. Learn more at **www.neb.com/MDx**.

Application		Products	Product Notes	Custom Formulations
PCR Applications	qPCR/ RT-qPCR	**DNA, Dye** • Luna® Universal qPCR Master Mix (NEB #M3003) **DNA, Probe** • Luna Universal Probe qPCR Master Mix (NEB #M3004)	• Compatible with automated liquid handling and reaction miniaturization • Room temperature stable for ≥ 24 hours	• Blue-dye-free • Lyo-compatible
		RNA (1-step), Dye • Luna Universal One-Step RT-qPCR Kit (NEB #E3005) **RNA (1-step), Probe** • Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) • Luna Probe One-Step RT-qPCR 4X Mix with UDG (No ROX) (NEB #M3029) • Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006) • Luna Probe One-Step RT-qPCR Kit (No ROX) (NEB #E3007) • Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit (NEB #E3019) • LyoPrime Luna® Probe One-Step RT-qPCR Mix with UDG (NEB #L4001)	• Luna WarmStart RT paired with Hot Start *Taq* increases reaction specificity and robustness • Compatible with automation and reaction miniaturization • Room temperature stable for ≥ 24 hours • Lyophilized format (NEB #L4001)	• Blue-dye-free • Lyo-compatible • Add primers and probes (NEB #L4001)
		RNA (2-step) • LunaScript® RT SuperMix (NEB #E3010/#M3010)	• Novel thermostable RT • Single-tube format • 13-minute cDNA synthesis protocol	• Blue-dye-free
	PCR/ RT-PCR	**Master Mixes** • Q5® Hot Start High-Fidelity 2X Master Mix (NEB #M0494) • Q5 High-Fidelity 2X Master Mix (NEB #M0492) **Standalone Enzyme & Buffer** • Q5 Hot Start High-Fidelity DNA Polymerase (NEB #M0493) • Q5 High-Fidelity DNA Polymerase (NEB #M0491)	• ~280X fidelity of *Taq* • Consistent, fast, reliable performance • Compatible with automation and reaction miniaturization • Room temperature stable for ≥ 24 hours	• High conc. • Glycerol-free • Custom mixes
		• Q5 Blood Direct 2X Master Mix (NEB #M0500)	• Amplification direct from blood	
		• Hot Start *Taq* DNA Polymerase (NEB #M0495) • Hot Start *Taq* 2X Master Mix (NEB #M0496)	• Unique aptamer-based enzyme control supports fast protocols	• High conc. • Glycerol-free
Isothermal Applications	LAMP	• WarmStart® Colorimetric LAMP 2X Master Mix (DNA & RNA) (NEB #M1800) • WarmStart Colorimetric LAMP 2X Master Mix with UDG (NEB #M1804) • Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit (NEB #E3019)	• Fast, clear pink-to-yellow visible detection of amplification • Results in approximately 30 minutes • Automation-compatible when coupled with absorbance plate reader	
		• WarmStart LAMP Kit (DNA & RNA) (NEB #E1700) • WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) (NEB #M1708) • WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG) (NEB #E1708) • LyoPrime WarmStart Fluorescent LAMP/RT-LAMP Mix (with UDG) (NEB #L4401)	• Master mix for LAMP and RT-LAMP workflows • Supports multiple detection methods, including fluorescence and turbidity • Lyophilized format (NEB #L4401)	• Lyo-compatible • High conc. • Fluorescent Dye-free (NEB # L4402)
		• *Tte* UvrD Helicase (NEB #M1202)	• Improves specificity of fluorescent LAMP reactions	• High conc.
		• *Bst* 2.0 WarmStart™ DNA Polymerase (NEB #M0538) • *Bst* 2.0 DNA Polymerase (NEB #M0537) • *Bst* 2.0 WarmStart DNA Polymerase (Glycerol-free) (NEB #M0402)	• Improved reaction properties compared to wild-type *Bst* DNA Polymerase • Increased dUTP tolerance enables carryover prevention	• Glycerol-free • High conc.
		• *Bst* 3.0 DNA Polymerase (NEB #M0374)	• DNA binding domain fusion supports robust performance • Significantly increased RT activity up to 72°C enables single enzyme RT-LAMP	• Glycerol-free • High conc.
		• *Bst*-XT WarmStart DNA Polymerase (NEB #M9204) • *Bst*-XT WarmStart DNA Polymerase (Glycerol-free) (NEB #M9205)	• Combines the fast polymerization spreed of *Bst* 3.0 and the high specificity of *Bst* 2.0	
		• WarmStart RTx Reverse Transcriptase (NEB #M0380) • WarmStart RTx Reverse Transcriptase (Glycerol-free) (NEB #M0439)	• *In-silico* designed RT for RT-LAMP with reversibly-bound aptamer that inhibits activity below 40°C	• Glycerol-free (NEB #M0439) • High conc.
	Strand Displacement	• Nt.BstNBI (NEB #R0607) • WarmStart Nt.BstNBI (NEB #R0725) • WarmStart Afu Uracil-DNA Glycosylase (UDG) (NEB #M1282)	• High purity, high quality nicking endonuclease • WarmStart Afu UDG digest uracil-containing amplification products at temperature greater than 40°C	• Glycerol-free • High conc.
	Recombinant Polymerase Amplification	• T4 UvsX Recombinase (Glycerol-free) (NEB #M3081) • T4 UvsY Protein (Glycerol-free) (NEB #M3082) • *Bsu* DNA Polymerase, Large Fragment (NEB #M0330) • T4 Gene 32 Protein (NEB #M0300)	• Enables low temperature isothermal applications • Facilitates active strand invasion and homology-based recombination • Can increase yield and efficiency of amplification reactions	• High conc. • Glycerol-free
	Other	• Deoxynucleotide (dNTP) Solution Mix (NEB #N0447)		• Custom conc.
		• Nuclease-free Water (NEB #B1500)		
		• Antarctic Thermolabile UDG (NEB #M0372)	• Unique thermolabile version is completely inactivated in typical isothermal and RT-qPCR workflows	• High conc.
		• Proteinase K, Molecular Biology Grade (NEB #P8107)		• Custom conc.
		• Thermolabile Proteinase K (NEB #P8111)	• Unique thermolabile version is completely inactivated in typical isothermal and RT-qPCR workflows	• Custom conc.

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/ WarmStart · Recombinant Albumin · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

PCR Polymerase Selection Chart

For over 50 years, New England Biolabs, Inc. has been a world leader in the discovery and production of reagents for the life science industry. NEB offers a wide range of DNA polymerases, and through our commitment to research, ensures the development of innovative and high quality tools for PCR and related applications. The following table simplifies the selection of a polymerase that best suits your experiment.

	Standard PCR		High-Fidelity PCR			Specialty PCR				
			Highest Fidelity		Moderate Fidelity	Long Amplicons	dU Tolerance		Blood Direct	
★ indicates recommended choice for application	One*Taq*®/ One*Taq* Hot Start	*Taq*/ Hot Start *Taq*	Q5®/Q5 Hot Start	Phusion™(4)/ Phusion (4) Flex	Vent®/ Deep Vent	LongAmp®/ LongAmp Hot Start *Taq*	Q5U®	EpiMark® Hot Start *Taq*	Q5 Blood	Hemo Klen*Taq*®
Properties										
Fidelity vs. Taq	2X	1X	~280X (2)	> 50X	5–6X	2X	ND	1X	ND	ND
Amplicon Size	< 6 kb	≤ 5 kb	≤ 20 kb	≤ 20 kb	≤ 6 kb	≤ 30 kb	App-specific	≤ 1 kb	≤ 7.5 kb	≤ 2 kb
Extension Time	1 kb/min	1 kb/min	6 kb/min	4 kb/min	1 kb/min	1.2 kb/min	2 kb/min	1 kb/min	2-4 kb/min	0.5 kb/min
Resulting Ends	3´ A/Blunt	3´ A	Blunt	Blunt	Blunt	3´ A/Blunt	Blunt	3´ A	Blunt	3´ A
3´→5´ exo	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No
5´→3´ exo	Yes	Yes	No	No	No	Yes	No	Yes	No	No
Units/50 µl Reaction	1.25	1.25	1.0	1.0	0.5–1.0	5.0	1.0	1.25	N/A	N/A
Annealing Temperature	Tm–5	Tm–5	Tm+3	Tm+3	Tm–5	Tm–5	Tm+3	Tm–5	Tm+3	Tm–5
Applications										
Routine PCR	★	•	•	•	•	•				
Colony PCR	★	•								
Enhanced Fidelity	•		★	•	•	•			•	
High Fidelity			★	•					•	
High Yield	★	•	★	•					•	
Fast			★	•					•	
Long Amplicon			★	•		★				
GC-rich Targets	★		★		•	•			•	
AT-rich Targets	★	•	★	•		•	★	•		
High Throughput	•	•	•	•			★	•		
Multiplex PCR	•	★(1)	•	•					•	
Extraction-free PCR									★	•
DNA Labeling		★								
Site-directed Mutagenesis			★	•						
Carryover Prevention							★	•		
USER® Cloning							★	•		
NGS Applications										
NGS Library Amplification			★(3)	•				★(5)		
Formats										
Hot Start Available	•	•	•	•		•	•	•	•	
Kit		•	•	•		•	•			
Master Mix Available	•	•	•	•		•	•		•	
Direct Gel Loading	•	•								

(1) Use Multiplex PCR 5X Master Mix.

(2) Due to the very low frequency of misincorporation events being measured, the error rate of high-fidelity enzymes like Q5 is challenging to measure in a statistically significant manner. We continue to investigate improved assays to characterize Q5's very low error rate to ensure that we present the most robust accurate fidelity data possible (Popatov, V. and Ong, J.L. (2017) *PLoS One*, 12(1):e0169774. doi 10.1371/journal. pone. 0169774).

(3) Use NEBNext High-Fidelity 2X PCR Master Mix.

(4) Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific.

(5) Use NEBNext Enzymatic Methyl-seq Kit (EM-seq™) for Illumina.

Tm Calculator

High-Fidelity polymerases benefit from a Tm+3 annealing temp. Use the **NEB Tm Calculator** to ensure successful PCR at **TmCalculator.neb.com**

Why choose Q5 for your PCR?

Q5® High-Fidelity DNA Polymerases

Q5® Hot Start High-Fidelity DNA Polymerase

Q5 High-Fidelity DNA Polymerase
#M0491S 100 units
#M0491L 500 units

Q5 High-Fidelity 2X Master Mix
#M0492S 100 reactions
#M0492L 500 reactions

Q5 Hot Start High-Fidelity DNA Polymerase
#M0493S 100 units
#M0493L 500 units

Q5 Hot Start High-Fidelity 2X Master Mix
#M0494S 100 reactions
#M0494L 500 reactions
#M0494X 500 reactions

Q5U® Hot Start High-Fidelity DNA Polymerase
#M0515S 100 units
#M0515L 500 units

Q5 High-Fidelity PCR Kit
#E0555S 50 reactions
#E0555L 200 reactions

Q5 Blood Direct 2X Master Mix
#M0500S 100 reactions
#M0500L 500 reactions

Description: Q5 High-Fidelity DNA Polymerase sets the standard for performance, ultra-low error rates and fidelity (~ 280 times higher than *Taq*). Q5 DNA Polymerase is composed of a novel polymerase that is fused to the processivity-enhancing Sso7d DNA binding domain, improving speed, fidelity and reliability.

The Q5 buffer system provides superior performance with minimal optimization across a broad range of amplicons, regardless of GC content. For routine or complex amplicons up to ~65% GC content, Q5 Reaction Buffer provides reliable and robust amplification. For amplicons with high GC content (>65% GC), addition of the Q5 High GC Enhancer ensures continued maximum performance.

Q5 Hot Start DNA Polymerase: In contrast to chemically-modified or antibody-based hot start polymerases, NEB's Q5 Hot Start utilizes a unique synthetic aptamer. This structure binds to the polymerase through non-covalent interactions, blocking activity during the reaction setup. The polymerase is activated during normal cycling conditions, allowing reactions to be set up at room temperature. Q5 Hot Start does not require a separate high temperature activation step, reducing the potential for sample degradation, shortening reaction times and increasing ease-of-use. Q5 Hot Start is an ideal choice for high specificity amplification and provides robust amplification of a wide variety of amplicons, regardless of GC content.

Q5U Hot Start High-Fidelity DNA Polymerase: A modified version of Q5 High-Fidelity DNA Polymerase capable of incorporating dUTP for carryover prevention. Q5U is also compatible with USER cloning methods and enables the amplification of bisulfite treated/ deaminated DNA.

Q5 Blood Direct 2X Master Mix: Amplify a wide variety of targets direct from dried blood spots or up to 30% whole human blood with this unique master mix.

Additional Formats: For added convenience, Q5 DNA Polymerase is available in master mix format or as a kit. Master mix formulations include dNTPs, Mg^{++} and all necessary buffer components. The Q5 High-Fidelity PCR Kit contains the Q5 High-Fidelity 2X Master Mix, nuclease-free water and the Quick-Load Purple 1 kb Plus DNA Ladder. The Q5 Site-Directed Mutagenesis Kit, with or without competent cells, is also available.

Concentration: 2,000 units/ml

Visit Q5PCR.com for more information.

Q5 POLYMERASE DETAILS

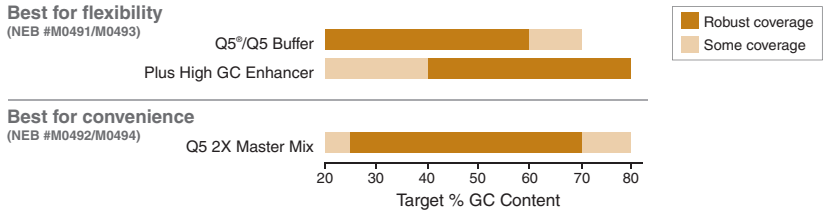
Extension Rate	6 kb/min
Amplicon Size	≤ 20 kb
Fidelity	~ 280X *Taq*
Units / 50 µl rxn	1 unit
Resulting Ends	Blunt
3´→5´ Exonuclease Activity	Yes
5´→3´ Exonuclease Activity	No
Supplied Buffer	Q5 Reaction Buffer
Supplied Enhancer	Q5 High GC Enhancer
Extraction-free PCR	Yes

PRODUCT FORMATS

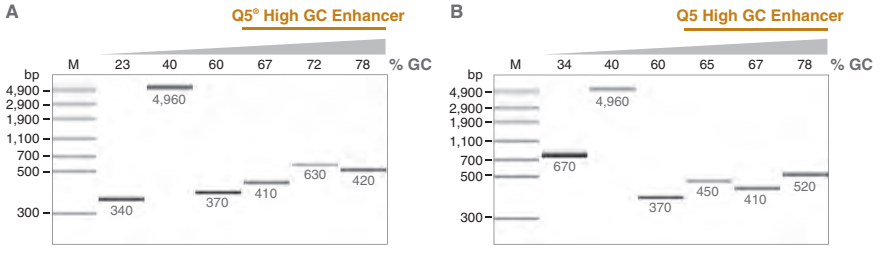
Hot Start Available Activation Required	Yes No
Master Mix Available	Yes
PCR Kit Available	Yes
NGS Version Available	Yes

APPLICATIONS

High-Fidelity PCR	Yes
Difficult PCR	Yes
High GC PCR	Yes
T/A, U/A Cloning	No
Colony PCR	No
Blunt Cloning	Yes
Multiplex PCR	Yes
USER Cloning	Yes (Q5U)
Carryover Prevention	Yes (Q5U)



The Q5 buffer system provides superior performance for a wide range of targets. *The stand-alone enzyme comes with a reaction buffer that supports robust amplification of high AT to routine targets. Addition of the High GC Enhancer allows amplification of GC rich and difficult targets. For added convenience, the master mix formats allow robust amplification of a broad range of targets with a single formulation.*



Robust amplification with Q5 (A) and Q5 Hot Start (B) High-Fidelity DNA Polymerases, regardless of GC content. *Amplification of a variety of human genomic amplicons from low-to-high GC content using either Q5 or Q5 Hot Start High-Fidelity DNA Polymerase. Reactions using Q5 Hot Start were set up at room temperature. All reactions were conducted using 30 cycles of amplification. and visualized by microfluidic LabChip® analysis.*

Q5 Hot Start High-Fidelity DNA Polymerase (NEB #M0493) and **Q5 Hot Start High-Fidelity 2X Master Mix (NEB #M0494)** are available as a **GMP-grade reagent**. See page 6 for details.

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/ WarmStart · Recombinant Albumin · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

Phusion™ High-Fidelity DNA Polymerase

RR NEBU HiFi PCR No Tm+3

Phusion™ Hot Start Flex DNA Polymerase

RR NEBU HiFi PCR Hot No Tm+3

Phusion High-Fidelity DNA Polymerase
#M0530S 100 units
#M0530L 500 units

Phusion High-Fidelity PCR Master Mix with HF Buffer
#M0531S 100 reactions
#M0531L 500 reactions

Phusion High-Fidelity PCR Master Mix with GC Buffer
#M0532S 100 reactions
#M0532L 500 reactions

Phusion Hot Start Flex DNA Polymerase
#M0535S 100 units
#M0535L 500 units

Phusion Hot Start Flex 2X Master Mix
#M0536S 100 reactions
#M0536L 500 reactions

Phusion High-Fidelity PCR Kit
#E0553S 50 reactions
#E0553L 200 reactions

Description: DNA polymerases with high fidelity are important for applications in which the DNA sequence needs to be correct after amplification. Manufactured and quality controlled at New England Biolabs, Thermo Scientific™ Phusion™ High-Fidelity DNA Polymerase offers both high fidelity and robust performance, and thus can be used for all PCR applications. Its unique structure, a novel Pyrococcus-like enzyme fused with a processivity-enhancing domain, increases fidelity and speed. Phusion Hot Start Flex DNA Polymerase is available as a standalone enzyme or in a master mix format, and enables high specificity amplification. Phusion DNA Polymerase is an ideal choice for cloning and can be used for long amplicons.

Additional Formats: Phusion and Phusion Hot Start Flex DNA Polymerases are also available in master mix format. Phusion master mixes are available with HF or GC Buffer. The Phusion PCR Kit contains Phusion Polymerase, Phusion HF and GC buffers, deoxynucleotides, $MgCl_2$, DMSO and DNA size standards.

Concentration: 2,000 units/ml

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. Phusion™ is a registered trademark and property of Thermo Fisher Scientific.

PHUSION POLYMERASE DETAILS

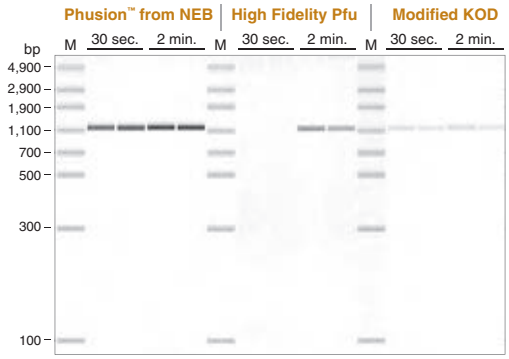
Extension Rate	4 kb/min
Amplicon Size	≤ 20 kb
Fidelity	> 50X *Taq*
Units / 50 µl rxn	1 unit
Resulting Ends	Blunt
3´→5´ Exonuclease Activity	Yes
5´→3´ Exonuclease Activity	No
Supplied Buffers	- 5X Phusion HF Buffer - 5X Phusion GC Buffer
Supplied Enhancer	100% DMSO

PRODUCT FORMATS

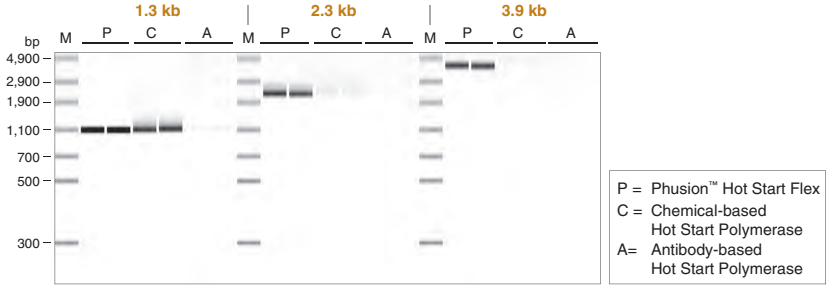
Hot Start Available	Yes
Activation Required	No
Master Mix Available	Yes
PCR Kit Available	Yes

APPLICATIONS

High-Fidelity PCR	Yes
T/A, U/A Cloning	No
Colony PCR	No
Blunt Cloning	Yes



Phusion DNA Polymerase generates robust amplification even with short extension times. *A 1.2 kb C. elegans genomic amplicon was analyzed using different polymerases. Reactions were set up in duplicate and visualized by microfluidic LabChip analysis. All reactions were conducted according to manufacturer's instructions using 30 cycles and extension times of either 30 seconds or 2 minutes as indicated.*



Phusion Hot Start Flex DNA Polymerase delivers robust amplification. *All amplicons are from human Jurkat template except for the 1.3 kb C. elegans amplicon. Amplicon sizes are indicated above gel. All reactions were conducted in duplicate according to manufacturer's instructions using 30 cycles and visualized after microfluidic LabChip analysis.*

Tm Calculator

High-Fidelity polymerases benefit from a Tm+3 annealing temp. Use the **NEB Tm Calculator** to ensure successful PCR at **TmCalculator.neb.com**

One*Taq*® DNA Polymerase

One*Taq*® Hot Start DNA Polymerase

One*Taq* DNA Polymerase
#M0480S 200 units
#M0480L 1,000 units
#M0480X 5,000 units

One*Taq* Hot Start DNA Polymerase
#M0481S 200 units
#M0481L 1,000 units
#M0481X 5,000 units

One*Taq* 2X Master Mix with Standard Buffer
#M0482S 100 reactions
#M0482L 500 reactions

One*Taq* Hot Start 2X Master Mix with Standard Buffer
#M0484S 100 reactions
#M0484L 500 reactions

One*Taq* Hot Start 2X Master Mix with GC Buffer
#M0485S 100 reactions
#M0485L 500 reactions

One*Taq* Quick-Load 2X Master Mix with Standard Buffer
#M0486S 100 reactions
#M0486L 500 reactions

One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer
#M0488S 100 reactions
#M0488L 500 reactions

One*Taq* Hot Start Quick-Load 2X Master Mix with GC Buffer
#M0489S 100 reactions
#M0489L 500 reactions

One*Taq* Quick-Load DNA Polymerase
#M0509L 500 units
#M0509X 2,500 units

One*Taq* RT-PCR Kit
#E5310S 30 reactions

One*Taq* One-Step RT-PCR Kit
#E5315S 30 reactions

Description: One*Taq* DNA Polymerase is an optimized blend of *Taq* and Deep Vent® DNA polymerases for use with routine and difficult PCR experiments. The 3´→5´ exonuclease activity of Deep Vent DNA Polymerase increases the fidelity and robust amplification of *Taq* DNA Polymerase. The One*Taq* reaction buffers and High GC Enhancer have been formulated for robust yields with minimal optimization, regardless of a template's GC content.

One*Taq* Hot Start DNA Polymerase: One*Taq* Hot Start DNA Polymerase utilizes an aptamer-based inhibitor. The hot start formulation combines convenience with decreased interference from primer-dimers and secondary products.

The aptamer-based inhibitor binds reversibly, blocking polymerase activity at temperatures below 45°C, allowing reactions to be set up at room temperature. One*Taq* Hot Start DNA Polymerase is activated during normal cycling conditions, eliminating the need for a separate high temperature incubation step to activate the enzyme. One*Taq* Hot Start DNA Polymerase can therefore be substituted into typical or existing *Taq*-based protocols.

One*Taq* and One*Taq* Hot Start are provided with Standard Reaction Buffer, GC Reaction Buffer and High GC Enhancer. Recommendations for buffer selection, based on % GC content, are shown below.

Quick-Load formats offer direct loading of PCR products onto gels, eliminating the need to add a separate loading/tracking dye.

Additional Formats: For added convenience, One*Taq* and One*Taq* Hot Start DNA Polymerases are available in master mix format. Master mixes are available with Standard or GC Buffer. High GC Enhancer is also provided with master mixes containing GC Buffer. For direct gel loading, Quick-Load versions of master mixes are also available. One*Taq*® RT-PCR Kit combines two powerful mixes, M-MuLV Enzyme Mix and One*Taq* Hot Start 2X Master Mix with Standard Buffer for 2-step RT-PCR applications. The One*Taq* One-Step RT-PCR Kit offers sensitive and robust end-point detection of RNA templates. cDNA synthesis and PCR amplification steps are performed in a single reaction using gene-specific primers, resulting in a streamlined RT-PCR protocol.

Concentration: 5,000 units/ml

ONE*Taq* POLYMERASE DETAILS

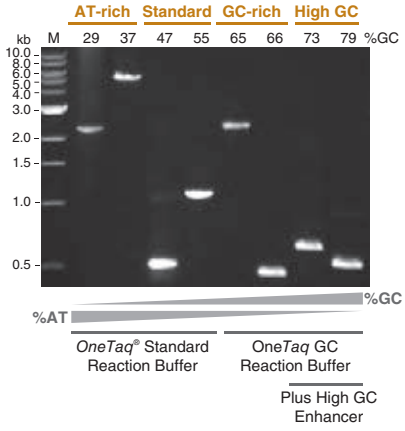
Extension Rate	1 kb/min
Amplicon Size	≤ 6 kb
Fidelity	> 2X *Taq*
Units / 50 µl rxn	1.25 units
Resulting Ends	3´ A/Blunt
3´→5´ Exonuclease Activity	Yes
5´→3´ Exonuclease Activity	Yes
Supplied Buffers	- One*Taq* Std Rxn Buffer - OneTaq GC Rxn Buffer
Supplied Enhancer	One*Taq* High GC Enhancer

PRODUCT FORMATS

Hot Start Available Activation Required	Yes No
Master Mix Available	Yes
Direct Gel-loading Available	Yes
PCR Kit Available	Yes

APPLICATIONS

Routine PCR	Yes
T/A, U/A Cloning	Yes
Colony PCR	Yes



Amplification of a selection of sequences with varying GC content from human and C. elegans genomic DNA using OneTaq DNA Polymerase. *GC content is indicated above gel. Marker M is the 1 kb DNA Ladder (NEB #N3232).*

One*Taq* Buffer Recommendations

Amplicon % GC Content	Recommended Default Buffer	Optimization Notes
< 50% GC	One*Taq* Standard Reaction Buffer	Adjust annealing temperature, primer/template concentration, etc., if needed.
50–65% GC	One*Taq* Standard Reaction Buffer	One*Taq* GC Reaction Buffer can be used to enhance performance of difficult amplicons.
> 65% GC	One*Taq* GC Reaction Buffer	One*Taq* GC Reaction Buffer with 10-20% One*Taq* High GC Enhancer can be used to enhance performance of difficult amplicons.

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Hot Start *Taq* DNA Polymerase

Taq DNA Polymerase with ThermoPol Buffer
#M0267S 400 units
#M0267L 2,000 units
#M0267X 4,000 units
#M0267E 20,000 units

Taq DNA Polymerase with Standard *Taq* Buffer
#M0273S 400 units
#M0273L 2,000 units
#M0273X 4,000 units
#M0273E 20,000 units

Taq DNA Polymerase with Standard *Taq* (Mg-free) Buffer
#M0320S 400 units
#M0320L 2,000 units

Taq PCR Kit
#E5000S 200 reactions

Taq 2X Master Mix
#M0270L 500 reactions

Quick-Load *Taq* 2X Master Mix
#M0271L 500 reactions

Taq 5X Master Mix
#M0285L 500 reactions

Multiplex PCR 5X Master Mix
#M0284S 100 reactions

Hot Start *Taq* DNA Polymerase
#M0495S 200 units
#M0495L 1,000 units

Hot Start *Taq* 2X Master Mix
#M0496S 100 reactions
#M0496L 500 reactions

Description: *Taq* DNA Polymerase is a thermostable DNA polymerase that possesses a 5´→ 3´ polymerase activity and a 5´ flap endonuclease activity. It is the most widely used enzyme for PCR. To accomodate a variety of PCR applications, *Taq* is available with different reaction buffers. Standard *Taq* Reaction Buffer is designed to support existing PCR platforms, and is an ideal choice for DHPLC and high-throughput applications. ThermoPol Reaction Buffer was designed at NEB, and is formulated to promote high product yields, even under demanding conditions. For additional convenience, *Taq* DNA Polymerase is also available in kit and master mix formats. For direct gel loading, a Quick-Load version of the *Taq* 2X Master Mix is also available.

Hot Start *Taq* DNA Polymerase: With value pricing and attractive commercial terms, Hot Start *Taq* is an ideal choice for research applications. In contrast to chemically modified or antibody-based hot start polymerases, NEB's Hot Start *Taq* utilizes an aptamer-based technology. The unique aptamer binds to the polymerase through non-covalent interactions, inhibiting polymerization at non-permissive temperatures. This novel method eliminates the need for an activation step, reducing the potential for sample degradation and decreasing overall reaction time.

Additional Formats: For added convenience, *Taq* and Hot Start *Taq* DNA Polymerase are available in master mix format. For direct gel loading, a Quick-Load version of the *Taq* 2X Master Mix is also available. The *Taq* PCR Kit contains *Taq* DNA Polymerase, dNTP Mix, Buffer, $MgCl_2$ and the Quick-Load Purple 1 kb Plus DNA Ladder. The Multiplex PCR 5X Master Mix formulation has been specifically optimized for enhanced performance in multiplex PCR reactions.

Concentration: 5,000 units/ml

NEB also offers a selection of Nucleotides and Deoxynucleotides, as well as Reaction Buffers sold separately.

Taq DNA POLYMERASE DETAILS

Extension Rate	1 kb/min
Amplicon Size	≤ 5 kb
Units / 50 µl rxn	1.25 units
Resulting Ends	3´ A
3´→5´ Exonuclease Activity	No
5´→3´ Exonuclease Activity	Yes
Supplied Buffer (product dependent)	- Std *Taq* Rxn Buffer or - ThermoPol Rxn Buffer

PRODUCT FORMATS

Hot Start Available Activation Required	Yes No
Master Mix Available	Yes
Direct Gel-loading Available	Yes
PCR Kit Available	Yes

APPLICATIONS

Routine PCR	Yes
SNP Detection	Yes
T/A, U/A Cloning	Yes
Colony PCR	Yes

***Taq* Buffer Selection Chart**

CHOICE OF BUFFER	AVAILABLE PRODUCTS
ThermoPol Reaction Buffer: Designed for optimal yield and specificity	*Taq* DNA Polymerase with ThermoPol Buffer (NEB #M0267)
Standard *Taq* Reaction Buffer: Detergent-free and designed to be compatible with existing assay systems	*Taq* DNA Polymerase with Standard Taq Buffer (NEB #M0273) *Taq* DNA Polymerase with Standard Taq (Mg-free) Buffer (NEB #M0320)

The Tanner Lab studies methods and tools for isothermal amplification, creating better ways for producing and detecting DNA and RNA for a variety of biotechnology applications.

LongAmp® *Taq* DNA Polymerase

LongAmp® Hot Start *Taq* DNA Polymerase

LongAmp *Taq* DNA Polymerase
#M0323S 500 units
#M0323L 2,500 units

LongAmp Hot Start *Taq* DNA Polymerase
#M0534S 500 units
#M0534L 2,500 units

LongAmp *Taq* 2X Master Mix
#M0287S 100 reactions
#M0287L 500 reactions

LongAmp Hot Start *Taq* 2X Master Mix
#M0533S 100 reactions
#M0533L 500 reactions

LongAmp *Taq* PCR Kit
#E5200S 100 reactions

LONGAMP *Taq* POLYMERASE DETAILS

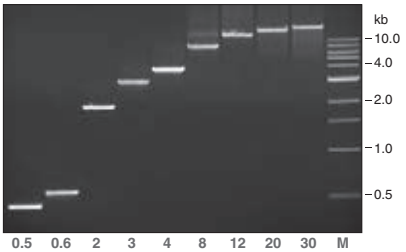
Extension Rate	1.2 kb/min
Amplicon Size	≤ 30 kb
Fidelity	2X *Taq* DNA Polymerase
Units / 50 µl rxn	5 units
Resulting Ends	3´ A/Blunt
3´→5´ Exonuclease Activity	Yes
5´→3´ Exonuclease Activity	Yes
Supplied Buffer (product dependent)	LongAmp *Taq* Rxn Buffer

PRODUCT FORMATS

Hot Start Available Activation Required	Yes No
Master Mix Available	Yes
Direct Gel-loading Available	No
PCR Kit Available	Yes

APPLICATIONS

Long Amplicons	Yes
Routine PCR	Yes
T/A, U/A Cloning	Yes
Colony PCR	Yes



Amplification of longer templates with LongAmp Taq. *Amplification of specific sequences from human genomic DNA using LongAmp Taq DNA Polymerase. Amplicon sizes are indicated below gel. Ladder (M) is NEB 1 kb DNA Ladder (NEB #N3232).*

Description: An optimized blend of *Taq* and Deep Vent DNA Polymerases, LongAmp *Taq* DNA Polymerase enables amplification of longer PCR products with higher fidelity than *Taq* DNA Polymerase alone.

LongAmp Hot Start *Taq* DNA Polymerase: LongAmp Hot Start *Taq* DNA Polymerase utilizes a unique synthetic aptamer. This structure binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 45°C, but releases the enzyme during normal PCR cycling conditions.

Additional Formats: For added convenience, LongAmp *Taq* and LongAmp Hot Start *Taq* are available in master mix format. The LongAmp *Taq* PCR Kit includes LongAmp *Taq* DNA Polymerase (2,500 units/ml), dNTP Mix (10 mM), LongAmp *Taq* Reaction Buffer Pack (5X), MgSO4 (100 mM) and nuclease-free water.

Concentration: 2,500 units/ml

Hemo KlenTaq®

#M0332S 200 reactions
#M0332L 1,000 reactions

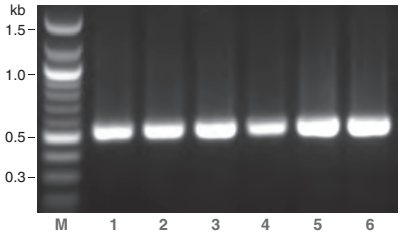
HEMO KLEN*Taq* DETAILS

Extension Rate	0.5 kb/min
Amplicon Size	≤ 2 kb
Units / 50 µl rxn	4 units
Resulting Ends	3´ A
3´→5´ Exonuclease Activity	No
5´→3´ Exonuclease Activity	No
Supplied Buffer	Hemo Klen*Taq* Rxn Buffer

APPLICATIONS

Extraction-free PCR	Yes
T/A, U/A Cloning	Yes
Colony PCR	Yes

Description: Hemo KlenTaq is a truncated version of *Taq* DNA Polymerase, lacking the first 280 amino acids. Hemo KlenTaq also contains mutations that make it resistant to inhibitors present in whole blood. It can amplify DNA from whole blood samples from humans and mice, without the need for DNA extraction. Hemo KlenTaq tolerates up to 20% whole blood in a 25 µl reaction (30% in a 50 µl reaction). Hemo KlenTaq works well with most common anticoagulants, including heparin, citrate and EDTA.



Amplification of human whole blood with Hemo KlenTaq. *Lane 1: 5% blood + Na-EDTA; Lane 2: 5% blood + K-EDTA; Lane 3: 5% blood + Na-Heparin; Lane 4: 5% blood + Na-Citrate; Lane 5: 1.2 mm² FTA Guthrie Card containing dried human blood + Na-Heparin; Lane 6: 1.2 mm² PTA Guthrie Card containing dried human blood + Na-Heparin (washed with 50 µl H2O at 50°C for 5 min.). Ladder (M) is the 1 kb Plus DNA Ladder (NEB #N3200).*

Source: An *E. coli* strain that carries a mutant *Taq* DNA polymerase gene. The protein lacks the N-terminal 5´→3´ exonuclease domain and the gene has three internal point mutations.

Heat Inactivation: Not Heat Inactivated

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EpiMark® Hot Start *Taq* DNA Polymerase

#M0490S 100 reactions
#M0490L 500 reactions

EPIMARK POLYMERASE DETAILS

Extension Rate	1 kb/min
Amplicon Size	≤ 1 kb
Units / 50 µl rxn	1.25 units
Resulting Ends	3´A
3´→5´ Exonuclease Activity	No
5´→3´ Exonuclease Activity	Yes
Supplied Buffer	EpiMark Hot Start *Taq* Rxn Buffer

APPLICATIONS

AT-rich Targets	Yes
Bisulfite-converted DNA	Yes
Routine PCR	Yes
T/A, U/A Cloning	Yes

Description: EpiMark Hot Start *Taq* DNA Polymerase is an excellent choice for use on bisulfite-converted DNA. It is a mixture of *Taq* DNA Polymerase and a temperature-sensitive, aptamer-based inhibitor. The inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 45°C, but releases the enzyme during normal PCR cycling conditions. This permits room temperature reaction assembly with no separate high-temperature incubation step to activate the enzyme.

Source: An *E. coli* strain that carries the *Taq* DNA Polymerase gene from *Thermus aquaticus* YT-1

Concentration: 5,000 units/ml

Heat Inactivation: Not Heat Inactivated

Vent® & Deep Vent® DNA Polymerases

Vent DNA Polymerase
#M0254S 200 units
#M0254L 1,000 units

Vent (exo-) DNA Polymerase
#M0257S 200 units
#M0257L 1,000 units

Deep Vent DNA Polymerase
#M0258S 200 units
#M0258L 1,000 units

Deep Vent (exo-) DNA Polymerase
#M0259S 200 units
#M0259L 1,000 units

VENT/DEEP VENT POLYMERASES DETAILS

Extension Rate	1 kb/min
Amplicon Size	≤ 6 kb
Fidelity	5-6X *Taq*
Resulting Ends	Blunt
3´→5´ Exonuclease Activity	Yes (M0254, M0258)
5´→3´ Exonuclease Activity	No
Supplied Buffer	ThermoPol Rxn Buffer

Description: Vent DNA Polymerase was the first high fidelity thermophilic DNA polymerase available for PCR. The fidelity is 5-fold higher than that observed for *Taq* DNA Polymerase, and is derived in part from an integral 3´→ 5´ proofreading exonuclease activity. Greater than 90% activity remains following a 1 hour incubation at 95°C.

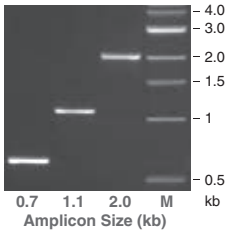
Deep Vent DNA Polymerase is a modified version of Vent DNA Polymerase. Deep Vent has similar fidelity with even more stability than Vent at temperatures of 95 to 100°C, with a half-life of 8 hours at 100°C.

Vent (exo–) DNA Polymerase has been genetically engineered to eliminate the 3´→ 5´ proofreading exonuclease activity associated with Vent DNA Polymerase. The fidelity of polymerization by this form is reduced to a level about 2-fold higher than that of *Taq* DNA Polymerase. Likewise, Deep Vent (exo–) DNA Polymerase has been genetically engineered to eliminate the 3´→ 5´ proofreading exonuclease activity associated with Deep Vent DNA Polymerase.

Source: Vent DNA Polymerase is purified from a strain of *E. coli* that carries the Vent DNA Polymerase gene from the archaea *Thermococcus litoralis*. Vent (exo–) is purified from an *E. coli* strain that carries the Vent (D141A/E143A) DNA Polymerase gene, a genetically engineered form of the native DNA polymerase.

Deep Vent DNA Polymerase is purified from a strain of *E. coli* that carries the Deep Vent DNA Polymerase gene from *Pyrococcus* species GB-D. Deep Vent (exo–) is purified from an *E. coli* strain that carries the Deep Vent (D141A/E143A) DNA Polymerase gene, a genetically engineered form of the native polymerase.

Concentration: 2,000 units/ml



Amplification of Jurkat genomic DNA with Vent DNA Polymerase. *Amplicon Sizes are indicated below gel. Marker (M) is the 1 kb DNA Ladder (NEB #N3232).*

Luna® qPCR and RT-qPCR

- *Convenient master mix and supermix formats with user-friendly protocols simplify reaction setup*
- *Non-interfering, visible tracking dye helps to eliminate pipetting errors*
- *Novel, thermostable reverse transcriptase (RT) improves performance*
- *One-Step RT-qPCR kits feature Luna WarmStart RT paired with Hot Start Taq for increased reaction specificity and robustness*

Fluorescence-based quantitative real-time PCR (qPCR) is the gold standard for the detection and quantification of nucleic acids due to its sensitivity and specificity. Luna products from NEB are optimized for qPCR or RT-qPCR, and are available for either intercalating dye or probe-based detection methods. Each Hot Start Taq-based Luna qPCR master mix has been formulated with a unique passive reference dye that is compatible across a wide variety of thermal cyclers, regardless of ROX requirements. No additional components are required to ensure compatibility. For two-step RT-qPCR, the LunaScript® RT SuperMix Kit offers a fast (less than 15 minute), robust, and sensitive option for cDNA synthesis upstream of your Luna qPCR experiment.

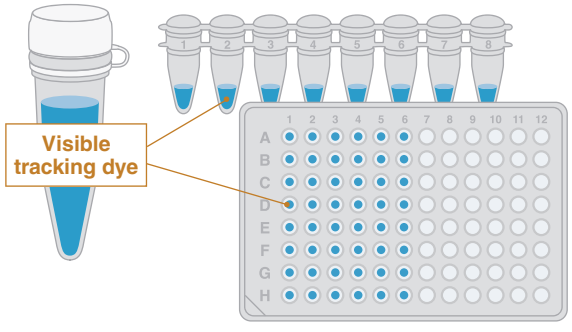
The Luna Probe One-Step RT-qPCR Mix with UDG is supplied at a 4X concentration and enables higher amounts of sample input, which is relevant for applications where increased sensitivity is needed, such as molecular diagnostics. Performance in multiplexing applications has been optimized, with linear detection achieved for up to 5 targets across a range of inputs.

The LyoPrime Luna® Probe One-Step RT-qPCR Mix with UDG offers the same versatile features and strong performance as the M3019 product in a lyophilized format. For more information on lyophilized products used for RNA detection, please visit **www.neb.com/lyoprime**.

Find the right Luna product for your application

1 Select your target	2 Select your detection method: Dye-based	Probe-based
Genomic DNA or cDNA	Luna® Universal qPCR Master Mix (NEB #M3003)	Luna Universal Probe qPCR Master Mix (NEB #M3004)*
Purified RNA One-Step RT-qPCR	Luna Universal One-Step RT-qPCR Kit (NEB #E3005)	Luna Universal Probe One-Step RT-qPCR: • Kit (NEB #E3006) • Kit (No ROX, NEB #E3007) • 4X Mix with UDG (NEB #M3019) • 4X Mix with UDG (No ROX, NEB #M3029) LyoPrime Luna™ Probe One-Step RT-qPCR Mix with UDG (NEB #L4001)
Two-Step RT-qPCR	LunaScript® RT SuperMix (NEB #E3010/M3010) + Luna Universal qPCR Master Mix (NEB #M3003)	LunaScript RT SuperMix (NEB #E3010/M3010) + Luna Universal Probe qPCR Master Mix (NEB #M3004)
RNA from cell lysate	Luna Cell Ready One-Step RT-qPCR Kit (NEB #E3030)	Luna Cell Ready Probe One-Step RT-qPCR Kit (NEB #E3031)

* No ROX version available (OEM)
For large volume, lyophilized or custom options, contact us at www.neb.com/CustomContactForm



Learn about "Dots in Boxes" visualization of qPCR data.

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/WarmStart · Recombinant Albumin · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

Luna Universal qPCR & Probe qPCR Master Mixes

Luna Universal qPCR Master Mix

#M3003S	200 reactions
#M3003L	500 reactions
#M3003X	1,000 reactions
#M3003E	2,500 reactions

Luna Universal Probe qPCR Master Mix

#M3004S	200 reactions
#M3004L	500 reactions
#M3004X	1,000 reactions
#M3004E	2,500 reactions

Companion Product:

Antarctic Thermolabile UDG

#M0372S	100 units
#M0372L	500 units

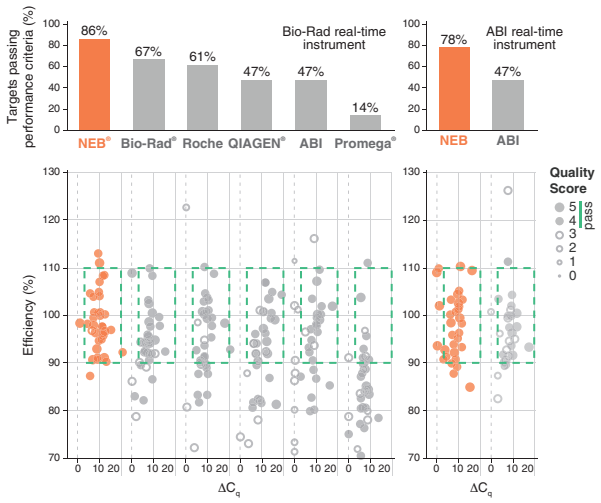
- *Convenient master mix formats and user-friendly protocols simplify reaction setup*
- *Non-interfering, visible tracking dye helps to eliminate pipetting errors*
- *Rigorously tested to optimize specificity, sensitivity, accuracy and reproducibility*
- *Unique passive reference dye for compatibility across wide range of instruments*

Learn more about our comprehensive qPCR/RT-qPCR testing and **"dots in boxes"** data visualization at **LUNAqPCR.com**.

Description: The Luna Universal qPCR Master Mix is an optimized 2X reaction mix for real-time qPCR detection and quantitation of target DNA sequences using the SYBR®/FAM channel of most real-time qPCR instruments.

The Luna Universal Probe qPCR Master Mix is a 2X reaction mix optimized for real-time qPCR detection and quantitation of target DNA sequences using hydrolysis probes.

Each Hot Start *Taq*-based Luna qPCR master mix has been formulated with a unique passive reference dye that is compatible across a wide variety of instrument platforms, including those that require a ROX reference signal. This means that no additional components are required to ensure machine compatibility. The mixes also contain dUTP, enabling carryover prevention when reactions are treated with Antarctic Thermolabile UDG. A blue visible dye assists in tracking the reagents when pipetting into clear, multi-welled PCR plates. These features, combined with rapid, sensitive and precise real-time qPCR performance, make Luna the universal choice for all your qPCR experiments.



Extensive performance evaluation of commercially available dye-based qPCR reagents demonstrates the robustness and specificity of Luna. *qPCR reagents from NEB and other manufacturers were tested across 16–18 qPCR targets varying in abundance, length and %GC, using either Jurkat genomic DNA or Jurkat-derived cDNA as input (10 genomic DNA targets and 8 cDNA targets on Bio-Rad real-time instrument, 9 genomic and 7 cDNA targets on ABI instrument). For each testing condition, data was collected by 2 users and according to manufacturer's specifications. Results were evaluated for efficiency, low input detection and lack of non-template amplification (where ΔCq = average Cq of non-template control – average Cq of lowest input). In addition, consistency, reproducibility and overall curve quality were assessed (Quality Score). Bar graph indicates % of targets that met acceptable performance criteria (indicated by green box on dot plot and Quality Score > 3). NEB's Luna Universal qPCR Master Mix outperformed all other reagents tested.*

LunaScript® RT SuperMix, SuperMix Kit & Master Mix Kit (Primer-free)

LunaScript RT SuperMix

#M3010L	100 reactions
#M3010X	500 reactions
#M3010E	2,500 reactions

LunaScript RT SuperMix Kit

#E3010S	25 reactions
#E3010L	100 reactions

LunaScript RT Master Mix Kit (Primer-free)

#E3025S	25 reactions
#E3025L	100 reactions

Description: The LunaScript RT SuperMix Kit is an optimized master mix containing all the necessary components for first strand cDNA synthesis in the context of a two-step RT-qPCR workflow. It features the thermostable Luna Reverse Transcriptase, which supports cDNA synthesis at elevated temperatures. Murine RNase Inhibitor is also included to protect template RNA from degradation. LunaScript RT SuperMix Kit contains random hexamer and poly-dT primers, which allow for even coverage across the length of the RNA targets. LunaScript RT SuperMix Kit also includes a No-RT Control Mix and Nuclease-free Water.

LunaScript RT Master Mix Kit (Primer-free) is the same formulation as the LunaScript RT SuperMix Kit but does not contain primers. It includes all components needed for carrying out first strand cDNA synthesis except for RNA template and user-supplied primers. It provides flexible options for using different primers (dT primers, random primers, gene-specific primers, modified primers, etc.) for first strand cDNA synthesis.

The LunaScript RT SuperMix Kit Includes:

- LunaScript RT SuperMix
- No-RT Control Mix
- Nuclease-free Water

The LunaScript RT Master Mix Kit (Primer-free) Includes:

- LunaScript RT Master Mix (Primer-free)
- No-RT Control Mix (Primer-free)
- Nuclease-free Water

Luna One-Step RT-qPCR Products

Luna Probe One-Step RT-qPCR 4X Mix with UDG

#M3019S	200 reactions
#M3019L	500 reactions
#M3019X	1,000 reactions
#M3019E	2,000 reactions

Luna Probe One-Step RT-qPCR 4X Mix with UDG (No ROX)

#M3029S	200 reactions
#M3029L	500 reactions
#M3029E	2,000 reactions

LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG

#L4001S	120 reactions
#L4001P	96 reactions

Luna Universal One-Step RT-qPCR Kit

#E3005S	200 reactions
#E3005L	500 reactions
#E3005X	1,000 reactions
#E3005E	2,500 reactions

Luna Universal Probe One-Step RT-qPCR Kit

#E3006S	200 reactions
#E3006L	500 reactions
#E3006X	1,000 reactions
#E3006E	2,500 reactions

Luna Probe One-Step RT-qPCR Kit (No ROX)

#E3007E	2,500 reactions

Companion Product:

Antarctic Thermolabile UDG

#M0372S	100 units
#M0372S	100 units

- *Novel, thermostable RT improves performance*
- *Luna WarmStart paired with Hot Start Taq increases reaction specificity and robustness*
- *Non-interfering, visible tracking dye helps to eliminate pipetting errors*
- *Products perform consistently across a wide variety of sample sources*
- *Lyophilized format (NEB #L4001) removes cold chain shipping requirements, enables room temperature storage, and can be quickly rehydrated*

Learn more about lyophilized reagents in our webinar.

Description: The Luna RT-qPCR kits contain a novel, *in silico*-designed reverse transcriptase (RT) engineered for improved performance. Both the Luna WarmStart Reverse Transcriptase and Hot Start *Taq* DNA Polymerase, included in these kits, utilize a temperature-sensitive, reversible aptamer, which inhibits activity below 45°C. This enables room temperature reaction setup and prevents undesired non-specific activity. Furthermore, the WarmStart RT has increased thermostability, improving performance at higher reaction temperatures.

The Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) supports robust, sensitive detection and quantitation of up to 5 targets in a multiplexed reaction. It is supplied at a 4X concentration and enables higher amounts of sample input, which is relevant for applications where RNA present in low abundance is of interest, such as pathogen detection. For simplified reaction setup, the single tube master mix format consolidates components for the one-step RT-qPCR reaction. It also includes dUTP and UDG in the mix for reduced risk of carryover contamination. This mix is also available without ROX (NEB #M3029) for instruments that do not require the ROX passive reference dye.

The LyoPrime Luna® Probe One-Step RT-qPCR Mix with UDG (NEB #L4001) is supplied in a lyophilized format, which can be rehydrated by simply adding nuclease-free water prior to use.

The Luna Universal One-Step RT-qPCR Kit (NEB #E3005) is optimized for dye-based real-time quantitation of target RNA sequences via the SYBR/FAM fluorescence channel of most real-time instruments.

The Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006) is optimized for real-time quantitation of target RNA sequences using hydrolysis probes.

For instruments that do not utilize ROX normalization, the Luna Probe One-Step RT-qPCR Kit (No ROX) (NEB #E3007) contains no reference dye. If ROX normalization is desired, ROX can be added; this is only necessary with the E3007 product.

The other Luna products contain dUTP and enable carryover prevention when reactions are treated with Antarctic Thermolabile UDG (NEB #M0372). A blue visible dye assists in tracking the reagents when pipetting into clear, multi-welled PCR plates. The reverse transcriptase, featured in the Luna One-Step RT-qPCR products is a novel, engineered WarmStart enzyme developed for robust performance and increased thermostability. These features, combined with rapid, sensitive and precise real-time qPCR performance, make Luna the universal choice for all your qPCR and RT-qPCR experiments.

The Luna Probe One-Step RT-qPCR Kit (No ROX) Includes:

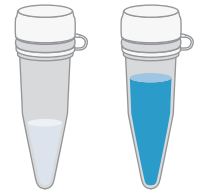
- Luna Universal Probe One-Step Reaction Mix (No ROX)
- Luna WarmStart RT Enzyme Mix
- Nuclease-free Water

The Luna Universal One-Step RT-qPCR Kit Includes:

- Luna Universal One-Step Reaction Mix
- Luna WarmStart RT Enzyme Mix
- Nuclease-free Water

The Luna Universal Probe One-Step RT-qPCR Kit Includes:

- Luna Universal Probe One-Step Reaction Mix
- Luna WarmStart RT Enzyme Mix
- Nuclease-free Water

	LyoPrime Luna® Probe One-Step RT-qPCR Mix with UDG	Luna® Probe One-Step RT-qPCR 4X Mix with UDG	Luna Universal Probe One-Step RT-qPCR Kit
	NEB #L4001	NEB #M3019	NEB #E3006
	Lyophilized Master Mix	4X Master Mix	20X RT Enzyme Mix; 2X Reaction Mix
			
Number of tubes	1	1	2
Sample-limiting concentration	2–4X	4X	2X
dUTP included	✓	✓	✓
UDG included	✓	✓	✗
Universal ROX included	✓	✓	✓
Storage temperature	Room temperature	–20°C	–20°C

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/WarmStart · Recombinant Albumin · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

Luna Cell Ready Probe One-Step RT-qPCR Kit

Luna Cell Ready One-Step RT-qPCR Kit
#E3030S 100 reactions

Luna Cell Ready Probe One-Step RT-qPCR Kit
#E3031S 100 reactions

Luna Cell Ready Lysis Module
#E3032S 100 reactions

Companion Product:

Antarctic Thermolabile UDG
#M0372S 100 units
#M0372L 500 units

- *Go direct from cells to RNA quantitation without purification*
- *Coordinated cell lysis, RNA release, and genomic DNA removal in a fast, 15-minute protocol*
- *Effective cell lysis preparation from 10-10,000 cells across numerous cell lines*
- *Features Luna WarmStart RT paired with HotStart Taq for increased thermostability and room temperature setup*

Description: The Luna Cell Ready One-Step RT-qPCR Kit provides all the necessary components for direct, dye-based RNA detection and quantitation, bypassing the need for RNA extraction and purification.

The Luna Cell Ready Probe One-Step RT-qPCR Kit provides all the necessary components for direct, probe-based RNA detection and quantitation, bypassing the need for RNA extraction and purification.

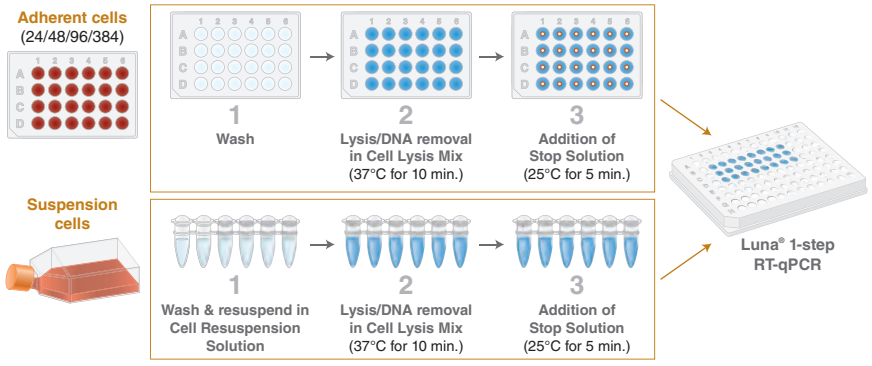
Cell cultures are often analyzed for gene expression or treatment responses as a proxy for a living organism. Traditionally, RNA is extracted and purified from treated cells via column-based or chemical methods. Coordinating the actions of DNase I and the Luna Cell Ready Protease, the Luna Cell Ready Lysis Module offers a simple alternative workflow resulting in effective cell lysis, RNA release, and genomic DNA removal simultaneously in a 15-minute protocol. The Lysis Module includes a unique Luna Cell Ready RNA Protection Reagent that maintains RNA integrity during cell lysis. The lysis capacity spans 10–100,000 cells in a 50 µl lysis reaction. Up to 2 µl of lysate (equivalent to RNA from 0.2–4,000 cells) can be transferred into 20 µl downstream RT-qPCR reactions. Similar to other Luna products, the lysis buffer includes an inert blue tracking dye for visual assistance throughout the workflow.

The Luna Cell Ready One-Step RT-qPCR Kit Includes:

- Luna Cell Ready Lysis Module
- Luna Universal One-Step RT-qPCR Kit (NEB #E3005)

The Luna Cell Ready Probe One-Step RT-qPCR Kit Includes:

- Luna Cell Ready Lysis Module
- Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006)



The Luna Cell Ready One-Step RT-qPCR Kit provides all the necessary components for direct RNA detection and quantitation from cultured cells (up to 100,000 cells per 50 µl lysis reaction). *By coordinating the actions of DNase I and the Luna Cell Ready Protease, the Luna Cell Ready Lysis Module offers a simple workflow resulting in effective cell lysis, RNA release and genomic DNA removal simultaneously in a 15-minute protocol. Up to 2 µl lysate (equivalent to RNA from 0.2 - 4,000 cells) can be transferred into 20 µl downstream RT-qPCR reactions.*

Harry joined NEB in 2020 and is a Program Manager in our Customized Solutions Group. Learn more about Harry's role at NEB in his video reel on Instagram.

#NEBiographies

SARS-CoV-2 Detection

Virus detection often utilizes nucleic acid amplification to identify the presence of specific sequences in SARS-CoV-2 viral RNA. These methods include RT-qPCR for real time quantitation and loop-mediated isothermal amplification (LAMP) for robust amplification performed at a single reaction temperature.

SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit

#E2019S 96 reactions

Companion Product:

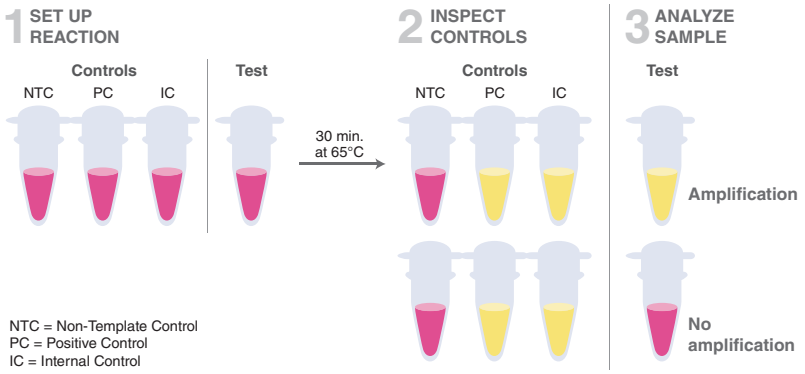
Control LAMP Primer Mix (rActin)
#S0164S 50 reactions

- *Colorimetric LAMP enables simple, visual detection (pink-to-yellow) of amplification of SARS-CoV-2 nucleic acid*
- *Set up reactions quickly and easily, using a simple heat source and unique WarmStart technology*
- *Reduce risk of carryover contamination, with UDG and dUTP included in the master mix*
- *Assay targets N and E regions of the SARS-CoV-2 genome, for optimized sensitivity and specificity*
- *Bring confidence to your results using the provided controls*

Description: The SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit utilizes Loop-Mediated Isothermal Amplification (LAMP) to detect SARS-CoV-2 nucleic acid. The kit is available for research use only and includes WarmStart Colorimetric LAMP 2X Master Mix with UDG and a primer mix targeting the N and E regions of the viral genome. Controls are provided to verify assay performance, and include an internal control primer set and a positive control template. Guanidine hydrochloride has been found to increase the speed and sensitivity of the RT-LAMP reaction and is also included.

Kit Includes:

- WarmStart Colorimetric LAMP 2X Master Mix with UDG
- SARS-CoV-2 Positive Control (N gene)
- Control LAMP Primer Mix (rActin)
- SARS-CoV-2 LAMP Primer Mix (N/E)
- Nuclease-free Water
- Guanidine Hydrochloride



In the SARS-CoV-2 Colorimetric LAMP Assay Kit, three control reactions are run with each test sample. *All reactions should be pink prior to incubation. The NTC reaction will contain all materials of the test sample (master mix, primers, etc.) except for the test input nucleic acid and serves as a measure of reaction contamination and primer-based mis-amplification. The NTC should stay pink throughout the experiment. The PC will contain master mix, a plasmid that contains the SARS-CoV-2 N-gene (GenBank: MN908947.3) and primers that will amplify this sequence. Amplification should be observed and the PC should become yellow after incubation. The IC will contain master mix, test input nucleic acid, and LAMP primers for rActin, an endogenous housekeeping gene. If reagents are active and samples have been handled appropriately, the IC should become yellow after incubation.*

Luna® SARS-CoV-2 RT-qPCR Multiplex Assay Kit

#E3019S 96 reactions

- *Multiplex detection of N1 and N2 targets*
- *Enables sample pooling of purified RNA*
- *Internal controls include a redesigned RNase P reverse primer for reduced background amplification*

Description: The Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit is optimized for real-time qualitative detection of SARS-CoV-2 nucleic acid using hydrolysis probes. It features the Luna Probe One-Step RT-qPCR 4X Mix with UDG, an optimized SARS-CoV-2 Primer/Probe mix containing primers and probes specific to two regions of the SARS-CoV-2 virus N-gene, and a positive control template. The probes have been modified to contain different fluorophores (N1, HEX; N2, FAM) to enable simultaneous observation on two different channels of a real-time instrument. To ensure the integrity of the input material and absence of inhibition, an internal control (IC) primer and probe set, designed to amplify the human RNase P gene, is also provided in the primer mix. The reverse primer of this target has been modified from the CDC design to target an exon/exon boundary to reduce background amplification from possible contaminating genomic DNA. Amplification of the IC is observed in the Cy5 channel.

Kit Includes:

- Luna Probe One-Step RT-qPCR 4X Mix with UDG
- SARS-CoV-2 Positive Control (N gene)
- SARS-CoV-2 Primer/Probe Mix (N1/N2/RP) (10X)
- Nuclease-free Water

View our loop mediated isothermal amplification tutorial.

Cloned at NEB | Recombinant Enzyme | High-Fidelity | PCR Enzyme | Hot Start/WarmStart | Recombinant Albumin | Heat Inactivation | Annealing Temperature | EpiMark | Extraction Free

WarmStart Fluorescent LAMP/RT-LAMP Kit (with or without UDG)

WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG)
#E1708S 100 reactions
#E1708L 500 reactions

NEW
LyoPrime WarmStart Fluorescent LAMP/RT-LAMP Mix (with UDG)
#L4401S 100 reactions
#L4401P 96 reactions

WarmStart LAMP Kit (DNA & RNA)
#E1700S 100 reactions
#E1700L 500 reactions

Companion Products:

WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)
#M1708S 100 reactions
#M1708S 100 reactions

LAMP Fluorescent Dye
#B1700S 0.25 ml

Control LAMP Primer Mix (rActin)
#S0164S 50 reactions

- *Reduce risk of carryover contamination with UDG and dUTP included in master mix*
- *Improve LAMP specificity and sensitivity with optimized master mixes*
- *Set up reactions at room temperature with our unique dual WarmStart formulation*
- *WarmStart Multi-Purpose LAMP/RT LAMP 2X Master Mix (with UDG) supports a variety of detection methods, including turbidity, visual detection and electrophoresis*

WarmStart Fluorescent LAMP/RT-LAMP kits are designed to provide a simple, one-step solution for Loop Mediated Isothermal Amplification (LAMP) of DNA or RNA (RT-LAMP) targets. LAMP and RT-LAMP are commonly used isothermal techniques that provide rapid detection of a target nucleic acid using LAMP-specific primers (supplied by the user) and a strand-displacing DNA polymerase.

The supplied master mixes contain an optimized blend of *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx Reverse Both enzymes have been engineered for improved performance in LAMP and RT-LAMP reactions. The inclusion of dUTP and UDG in the master mix (as noted in the product name) reduces the possibility of carryover contamination between reactions. A fluorescent dye is also supplied to enable real-time fluorescence measurement of LAMP. All WarmStart LAMP/RT-LAMP kits are compatible with multiple detection methods, including turbidity detection, real-time fluorescence detection (when used with LAMP fluorescent dye) and end-point visualization.

The LyoPrime WarmStart™ Fluorescent LAMP/RT-LAMP Mix (with UDG) is supplied in a lyophilized format, which can be rehydrated by simply adding nuclease-free water prior to use.

The WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG) includes:

- WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)
- LAMP Fluorescent Dye

The WarmStart Fluorescent LAMP/RT-LAMP Kit includes:

- WarmStart LAMP 2X Master Mix
- LAMP Fluorescent Dye (50X)

The LyoPrime WarmStart™ Fluorescent LAMP/RT-LAMP Mix (with UDG) includes:

- LyoPrime WarmStart Fluorescent LAMP/RT-LAMP Mix (with UDG)

WarmStart® Colorimetric LAMP/RT-LAMP 2X Master Mix

WarmStart® Colorimetric LAMP/RT-LAMP 2X Master Mix (with UDG)

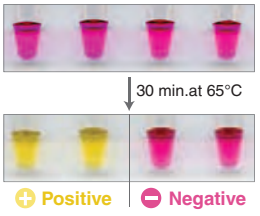
WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA)
#M1800S 100 reactions
#M1800L 500 reactions

WarmStart Colorimetric LAMP 2X Master Mix with UDG
#M1804S 100 reactions
#M1804L 500 reactions

- *Simple, one-step solution for Loop-Mediated Isothermal Amplification (LAMP) of DNA or RNA (RT-LAMP) targets*
- *Fast, clear, pink-to-yellow visible detection of amplification*
- *WarmStart feature inhibits enzyme activity at room temperature allowing for flexible reaction setup*
- *Reduce risk of carryover contamination with UDG and dUTP included in the master mix (NEB #M1804)*

Description: The WarmStart Colorimetric LAMP/RT-LAMP 2X Master Mix is an optimized formulation of *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx in a special low-buffer reaction solution containing a visitble pH indicator for rapid and easy detection of Loop Mediated Isothermal Amplification (LAMP) and RT-LAMP reactions. WarmStart Colorimetric LAMP/RT-LAMP 2X Master Mix (with UDG) contains dUTP and UDG in the master mix, which reduces the possibility of carryover contamination between reactions.

This system is designed to provide a fast, clear visual detection of amplification based on the production of protons and subsequent drop in pH that occurs from the extensive DNA polymerase activity in a LAMP reaction, producing a change in solution color from pink to yellow (an overview of LAMP and primer design can be found in the Featured Videos section). This mix can be used for any LAMP or RT-LAMP reaction and requires only a heated chamber and samples, with readout of positive amplification judged by eye in 15–40 minutes.



How is colorimetric LAMP used in point of care diagnostics?

Bst DNA Polymerase-based Products for Isothermal DNA Amplification

Product	5´→ 3´ Exo Activity	Amplification Speed	Room Temp. Setup	Reverse Transcriptase Actvity	Inhibitor Tolerance	Amplification Specificity	Applications
***Bst* DNA Polymerase, Full Length**	★★	N/A	N/A	N/A	★	N/A	• Nick translation reactions at elevated temperatures • Primer extension
***Bst* DNA Polymerase, Large Fragment**	N/A	★	N/A	★	★	★	• General strand-displacement reactions
***Bst* 2.0 DNA Polymerase**	N/A	★★	N/A	★★	★	★★	• Improved LAMP, SDA, and other amplification reactions • Minimal effect of substitution of dTTP with dUTP
***Bst* 2.0 WarmStart DNA Polymerase**	N/A	★★	★★★	★★	★★	★★★	• Consistent, room-temperature, and high-throughput amplification assays • Minimal effect of substitution of dTTP with dUTP
***Bst* 3.0 DNA Polymerase**	N/A	★★★	★★	★★★	★★★	★★	• Fused to novel nucleic acid binding domain for enhanced performance • Fastest, most robust LAMP and RT-LAMP reactions • High reverse transcriptase activity up to 72°C • Strand displacement DNA synthesis
***Bst*-XT WarmStart DNA Polymerase**	N/A	★★★	★★★	★★★	★★★	★★★	• Supports LAMP at lower temperatures (optimal temperature range is 50–70°C)

★★★ Optimal, recommended product for selected application
★★ Works well for selected application
★ Will perform selected application, but is not recommended
N/A Not applicable to this application

Bst DNA Polymerases

Bst DNA Polymerase, Large Fragment
#M0275S 1,600 units
#M0275L 8,000 units

for high (15X) concentration
#M0275M 8,000 units

Bst DNA Polymerase, Full Length
#M0328S 500 units

Bst 2.0 DNA Polymerase
#M0537S 1,600 units
#M0537L 8,000 units

for high (15X) concentration
#M0537M 8,000 units

Bst 2.0 WarmStart DNA Polymerase
#M0538S 1,600 units
#M0538L 8,000 units

for high (15X) concentration
#M0538M 8,000 units

NEW
Bst 2.0 WarmStart DNA Polymerase (Glycerol-free)
#M0402L 8,000 units

Bst 3.0 DNA Polymerase
#M0374S 1,600 units
#M0374L 8,000 units

for high (15X) concentration
#M0374M 8,000 units

NEW
Bst-XT WarmStart DNA Polymerase
M9204S.....1,600 units
M9204L.....8,000 units

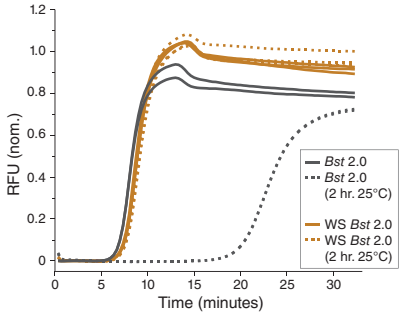
NEW
Bst-XT WarmStart DNA Polymerase (Glycerol-free)
M9205L 8,000 units

Description: *Bst* DNA Polymerase, Large Fragment, is the portion of the Bacillus *stearothermophilus* DNA Polymerase protein that contains the 5´→ 3´ polymerase activity, but lacks 5´→ 3´ exonuclease activity.

Bst DNA Polymerase, Full Length is the full length polymerase from Bacillus *stearothermophilus*. It has 5´→ 3´ polymerase and double-strand specific 5´→ 3´ exonuclease activities, but lacks 3´→ 5´ exonuclease activity.

Bst 2.0 DNA Polymerase is an *in silico* designed homologue of *Bst* DNA Polymerase, Large Fragment. It contains 5´→ 3´ DNA polymerase activity and strong strand displacement activity but lacks 5´→ 3´ exonuclease activity. It has improved amplification speed, yield, salt tolerance and thermostability compared to wild-type *Bst* DNA Polymerase, Large Fragment.

Bst 2.0 WarmStart DNA Polymerase utilizes aptamer technology to inhibit activity at non-permissive temperatures (< 50°C). Like "Hot Start" PCR polymerases, the WarmStart feature enables room temperature set up and prevents non-templated addition of nucleotides, increasing reaction efficiencies. Additionally, no separate activation step is required to release the aptamer from the enzyme. *Bst* 2.0 WarmStart DNA Polymerase permits reaction temperatures from 60–72°C. *Bst* 2.0 WarmStart DNA Polymerase is also available in a glycerol-free format to support lyophilization and automation workflows.



Benefits of Bst 2.0 WarmStart. *Identical LAMP reactions were run either immediately after setup (solid line) or after a 2 hour incubation at 25°C: Without the protection from Bst 2.0 WarmStart, this room temperature incubation results in variable LAMP performance. Bst 2.0 WarmStart provides more consistent amplification reaction and enables room-temperature and high-throughput setup.*

Bst 3.0 DNA Polymerase is a similarly designed *in silico* homologue engineered and fused to a novel nucleic acid binding domain for improved isothermal amplification performance and increased reverse transcription activity. *Bst* 3.0 DNA Polymerase contains 5´→ 3´ DNA polymerase activity with either DNA or RNA templates but lacks 5´→ 3´ and 3´→ 5´ exonuclease activity. It demonstrates robust performance in the presence of inhibitors and significantly increased reverse transcriptase activity compared to *Bst* DNA Polymerase.

Bst-XT WarmStart DNA Polymerase combines the fast polymerization speed of *Bst* 3.0 and the high specificity of *Bst* 2.0.

Concentration: *Bst* DNA Polymerase, Full Length: 5,000 units/ml. All others: 8,000 and 120,000 units/ml. NEB #M0402 only sold at 120,000 units/ml.

Heat Inactivation: 80°C for 20 minutes

Usage Notes: No *Bst* DNA Polymerase-based products can be used for thermal cycle sequencing or PCR. *Bst* 2.0 WarmStart DNA Polymerase permits reaction temperatures from 60–72°C. *Bst*-XT WarmStart DNA Polymerase has optimal reaction performance from 50–70°C. Generally, reaction temperatures above 72°C are not recommended for any *Bst* DNA Polymerase-based product.

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NEW

phi29-XT WGA Kit

#E1604S	25 reactions
#E1604L	100 reactions

Companion Products:

T7 Endonuclease I

#M0302S	250 units
#M0302S	250 units

NEBNext Ultra II FS DNA Library Prep Kit for Illumina

#E7805S	24 reactions
#E7805S	24 reactions

Deoxynucleotide (dNTP) Solution Mix

#N0447S	8 µmol
#N0447S	8 µmol

phi29 DNA Polymerase

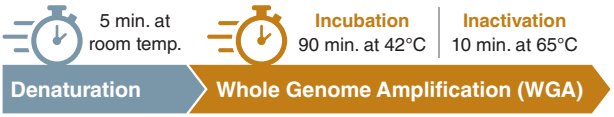
#M0269S	250 units
#M0269S	250 units

- *High sensitivity: amplify from as little as 10 fg of input DNA*
- *Flexible protocol offers compatibility with different types of sample input material*
- *Simple workflow produces high yield in a short reaction time (less than 2 hours)*
- *Whole genome representation for human and microbial DNA*

Description: The phi29-XT WGA Kit is optimized for sensitive, fast, and robust whole genome amplification (WGA), an isothermal method for amplification of an entire genome, starting from femtogram quantities of DNA and resulting in microgram quantities of amplified products. The kit features phi29-XT DNA Polymerase, an engineered polymerase with improved thermostability, sensitivity, and capable of generating a higher yield in a shorter reaction time than wild-type phi29 DNA Polymerase. Also included are exonuclease-resistant random primers (containing phosphorothioate bonds) to universally amplify any DNA sequence, neutralization buffer, and dNTPs. Input material can be purified genomic DNA, cells, or microbiomes. WGA enables several downstream applications, including single cell whole genome sequencing (WGS), genomic DNA enrichment, and environmental DNA (eDNA) enrichment and sequencing.

Kit includes:

- phi29-XT DNA Polymerase for WGA
- phi29-XT Reaction Buffer for WGA
- Neutralization Buffer
- Exonuclease-Resistant Random Primers
- Deoxynucleotide (dNTP) Solution Mix



NEW

phi29-XT RCA Kit

#E1603S	100 reactions
#E1603L	500 reactions

Companion Product:

NEBExpress Cell-free *E. coli* Protein Synthesis System

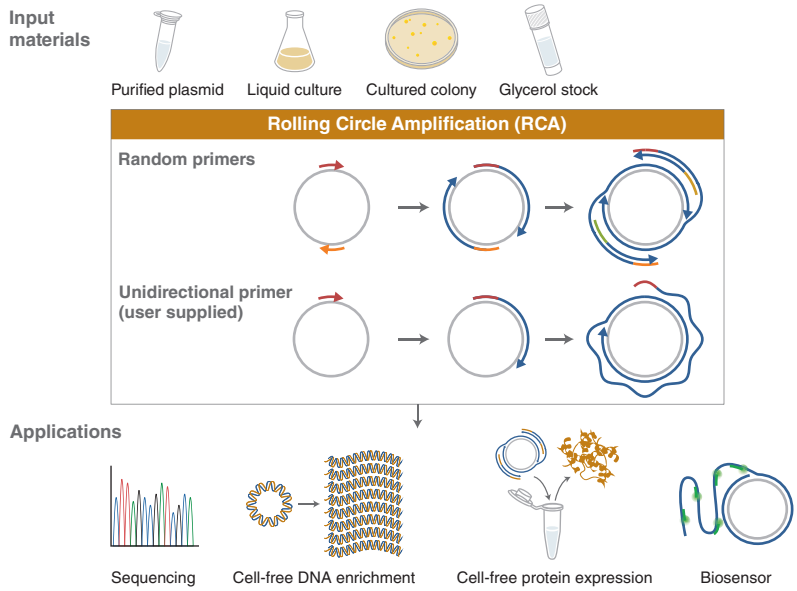
#E5360S	10 reactions
#E5360S	10 reactions

- *High sensitivity: as little as 1 fg of input plasmid DNA needed*
- *Robust and simple workflow generates high yield in a short reaction time*
- *Flexible input material format: purified circular DNA or plasmid/fosmid containing bacterial colony, glycerol stock, or liquid culture*
- *Optimal reaction temperature of 42°C*

Description: Rolling Circle Amplification (RCA) is a robust and highly sensitive isothermal amplification approach to continuously amplify circular DNA, generating long, repetitive copies of the circular sequence.This kit features phi29-XT DNA Polymerase, an engineered polymerase that generates more product in a shorter amount of time than wild-type phi29 DNA polymerase. It also possesses greater sensitivity than the wild-type enzyme while sharing the high processivity, strong strand-displacement, and high-fidelity qualities that are ideal for RCA applications. It is also more thermostable, with an optimal reaction temperature of 42°C. This kit includes exonuclease-resistant random hexamer primers to universally amplify any circular DNA sequence.

Kit Includes:

- phi29-XT DNA Polymerase
- phi29-XT Reaction Buffer
- Exonuclease-resistant Random Primers
- Deoxynucleotide (dNTP) Solution Mix



Overview of the phi29-XT RCA DNA Amplification Kit. *The phi29-XT RCA DNA Amplification Kit (NEB #E1603) is a fast, simple to use and highly versatile kit containing all the required components for rolling circle amplification (RCA) using a random primer mix. The kit delivers high yields of DNA products from a variety of starting materials including purified circular DNA or bacterial cells. This kit is ideal for various DNA applications such as DNA sequencing, cell-free DNA enrichment, cell-free protein expression and DNA biosensors.*

phi29 DNA Polymerase

#M0269S 250 units
#M0269L 1,250 units

- *Extreme processivity*
- *Extreme strand displacement*
- *Replication requiring a high degree of strand displacement and/or processive synthesis*
- *High-fidelity replication at moderate temperatures*

GMP-reagent now available.
See page 6 for details.

Description: phi29 DNA Polymerase is the replicative polymerase from the *Bacillus subtilis* phage phi29 (φ29). This polymerase has exceptional strand displacement and processive synthesis properties. The polymerase has an inherent 3´→ 5´ proofreading exonuclease activity.

Applications:

- Replication requiring a high degree of strand displacement and/or processive synthesis
- High fidelity replication at moderate temperatures

Reagents Supplied:

- phi29 DNA Polymerase Reaction Buffer
- Recombinant Albumin, Molecular Biology Grade

Source: An *E. coli* strain that carries the phi29 DNA Polymerase gene from bacteriophage phi29

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 0.5 pmol of dNTP into acid insoluble material in 10 minutes at 30°C.

Concentration: 10,000 units/ml

Heat Inactivation: 65°C for 10 minutes

LunaScript® Multiplex One-Step RT-PCR Kit

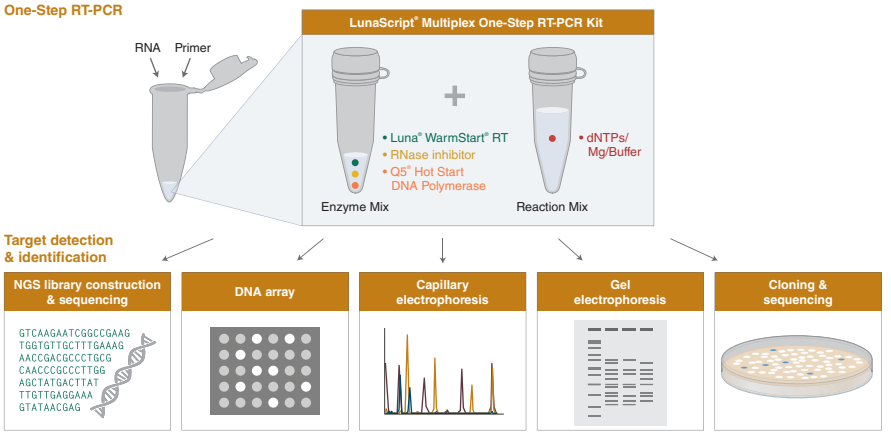
#E1555S 50 reactions
#E1555L 250 reactions

- *Detect as low as 0.01 pg of human total RNA*
- *Multiplexing capacity supports use in ARTIC workflows*
- *Set up reactions at room temperature*
- *Save time, plastics, and minimize contamination with a closed-tube, one-step RT-PCR Protocol*

The LunaScript Multiplex One-Step RT-PCR Kit offers a streamlined protocol for cDNA synthesis and PCR amplification in a single reaction. The 5X reaction mix contains dNTPs and is optimized for multiple target detection in a simple workflow. The 25X enzyme mix features Luna WarmStart Reverse Transcriptase and Q5 Hot Start High-Fidelity DNA Polymerase. The dual-temperature control of enzyme activities by aptamer-based inhibition enables room temperature reaction setup, with preassembled reactions stable at room temperature for up to 24 hours.

Kit Includes:

- LunaScript® Multiplex One-Step RT-PCR Enzyme Mix
- LunaScript® Multiplex One-Step RT-PCR Reaction Mix
- Nuclease-free Water



Multiplex RNA target detection and identification from a single RT-PCR reaction. *The LunaScript Multiplex One-Step RT-PCR Kit (NEB #E1555) requires only RNA template and gene-specific primers to enable multiplex cDNA target synthesis and amplification in a single reaction. Amplified cDNA products can be detected or identified by downstream applications including next-generation sequencing, DNA arrays, fragment analysis, electrophoresis and traditional cloning/sequencing workflows.*

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One*Taq*® One-Step RT-PCR Kit

#E5315S 30 reactions

Companion Products:

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

ProtoScript II First Strand cDNA Synthesis Kit
#E6560S 30 reactions
#E6560L 150 reactions

ProtoScript II Reverse Transcriptase
#M0368S 4,000 units
#M0368L 10,000 units
#M0368X 40,000 units

- *Combine cDNA synthesis and PCR in a single reaction*
- *Detect as little as 0.1 pg of a GAPDH target*
- *Robust amplification from 100 bp to 9 kb*
- *Faster protocols with less hands-on time*
- *Quick-Load Reaction Mix allows direct gel loading*

The One*Taq* One-Step RT-PCR Kit offers sensitive and robust end-point detection of RNA templates. cDNA synthesis and PCR amplification steps are performed in a single reaction using gene-specific primers, resulting in a streamlined RT-PCR protocol.

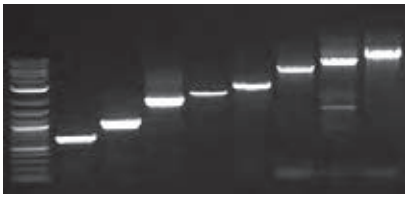
The kit combines three optimized mixes: One*Taq* OneStep Enzyme Mix, One*Taq* One-Step Reaction Mix and One*Taq* One-Step Quick-Load Reaction Mix. The Enzyme Mix combines ProtoScript II Reverse Transcriptase, Murine RNase Inhibitor and One*Taq* Hot Start DNA Polymerase. ProtoScript II Reverse Transcriptase is a mutant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. One*Taq* Hot Start DNA Polymerase is an optimized blend of *Taq* and Deep Vent DNA polymerases combined with an aptamer-based inhibitor.The One*Taq* One-Step RT-PCR Kit is capable of amplifying long transcripts up to 9 kb in length.

Two optimized reaction mixes are included, One*Taq* OneStep Reaction Mix and Quick-Load One*Taq* One-Step Reaction Mix. The reaction mixes offer robust conditions for both cDNA synthesis and PCR amplification. The unique Quick-Load One*Taq* One-Step Reaction Mix contains additional dyes, offering color indication for reaction setup as well as direct gel loading.

Both total RNA and mRNA can be used as template. The kit can detect a GAPDH target as low as 0.1 pg per reaction. It can routinely detect RNA targets up to 9 kb. The OneTaq One-Step RT-PCR Kit is capable of multiplex detection of two or three targets.

Kit Includes:

- One*Taq* One-Step Enzyme Mix
- One*Taq* One-Step Reaction Mix
- Quick-Load One*Taq* One-Step Reaction Mix
- Nuclease-free Water



Detection of RNA templates of different length. *About 100 ng of Jurkat total RNA was used in 50 µl reactions following the standard protocol. The target sizes were Lane 1: 0.7 kb, Lane 2: 1.1 kb, Lane 3: 1.9 kb, Lane 4: 2.3 kb, Lane 5: 2.5 kb, Lane 6: 5.5 kb, Lane 7: 7.6 kb and Lane 8: 9.3 kb. The marker lane (M) contains Quick-Load 1 kb Plus DNA Ladder (NEB #N0469).*

One*Taq*® RT-PCR Kit

#E5310S 30 reactions

Companion Products:

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314S 3,000 units

M-MuLV Reverse Transcriptase
#M0253S 10,000 units
#M0253S 10,000 units

One*Taq* Hot Start 2X Master Mix with Standard Buffer
#M0484S 100 reactions
#M0484S 100 reactions

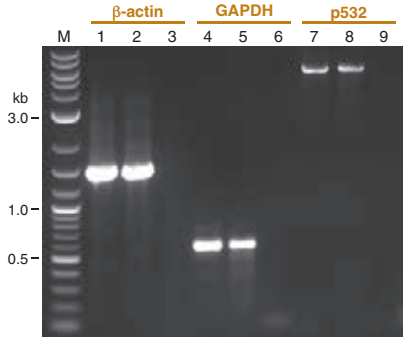
- *Robust RT-PCR reactions*
- *Convenient master mix formats*
- *OneTaq DNA Polymerase offers robust amplification for a wide range of templates*

One*Taq* RT-PCR Kit combines two powerful mixes, M-MuLV Enzyme Mix and One*Taq* Hot Start 2X Master Mix with Standard Buffer for 2-step RT-PCR applications. The two mixes require minimal handling during reaction setup and yet offer consistent and robust RT-PCR reactions.

The first strand cDNA synthesis is achieved by using two optimized mixes, M-MuLV Enzyme Mix and M-MuLV Reaction Mix. M-MuLV Enzyme Mix combines M-MuLV Reverse Transcriptase and RNase Inhibitor, Murine while M-MuLV Reaction Mix contains dNTPs and an optimized buffer. The kit also contains two optimized primers for reverse transcription and nuclease-free water. An anchored Oligo-dT primer [$d(T)_{23}$VN] forces the primer to anneal to the beginning of the polyA tail. The optimized Random Primer Mix provides random and consistent priming sites covering the entire RNA templates including both mRNAs and non-polyadenylated RNAs.

Kit Includes:

- M-MuLV Enzyme Mix
- M-MuLV Reaction Mix
- One*Taq* Hot Start 2X Master Mix with Standard Buffer
- Random Primer Mix
- Oligo $d(T)_{23}$ VN
- Nuclease-free Water



First strand cDNA synthesis. *Synthesis was carried out in the presence of 1X M-MuLV Enzyme Mix at 42°C using 0.5 µg of human spleen total RNA in the presence of dT23VN (lanes 1, 4 and 7) or Random Hexamer Mix (lanes 2, 5 and 8). No-RT controls were lanes 3, 6 and 9. OneTaq Hot Start Master Mix was used to amplify a 1.5 kb fragment of beta-actin gene, a 0.6 kb fragment of GAPDH gene, and a 5.5 kb fragment from p532 gene in 35 cycles. The marker lane (M) contains 1 kb Plus DNA Ladder (NEB #N3200).*

PreCR® Repair Mix

#M0309S 30 reactions
#M0309L 150 reactions

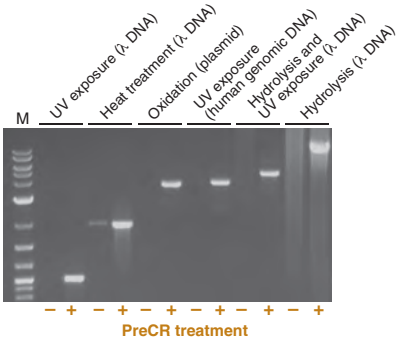
Companion Product:

β-Nicotinamide adenine dinucleotide (NAD+)
#B9007S 0.2 ml

- *Repair DNA prior to its use in DNA-related technologies*
- *Easy-to-use protocols*
- *Does not harm template*

Need to repair FFPE-treated DNA prior to next gen sequencing?
Try our NEBNext FFPE DNA Repair v2 Module (NEB #E7360).

Description: The PreCR Repair Mix is an enzyme cocktail formulated to repair damaged template DNA prior to its use in the polymerase chain reaction (PCR), microarrays, or other DNA technologies. The PreCR Repair Mix is active on a broad range of DNA damage, including those that block PCR (e.g., apurinic/apyrimidinic sites, thymidine dimers, nicks and gaps) and those that are mutagenic (e.g., deaminated cytosine and 8-oxo-guanine). In addition, it will remove a variety of moieties from the 3´ end of DNA leaving a hydroxyl group. The PreCR Repair Mix will not repair all damage that inhibits/interferes with PCR. It can be used in conjunction with any thermophilic polymerase.



Repair of different types of DNA damage with the PreCR Repair Mix. *The gel shows amplification of damaged DNA that was either not treated (–) or treated (+) with the PreCR Repair Mix. Type of DNA damage is shown. Note: heat treated DNA is incubated at 99°C for 3 minutes. Marker (M) is the 1 kb Plus DNA Ladder (NEB #N3200).*

Applications:

- Repair of DNA prior to its use as a template in PCR or other DNA technologies.

Types of DNA Damage

DNA Damage	Cause	Repaired By PreCR Repair Mix?
Abasic sites	• Hydrolysis	Yes
Nicks	• Hydrolysis • Nucleases • Shearing	Yes
Thymidine dimers	• UV radiation	Yes
Blocked 3´ends	• Multiple	Yes
Oxidized guanine	• Oxidation	Yes
Oxidized pyrimidines	• Oxidation	Yes
Deaminated cytosine	• Hydrolysis	Yes
Fragmentation	• Hydrolysis • Nucleases • Shearing	No
Protein-DNA crosslinks	• Formaldehyde	No

Sulfolobus DNA Polymerase IV

#M0327S 100 units

- *Synthesis of DNA through DNA lesions (lesion bypass)*
- *DNA Repair*

Description: *Sulfolobus* DNA Polymerase IV is a thermostable Y-family lesion-bypass DNA Polymerase that efficiently synthesizes DNA across a variety of DNA template lesions.

Source: An *E. coli* strain that carries the gene encoding DNA polymerase IV from *Sulfolobus islandicus.*

Concentration: 2,000 units/ml

Therminator™ DNA Polymerase

#M0261S 200 units
#M0261L 1,000 units

- *Incorporation of modified nucleotides*
- *DNA sequencing by partial ribosubstitution*
- *DNA sequencing or SNP analysis using dideoxy or acyclo chain terminators*

Description: Therminator DNA Polymerase is a 9°N™ DNA Polymerase variant with an enhanced ability to incorporate modified substrates such as dideoxynucleotides, ribonucleotides and acyclonucleotides.

Source: An *E. coli* strain that carries the 9°N (D141A / E143A / A485L) DNA Polymerase gene, a genetically engineered form of the native DNA polymerase from *Thermococcus* species 9°N-7.

Concentration: 2,000 units/ml

Note: Amplification of extended regions may require optimization of reaction conditions.

DNA Polymerase I (*E. coli*)

#M0209S 500 units
#M0209L 2,500 units

- *Nick translation of DNA*
- *Second strand cDNA synthesis*

Description: DNA Polymerase I (*E. coli*) is a DNA-dependent DNA polymerase with inherent 3´→ 5´ and 5´→ 3´ exonuclease activities. The 5´→ 3´ exonuclease activity removes nucleotides ahead of the growing DNA chain, allowing nick translation.

Source: An *E. coli* strain that carries an overexpressed copy of the *polA* gene.

Concentration: 10,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Note: DNase I is not included with this enzyme and must be added for nick translation reactions.

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DNA Polymerase I, Large (Klenow) Fragment

#M0210S 200 units
#M0210L 1,000 units

for high (10X) concentration
#M0210M 1,000 units

- *Generates probes using random primers*
- *Removal of 3´ overhangs or fill-in of 5´ overhangs to form blunt ends*
- *Second strand cDNA synthesis*

Description: DNA Polymerase I, Large (Klenow) Fragment was originally derived as a proteolytic product of *E. coli* DNA Polymerase I that retains polymerase and 3´→ 5´ exonuclease activity, but lacks 5´→ 3´ exonuclease activity. Klenow retains the polymerization fidelity of the holoenzyme without degrading 5´ termini.

Source: An *E. coli* strain that contains the *E. coli polA* gene that has had its 5'→3' exonuclease domain removed.

Concentration: 5,000 and 50,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Note: Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times may result in recessed ends due to the 3´→ 5´ exonuclease activity of the enzyme.

Klenow Fragment (3´→5´ exo-)

#M0212S 200 units
#M0212L 1,000 units

for high (10X) concentration
#M0212M 1,000 units

- *Generates probes using random primers*
- *Random priming labeling*
- *Second strand cDNA synthesis*

Description: Klenow Fragment (3´→ 5´ exo$^-$) is an N-terminal truncation of DNA Polymerase I that retains polymerase activity, but has lost the 5´→ 3´ exonuclease activity, and has mutations (D355A, E357A) that abolish the 3´→ 5´ exonuclease activity.

Source: An *E. coli* strain containing a plasmid with a fragment of the *E.coli polA* (D355A, E357A) gene starting at codon 324.

Concentration: 5,000 and 50,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Note: Klenow Fragment (3´→ 5´ exo-) is not suitable for generating blunt ends because it lacks the 3´→ 5´ exonuclease necessary to remove non-templated 3´ additions.

T4 DNA Polymerase

#M0203S 150 units
#M0203L 750 units

Companion Product:

Quick Blunting Kit
#E1201S 20 reactions
#E1201L 100 reactions

- *Gap filling (no strand displacement activity)*
- *Removal of 3´ overhangs or fill-in of 5´ overhangs to form blunt ends*
- *Probe labeling using replacement synthesis*
- *Single-strand deletion subcloning*

Description: T4 DNA Polymerase catalyzes the synthesis of DNA in the 5´→ 3´ direction and requires the presence of template and primer. This enzyme has a 3´→ 5´ exonuclease activity which is much more active than that found in *E. coli* DNA Polymerase I. Unlike DNA Polymerase I, T4 DNA Polymerase does not have a 5´→ 3´ exonuclease function.

Source: Purified from a strain of *E. coli* that carries the T4 DNA Polymerase gene.

Concentration: 3,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Note: Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times may result in recessed ends due to the 3´→ 5´ exonuclease activity of the enzyme.

T7 DNA Polymerase (unmodified)

#M0274S 300 units
#M0274L 1,500 units

- *Gap-filling reaction (no strand displacement)*

Description: T7 DNA Polymerase catalyzes the replication of T7 phage DNA during infection. The protein dimer has two catalytic activities: DNA polymerase activity and strong 3´→ 5´ exonuclease. The high fidelity and rapid extension rate of the enzyme make it particularly useful in copying long stretches of DNA template.

Source: T7 DNA Polymerase consists of two subunits: T7 gene 5 protein (84 kilodaltons) and *E.coli* thioredoxin (12 kilodaltons) (1,4-7). Each protein is cloned and overexpressed in a T7 expression system in *E. coli* (4).

Concentration: 10,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Note: The high polymerization rate of the enzyme makes long incubations unnecessary. T7 DNA Polymerase is not suitable for DNA sequencing.

NEB also offers a selection of Nucleotides and Deoxynucleotides, as well as Reaction Buffers sold separately.

Bsu DNA Polymerase, Large Fragment

#M0330S 200 units
#M0330L 1,000 units

- *Random primer labeling*
- *Second strand cDNA synthesis*
- *Single dA tailing*
- *Strand displacement DNA synthesis*

Description: *Bsu* DNA Polymerase, Large Fragment retains the 5´→ 3´ polymerase activity of the *Bacillus subtilis* DNA polymerase, but lacks the 5´→ 3´ exonuclease domain. This large fragment naturally lacks 3´→ 5´ exonuclease activity.

Source: An *E. coli* strain that contains the Bacillus subtilis DNA polymerase gene (starting from codon 297 thus lacking the 5´→ 3´ exonuclease domain).

Concentration: 5,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Note: *Bsu* DNA Polymerase, Large Fragment is not suitable for generating blunt ends because it lacks the 3´→ 5´ exonuclease necessary to remove non-templated 3´ additions. *Bsu* DNA Polymerase, Large Fragment retains 50% activity at 25°C and is twice as active as Klenow Fragment (3´→ 5´ exo–) at this temperature.

Terminal Transferase

#M0315S 500 units
#M0315L 2,500 units

- *Addition of homopolymer tails to the 3´ ends of DNA*
- *Labeling the 3´ ends of DNA with modified nucleotides (e.g., ddNTP, DIG-dUTP)*
- *TUNEL assay (in situ localization of apoptosis)*
- *TdT dependent PCR*

Description: Terminal Transferase (TdT) is a template independent polymerase that catalyzes the addition of deoxynucleotides to the 3´ hydroxyl terminus of DNA molecules. Protruding, recessed or blunt-ended double or single-stranded DNA molecules serve as a substrate for TdT. The 58.3 kDa enzyme does not have 5´ or 3´ exonuclease activity. The addition of Co^{2+} in the reaction makes tailing more efficient.

Source: An *E. coli* strain that carries the cloned Terminal Transferase gene from calf thymus.

Concentration: 20,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Polymerase Reaction Buffers

Q5 Reaction Buffer Pack
#B9027S 6 ml

Phusion HF Buffer Pack
#B0518S 6 ml

Phusion GC Buffer Pack
#B0519S 6 ml

Standard *Taq* Reaction Buffer Pack
#B9014S 6 ml

Standard *Taq* (Mg-free) Reaction Buffer Pack
#B9015S 6 ml

ThermoPol Reaction Buffer
#B9004S 6 ml

Isothermal Amplification Buffer Pack
#B0537S 6 ml

Isothermal Amplification Buffer II Pack
#B0374S 6 ml

Description: Q5 Reaction Buffer and High GC Enhancer are provided with both Q5 and Q5 Hot Start High-Fidelity DNA Polymerases.

Phusion High-Fidelity DNA Polymerase is supplied with 5X Phusion HF Buffer, 5X Phusion GC Buffer, DMSO, and 50 mM MgCl2.

Standard *Taq* Reaction Buffer is provided with Taq DNA Polymerase as an alternative to the ThermoPol Reaction Buffer.

ThermoPol Reaction Buffer is provided with *Taq*, Vent, Deep Vent, *Bst* Full Length and *Bst* Large Fragment, Sulfolobus IV and Therminator DNA Polymerases; this buffer contains 2 mM MgSO4 when the buffer is diluted to its final 1X concentration.

Isothermal Amplification Buffer is supplied with *Bst* 2.0 and *Bst* 2.0 WarmStart DNA Polymerases.

Isothermal Amplification Buffer II is supplied with *Bst* 3.0 DNA Polymerase.

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. Phusion® and Thermo Scientific® are registered trademarks and property of Thermo Fisher Scientific.

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Nucleotides

Acyclonucleotide Set
#N0460S 0.5 µmol

Deoxynucleotide (dNTP) Solution Set
#N0446S 25 µmol

Deoxynucleotide (dNTP) Solution Mix
#N0447S 8 µmol
#N0447L 40 µmol

Ribonucleotide Solution Set
#N0450S 10 µmol
#N0450L 50 µmol

Ribonucleotide Solution Mix
#N0466S 10 µmol
#N0466L 50 µmol

7-deaza-dGTP
#N0445S 0.3 µmol
#N0445L 1.5 µmol

Adenosine 5'-Triphosphate (ATP)
#P0756S 1 ml
#P0756L 5 ml

5-methyl-dCTP
#N0356S 1 µmol

NEW
Pseudouridine-5'-Triphosphate (Pseudo-UTP)
#N0433S 0.1 ml

NEW
N1-Methyl-Pseudouridine-5'-Triphosphate (N1-Methyl-Pseudo-UTP)
#N0431S 0.1 ml

NEW
5-Methyl-Cytidine-5'-Triphosphate (5-Methyl-CTP)
#N0432S 0.1 ml

NEW
5-Methoxy-Uridine-5'-Triphosphate (5-Methoxy-UTP)
#N0434S 0.1 ml

dATP Solution
#N0440S 25 µmol

dUTP Solution
#N0459S 25 µmol

dGTP Solution
#N0442S 25 µmol

Deoxynucleotide (dNTP) Solution Mix (NEB #N0447) is available as a **GMP-grade reagent**. See page 6 for details.

Description:
Deoxynucleotide Solution Set:
Four separate solutions of ultrapure deoxynucleotide (dATP, dCTP, dGTP and dTTP). Each deoxynucleotide is supplied at a concentration of 100 mM.

Deoxynucleotide Solution Mix:
An equimolar solution of ultrapure dATP, dCTP, dGTP and dTTP. Each deoxynucleotide is supplied at a concentration of 10 mM.

Ribonucleotide Solution Set:
Four separate solutions of ATP, CTP, GTP and UTP, pH 7.5, as sodium salts.

Ribonucleotide Solution Mix:
A buffered equimolar solution of ribonucleotide triphosphates rATP, rCTP, rGTP and rUTP. Each nucleotide is supplied at a concentration of 25 mM (total rNTP concentration equals 100 mM).

7-deaza-dGTP:
7-deaza contains a 5 mM solution of 7-deaza-dGTP as a dilithium salt.

5-methyl-dCTP:
5mdCTP is supplied as a triethylammonium salt in Milli-Q® water.

Pseudouridine-5'-Triphosphate (Pseudo-UTP):
Supplied as a sodium salt, pH 7.0.

N1-Methyl-Pseudouridine-5'-Triphosphate (N1-Methyl-Pseudo-UTP):
Supplied as a sodium salt, pH 7.5.

5-Methyl-Citidine-5'-Triphosphate **(5-Methyl-CTP):**
Supplied as a sodium salt, pH 7.3.

5-Methoxy-Uridine-5'-Triphosphate **(5-Methoxy-UTP):**
Supplied as a sodium salt, pH 7.1.

dATP Solution:
dATP Solution contains a 100 mM solution of dATP as a sodium salt at pH 7.4.

dUTP Solution:
dUTP Solution contains a 100 mM solution of dUTP as a sodium salt at pH 7.5.

dGTP Solution:
dGTP Solution contains a 100 mM solution of dGTP as a sodium salt at pH 7.5.

Acyclonucleotide Set: Four separate tubes of acyNTPs (acyATP, acyCTP, acyGTP and acyTTP). Acyclonucleotides are supplied dry, as the triethylammonium salt. Addition of 50 µl of distilled or de-ionized (Milli-Q) water will result in a final concentration of 10 mM acyNTP.

Acyclonucleotides (acyNTPs) act as chain terminators and are thus useful in applications that normally employ dideoxynucleotides such as DNA sequencing and SNP detection. AcyNTPs are especially useful in applications with archaeal DNA Polymerases, more specifically with Therminator DNA Polymerase. Therminator DNA Polymerase is an engineered enzyme with an increased capacity to incorporate analogs with altered sugars, such as ribonucleotides, dideoxynucleotides, 2´ deoxynucleotides and especially acyclo-base analogs.

Team members of our subsidiary office in France.

cDNA Synthesis Selection Chart

Product	NEB #	Size	Features
LunaScript RT SuperMix Kit	E3010S E3010L	25 reactions 100 reactions	• Ideal for cDNA synthesis of targets up to 3 kb (two-step RT-qPCR, amplicon sequencing) • Single tube supermix contains random hexamer and oligo-dT primers, dNTPs, Murine RNase Inhibitor, and Luna Reverse Transcriptase • Visible blue tracking dye for easy reaction setup • Fast 13-minute protocol
LunaScript RT Master Mix Kit (Primer-free)	E3025S E3025L	25 reactions 100 reactions	• Ideal for first strand cDNA synthesis • Compatible with random primers, oligo dT primers and gene-specific primers • 5X master mix contains dNTPs, Murine RNase Inhibitor and Luna Reverse Transcriptase • Visible blue tracking dye for easy reaction setup • Fast 13-minute protocol
ProtoScript II First Strand cDNA Synthesis Kit	E6560S E6560L	30 reactions 150 reactions	• Generates cDNA up to 10 kb in length • Contains ProtoScript II Reverse Transcriptase, an enzyme with increased thermostability and reduced RNase H activity • Convenient 2-tube kit • Includes dNTPs, Oligo-dT primer and Random Primer Mix
ProtoScript First Strand cDNA Synthesis Kit	E6300S E6300L	30 reactions 150 reactions	• Generates cDNA at least 5 kb in length • Contains M-MuLV Reverse Transcriptase • Convenient 2-tube kit • Includes dNTPs, Oligo-dT primer and Random Primer Mix
Template Switching RT Enzyme Mix	M0466S M0466L	20 reactions 100 reactions	• Incorporates a universal adaptor sequence at the 3´ end of cDNA during the RT reaction • Enzyme mix and buffer are optimized for efficient template switching • RT enzyme mix includes RNase Inhibitor • High sensitivity for cDNA amplification – enables transcriptome analysis by RNA-seq from single cells or as low as 2 pg of human total RNA • Robust and simple workflow for 5´ Rapid Amplification of cDNA Ends (RACE) • Retains the complete 5´ end of transcripts for 2nd Strand cDNA Synthesis
Induro Reverse Transcriptase	M0681S M0681L	4,000 units 10,000 units	• Fast and processive intron-encoded RT for generating long transcripts (> 12 kb in under 10 mn.) • Increased reaction temperatures (50–60°C) • Increased inhibitor tolerance
ProtoScript II Reverse Transcriptase	M0368S M0368L M0368X	4,000 units 10,000 units 40,000 units	• RNase H– mutant of M-MuLV Reverse Transcriptase with increased thermostability and reduced RNase H activity • Increased reaction temperatures (37–50°C)
M-MuLV Reverse Transcriptase	M0253S M0253L	10,000 units 50,000 units	• Robust reverse transcriptase for a variety of templates • Standard reaction temperatures (37–45°C)
AMV Reverse Transcriptase	M0277S M0277L	200 units 1,000 units	• Robust reverse transcriptase for a broad temperature range (37–52°C) • Can be used for templates requiring higher reaction temperatures
WarmStart RTx Reverse Transcriptase	M0380S M0380L	400 units 2,000 units	• Permits room temperature reaction setup • Increased reaction temperatures (50–65°C) • Optimized for RT-LAMP isothermal detection
WarmStart RTx Reverse Transcriptase (Glycerol-free)	M0439L	2,000 units	• Permits room temperature reaction setup • Increased reaction temperatures (50–65°C) • Optimized for RT-LAMP isothermal detection

More information about our reverse transcriptases and cDNA synthesis kits can be found in the RNA analysis chapter.

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/WarmStart · Recombinant Albumin · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

NEW

Monarch® Spin PCR & DNA Cleanup Kit (5 µg)

#T1130S 50 preps
#T1130L 250 preps

Companion Products:

Monarch Spin DNA Gel Extraction Kit
#T1120S 50 preps
#T1120S 50 preps

Monarch Spin Columns S1A and Tubes
#T2037L 100 columns

Monarch Spin Plasmid Miniprep Kit
#T1110S 50 preps
#T1110S 50 preps

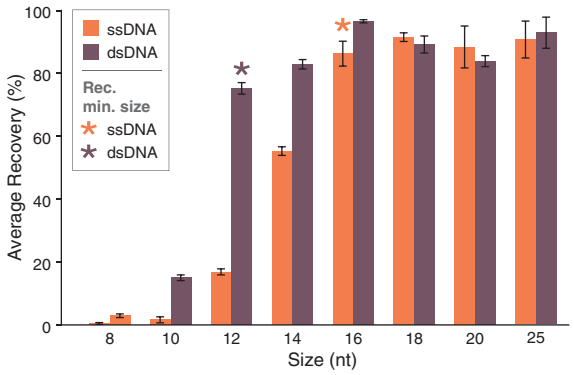
Monarch Spin Collection Tubes
#T2118L 100 tubes

- *New version of PCR and DNA cleanup kit, featuring upgraded spin columns precision-engineered for high performance*
- *Elute in as little as 5 µl for highly concentrated DNA, with yields up to 5 µg*
- *Prevent buffer retention and salt carry over with unique, optimized column design*
- *No need to monitor pH*
- *Protocol modification for oligonucleotide cleanup is provided, allowing purification of ssDNA, oligonucleotide and other small DNA fragments*
- *Reduce hands-on time with faster protocols and less spin time*
- *Significantly less plastic by design in columns and kit compared to leading suppliers*

The Monarch Spin PCR & DNA Cleanup Kit (5 µg) offers an efficient method for purification and concentration up to 5 µg of high-quality, double-stranded and single-stranded DNA from enzymatic reactions such as PCR, restriction digestion, ligation, and reverse transcription. This new version features upgraded spin columns precision-engineered to uniquely allow for low elution, in as little as 5 µl, for highly concentrated DNA in downstream applications such as sequencing, restriction digestion, library construction, labeling and other enzymatic manipulations. The kit includes spin columns, collection tubes, buffers, and other reagents in compact and streamlined packaging for silica membrane-based PCR purification and DNA cleanup. Our optimized buffer system allows efficient binding and purification of the DNA, without a need to monitor pH. These kits contain uniquely designed columns and bottles to significantly reduce plastic usage compared to leading suppliers, while maintaining structural integrity for centrifugation, vacuum manifolds and other steps. The kit features Monarch spin columns that are manufactured using state of the art automation machinery for high quality and consistency.

Kit Includes:

- Monarch Spin Columns S1A
- Monarch Spin Collection Tubes
- Monarch Buffer BZ
- Monarch Buffer WZ
- Monarch Buffer EY



Monarch Spin PCR & DNA Cleanup Kit (5 µg) effectively cleans up oligonucleotide DNA using the oligonucleotide cleanup protocol. *Synthesized ssDNA (≥ 16 nt) and dsDNA (≥12 bp) oligonucleotides can be effectively purified and recovered using Monarch Spin PCR & DNA Cleanup Kit (5 µg). The provided oligonucleotide cleanup protocol was followed using 1 µg of oligonucleotides of varying lengths (8–25 nt/bp) as an input. DNA was eluted in 20 µl of Monarch Buffer EY. Concentrations of DNA were measured using a Trinean DropSense 16 and percent recovery calculations are based on the eluted DNA concentration and elution volume used. The minimum sized ss- and ds- oligonucleotides that can be used are marked with a star (*).*

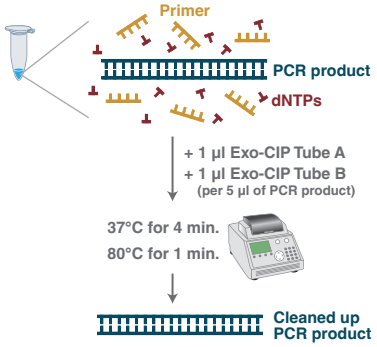
Exo-CIP™ Rapid PCR Cleanup Kit

#E1050S 100 reactions
#E1050L 400 reactions

Description: The Exo-CIP Rapid PCR Cleanup Kit contains optimized formulations of thermolabile Exonuclease I and thermolabile Calf Intestinal Phosphatase and is used to rapidly degrade residual PCR primers and dephosphorylate excess dNTPs after amplification. Degradation occurs in only 4 minutes at 37°C, and is immediately followed by rapid inactivation of the enzymes by heating for 1 minute at at 80°C. In just 5 minutes, the PCR product is ready for downstream analysis such as Sanger sequencing, SNP detection, or library preparation for NGS. The Exo-CIP Rapid PCR Cleanup Kit is compatible with all commonly-used reaction buffers.

Kit Includes:

- Exo-CIP Tube A
- Exo-CIP Tube B



Exo-CIP Rapid PCR Cleanup Kit workflow. *1 µl of Exo-CIP Tube A (thermolabile Exo I) and 1 µl of Exo-CIP Tube B (thermolabile CIP) are added to the PCR product to degrade excess primers and dNTPs. The mixture is incubated at 37°C for 4 minutes, followed by a 1 minute incubation at 80°C to irreversibly inactivate both enzymes. The cleaned PCR product is ready for downstream applications or analysis.*

Listen to hear more on this topic from Adewunmi.

LISTEN

Health official takes blood sample of a mother for testing
Credit: PIUS UTOMI EKPEI/AFP via Getty Images

Protecting Communities in the Fight Against Hepatitis B

The "Community Hepatitis B Shield Project," led by HealthDrive Nigeria, is an extraordinary initiative dedicated to combating viral hepatitis in underserved communities across Nigeria. Founded by Adewunmi Akingbola, the project addresses the pressing need for education, testing and vaccination against hepatitis B and C, particularly in low-income areas where access to healthcare is limited.

Akingbola's journey began with personal experiences of loss and intensified while attending medical school, where the prevalence of hepatitis among patients in gastroenterology clinics was alarmingly high — out of every 10 patients, six tested positive for hepatitis B, and at least one for hepatitis C. This realization, coupled with the general lack of public knowledge about the disease, sparked a profound motivation to drive awareness and change.

Recognizing that education alone was not enough to prevent infections, Akingbola initiated a comprehensive campaign. The effort began with public speaking engagements and social media campaigns, which later evolved into offering rapid diagnostic screenings for hepatitis B and C. The screenings were particularly crucial, as hepatitis B, while manageable, is incurable, and hepatitis C, though treatable, remains a significant health threat.

HealthDrive Nigeria's innovative "B-Safe Model" further expanded to establish screening points in various communities, ensuring that community members were regularly tested and educated about hepatitis. Akingbola quickly realized that the risk of new infections persisted. This led to the formation of partnerships with local health clinics, hospitals and pharmaceutical companies to make hepatitis vaccines more affordable and accessible. Through these collaborations, HealthDrive Nigeria was able to launch vaccination campaigns, offering subsidized hepatitis vaccines that significantly increased community uptake.

The project has since grown to include over 100 volunteers, including medical students from various states, all committed to eradicating hepatitis through community engagement and awareness. Professional nurses administer the vaccines, while trained volunteers conduct the tests, creating a comprehensive network of care and support.

The project's impact has been profound, with over 15,000 people screened and more than 10,000 vaccinated in southwestern Nigeria alone. Beyond the immediate health benefits, the project has also increased public awareness about hepatitis in the region, encouraging preventive measures in communities that were previously unaware of the disease's dangers. For example, it addresses unsafe local practices, that significantly increase the risk of hepatitis transmission, such as unsterilized equipment in barbershops.

Akingbola acknowledges the ongoing challenges, particularly in securing sustainable funding. However, the project continues to innovate, integrating other community services such as food and clothing distribution to enhance engagement and ensure the longevity of their health messages.

Operating from the UK, Akingbola provides guidance and support to the local team in Nigeria, ensuring the project's continued growth and impact. As the campaign expands its social media presence and seeks further partnerships with news agencies, the goal remains clear: to raise awareness about hepatitis B and C, reduce infections and ultimately save lives in the communities that need it most.

Dr. Adewunmi Akingbola
Kings College, Cambridge, UK
2024 Passion in Science Humanitarian Duty Award

DNA Modifying Enzymes & Cloning Technologies

The trusted source for DNA-modifying enzymes & cloning technologies.

Molecular biology, which also includes synthetic biology, is a fundamental area of research and development. Central to these advances has been the use of DNA modifying enzymes and novel cloning technologies. Some common examples of DNA-modifying enzymes include kinases, ligases, methylases, exonucleases and endonucleases, while newer cloning technologies include NEBuilder® HiFi DNA Assembly and NEBridge® Golden Gate Assembly.

Over 50 years as a leader in enzyme technologies gives you confidence in the products and support you'll receive. NEB continues to serve the scientific community by providing the tools to carry out the most innovative research, from start to finish. All NEB products pass stringent quality control assays to ensure the highest level of functionality and purity.

NEB offers several online tools to aid in your cloning experiments, including:

- **NEBcloner®** – find the right products and protocols for each step of your traditional cloning experiment, including double digests and mutagenesis
- **NEBioCalculator®** – use this tool for your scientific calculations and conversions
- **NEBuilder Assembly Tool** – use this tool for help with your DNA assembly primer design
- **NEBuilder Protocol Calculator** – use this tool to calculate the optimal amounts of input DNA sequences for your DNA assembly reaction with NEBuilder
- **Exo Selector** – find the right exonuclease for your workflows
- **NEBridge Golden Gate Assembly Tool** – use this tool for help with construct design for Golden Gate Assembly
- **NEBridge Ligase Fidelity Tools** – utilize ligation preferences for the design of high-fidelity Golden Gate Assembly

To view the full list of online tools available, visit **www.neb.com/nebtools**.

Featured Products

Featured Tools & Resources

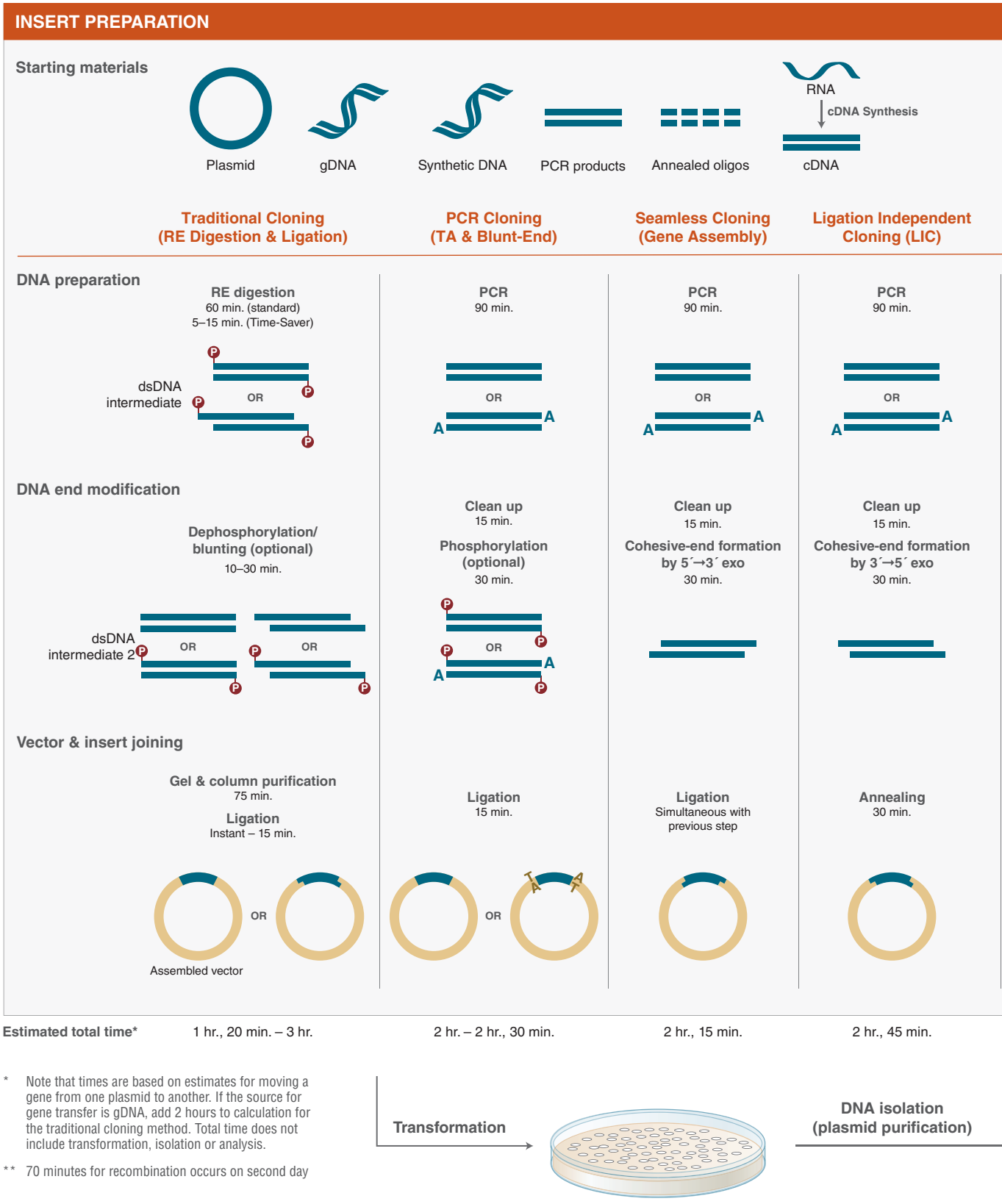
Visit **www.neb.com/FastCloning** to accelerate your cloning experiments with reagents from NEB.

Visit **ClonewithNEB.com** to view our online tutorials explaining each of the steps in the cloning workflow.

RI Recombinant Enzyme

Cloning Workflow Comparison

The figure below compares the steps for the various cloning methodologies, along with the time needed for each step in the workflows.



* Note that times are based on estimates for moving a gene from one plasmid to another. If the source for gene transfer is gDNA, add 2 hours to calculation for the traditional cloning method. Total time does not include transformation, isolation or analysis.

** 70 minutes for recombination occurs on second day

rCutSmart r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · rAlbumin Recombinant Albumin · SAM Requires SAM · 37° Incubation Temperature · 65° Heat Inactivation

Recombinational (Gateway/Creator/Univector)

PCR
90 min.

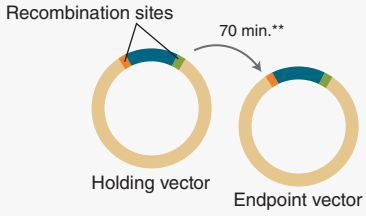
Clean up
15 min.

RE digestion
60 min. (standard)
5–15 min. (Time-Saver)

Clean up
15 min.

Site-specific recombination
60 min.

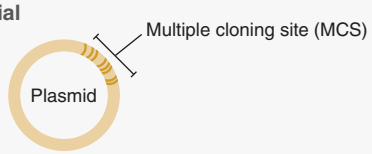
Proteinase K treatment
10 min.

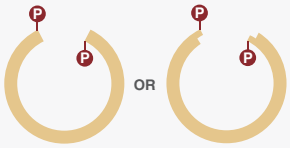


3 hr., 15 min. – 5 hr., 20 min.

VECTOR PREPARATION

Starting material



	Restriction Enzyme (RE) Digestion	PCR
DNA preparation	**RE digestion** 60 min. (standard) 5–15 min. (Time-Saver)	**PCR** 2 hr.
		**Clean up** 15 min.
	Linear vector	
DNA end modification	**Dephosphorylation (optional)** 10–30 min.	**T-addition (optional)** 1.5 hr.
	Clean up 15 min. OR **Gel & column purification** 75 min.	
		
	Linear vector, ready for joining	
Estimated total time	20 min. – 2 hr., 25 min.	2 hr., 15 min – 3 hr., 45 min.

NEBcloner®
For help with choosing the right product for each step in the cloning workflow, visit **NEBcloner.neb.com**

GMP-grade* Products for Nucleic Acid Therapeutic Manufacturing

NEB's portfolio of research-grade (RUO) and GMP-grade products enables bench to commercial-scale production batch sizes. When it is time to scale up and optimize reaction components, our standalone reagents are readily available in formats matching our GMP-grade offering, enabling a seamless transition to large-scale manufacturing.

NEB manufactures and inventories the following enzymes at GMP-grade to support the production of commercially approved mRNA and DNA product(s) meeting customer needs with short lead times

Product	NEB #	Description
Amplification		
phi29 DNA Polymerase	M0269	Replicative polymerase from the Bacillus subtillis phage phi29 and has exceptional strand displacement and processive synthesis properties with inherent 3´-5´ proofreading exonuclease activity
Deoxynucleotide (dNTP) Solution Mix	N0447	An equimolar (25mM) solution of ultrapure dATP, dGTP, dCTP, and dTTP
COMING SOON **Q5® Hot Start High-Fidelity Master Mix**	M0494	Q5 is composed of a novel polymerase that is fused to the processivity-enhancing Sso7d binding domain, improving speed, fidelity and reliability of performance. The formulation of the GMP-grade product is different than the catalog product.
mRNA Synthesis		
T7 RNA Polymerase	M0251	RNA Polymerase used for in vitro mRNA synthesis, and is highly specific for the T7 phage promoter
Inorganic Pyrophosphatase (*E. coli*)	M0361	Catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate
RNase inhibitor (Murine)	M0314	Specifically inhibits RNases A,B and C
DNAse I (RNase-free)	M0303	NA specific endonuclease used for removal of contaminating genomic DNA from RNA samples and degradation of DNA templates in transcription reactions
Vaccinia Capping Enzyme	M2080	Adds the m7G-cap (Cap-0) to the 5´ end of the triphosphorylated and dephosphorylated RNA
Faustovirus Capping Enzyme	M2081	Adds the m7G-cap (Cap-0) to the 5´ end of the triphosphorylated and dephosphorylated RNA
mRNA Cap 2´-O-Methyltransferase	M0366	Adds a methyl group at the 2´-O position of the first nucleotide adjacent to the cap structure at the 5' end of the RNA
HiScribe® T7 RNA Polymerase Mix	E2040	Separate components available in GMP-grade format
Nucleic Acid Therapeutic Manufacturing		
BspQI	R0712	Type IIS restriction enzyme and Isoschizomer of LguI and SapI used to linearize plasmid DNA for mRNA therapeutics
T4 DNA Ligase	M0202	Catalyzes the formation of a phosphodiester bond between juxtaposed 5´ phosphate and 3' hydroxyl termini in duplex DNA or RNA. Joins blunt end and cohesive end termini as well as repair single stranded nicks in duplex DNA and some DNA/RNA hybrids
T5 Exonuclease	M0663	Double-stranded DNA specific exonuclease and single-stranded DNA endonuclease, initiates at the 5´ termini of linear or nicked double-stranded DNA
TelN Protelomerase	M0651	Cuts dsDNA at a TelN recognition sequence and leaves covalently closed ends at the site of cleavage

**"GMP-grade" is a branding term NEB uses to describe reagents manufactured or finished at NEB's Rowley facility. The Rowley facility was designed to manufacture reagents under more rigorous infrastructure and process controls to achieve more stringent product specifications and customer requirements. Reagents manufactured at NEB's Rowley facility are manufactured in compliance with ISO 9001 and ISO 13485 quality management system standards. However, at this time, NEB does not manufacture or sell products known as Active Pharmaceutical Ingredients (APIs), nor does NEB manufacture its products in compliance with all of the Current Good Manufacturing Practice regulations.*

NEB was honored to host Nobel laureate Dr. Ardem Patapoutian (center) to present his work as part of our research seminar series. He is pictured here with NEB's CEO Sal Russello (left) and NEB's CSO and Nobel laureate Sir Richard Roberts (right).

rCutSmart r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · rAlbumin Recombinant Albumin · SAM Requires SAM · 37° Incubation Temperature · 65° Heat Inactivation

NEBuilder® HiFi DNA Assembly Master Mix & Cloning Kit

NEBuilder HiFi DNA Assembly Master Mix
#E2621S 10 reactions
#E2621L 50 reactions
#E2621X 250 reactions

NEBuilder HiFi DNA Assembly Cloning Kit
#E5520S 10 reactions

NEBuilder HiFi DNA Assembly Bundle for Large Fragments
#E2623S 20 reactions

- *Simple and fast seamless cloning in as little as 15 minutes*
- *Use one system for both "standard-size" cloning and larger gene assembly products (up to 11 fragments and 20+ kb)*
- *Clone into any vector with no additional sequence added (scarless)*
- *DNA can be used immediately for transformation or as template for PCR or RCA*
- *Adapts easily for multiple DNA manipulations, including site-directed mutagenesis*
- *Enjoy less screening/re-sequencing of constructs, with virtually error-free, high-fidelity assembly*
- *Use NEBuilder HiFi in successive rounds of assembly, as it removes 5´- and 3´-end mismatches*
- *Bridge two ds-fragments with a synthetic ssDNA oligo for simple and fast construction (e.g., linker insertion or gRNA library)*
- *No PCR cleanup step required*
- *No licensing fee requirements from NEB for NEBuilder products*

To learn how simple NEBuilder HiFi is, visit **NEBuilderHiFi.com**

NEBuilder® Assembly Tool

Get started designing primers at **NEBuilder.neb.com**

NEBuilder® Protocol Calculator

Generate a custom DNA assembly protocol at **NEBuilderCalculator.neb.com**

Description: NEBuilder HiFi DNA Assembly Master Mix was developed to improve the efficiency and accuracy of DNA assembly. This method allows for seamless assembly of multiple DNA fragments, regardless of fragment length or end compatibility. This method has been used to assemble either single-stranded oligonucleotides or different sizes of DNA fragments with varied overlaps (15–80 bp). It has utility for the synthetic biology community, as well as those interested in one-step cloning of multiple fragments due to its ease of use, flexibility and simple master-mix format. The reaction features different enzymes that perform in the same buffer. The end result is a double-stranded, fully sealed DNA molecule that can serve as template for PCR, RCA or a variety of other molecular biology applications, including direct transformation of *E. coli*.

NEBuilder HiFi Kits can be purchased with NEB 5-alpha Competent *E. coli* (Cloning Kit, NEB #E5520) or as a bundle with NEB 10-beta Competent ***E. coli*** (Bundle for Large Fragments, NEB #E2623). NEB 5-alpha competent cells are excellent for routine assemblies of 15 kb or less. NEB recommends NEB 10-beta competent cells for assemblies larger than 15 kb.

The NEBuilder HiFi DNA Assembly Master Mix Includes:

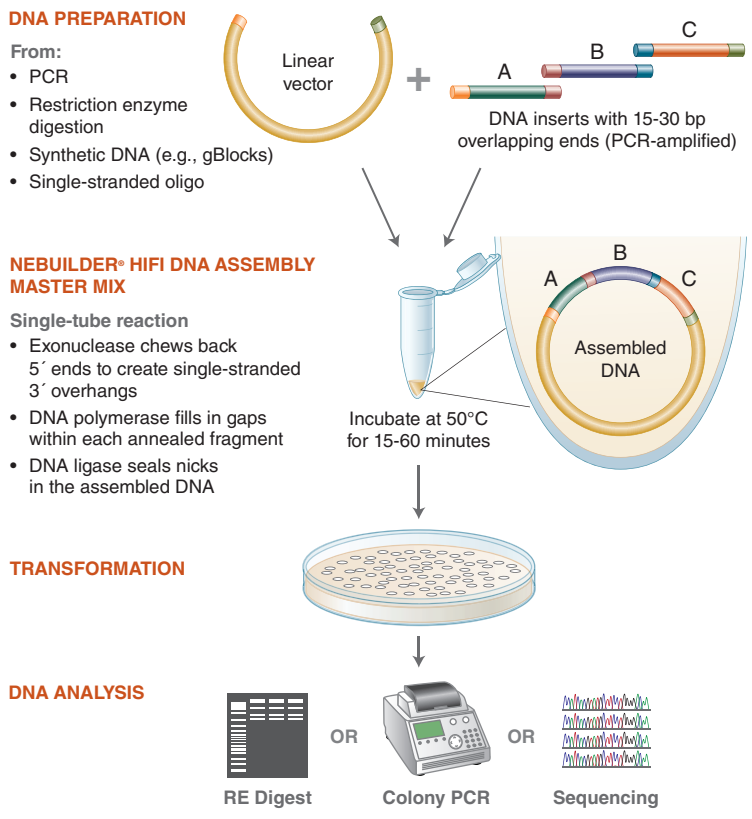
- NEBuilder HiFi DNA Assembly Master Mix
- NEBuilder Positive Control

The NEBuilder HiFi DNA Assembly Cloning Kit Includes:

- NEBuilder HiFi DNA Assembly Master Mix
- NEBuilder Positive Control
- NEB 5-alpha Competent *E. coli* (High Efficiency)
- SOC Outgrowth Medium
- pUC19 Control DNA

The NEBuilder HiFi DNA Assembly Bundle for Large Fragments Includes:

- NEBuilder HiFi DNA Assembly Master Mix
- NEBuilder Positive Control
- NEB 10-beta Competent *E. coli* (High Efficiency)
- NEB 10-beta/Stable Outgrowth Medium
- pUC19 Control DNA



Overview of the NEBuilder HiFi DNA Assembly Cloning Method.

How does NEBuilder HiFi DNA Assembly work?

Gibson Assembly® Master Mix & Cloning Kit

Gibson Assembly Master Mix
#E2611S 10 reactions
#E2611L 50 reactions

Gibson Assembly Cloning Kit
#E5510S 10 reactions

- *High efficiency assembly, particularly for longer or greater numbers of fragments*
- *Flexible sequence design with no need to engineer cloning sites*
- *Assemble multiple DNA fragments and transform in just under 2 hours*
- *Clone into any vector with no additional sequence added (scarless)*
- *No PCR clean-up step required*

Description: Gibson Assembly Master Mix allows for successful assembly of multiple DNA fragments, regardless of fragment length or end compatibility.

Gibson Assembly efficiently joins multiple overlapping DNA fragments in a single-tube isothermal reaction. The Gibson Assembly Master Mix includes three different enzymatic activities that perform in a single buffer:

- The exonuclease creates a single-stranded 3´ overhang that facilitates the annealing of fragments that share complementarity at one end
- The polymerase fills in gaps within each annealed fragment
- The DNA ligase seals nicks in the assembled DNA

The Gibson Assembly Cloning Kit has been optimized for the assembly and cloning of up to 6 fragments.

The Gibson Assembly Master Mix Includes:

- Gibson Assembly Master Mix
- NEBuilder Positive Control

The Gibson Assembly Cloning Kit Includes:

- Gibson Assembly Master Mix
- NEBuilder Positive Control
- NEB 5-alpha Competent *E. coli* (High Efficiency)
- SOC Outgrowth Medium
- pUC19 Control DNA

View our online tutorials at **NEBGibson.com**

Synthetic Biology/DNA Assembly Selection Chart

	NEBuilder HiFi DNA Assembly NEB #E2621 NEB #E5520 NEB #E2623	**NEB Gibson Assembly** NEB #E2611 NEB #E5510	**NEBridge Golden Gate Assembly Kits (BsaI-HFv2/BsmBI-v2)** NEB #E1601 NEB #E1602 **NEBridge Ligase Master Mix** NEB #M1100	**USER Enzyme** NEB #M5505 **Thermolabile USER II Enzyme** NEB #M5508
Properties				
Removes 5´ or 3´ End Mismatches	★★★	★	N/A	N/A
Assembles with High Fidelity at Junctions	★★★	★★	★★★	★★★
Tolerates Repetitive Sequences at Ends	★	★	★★★	★★★
Generates Fully Ligated Product	★★★	★★★	★★★	NR
Joins dsDNA with Single-stranded Oligo	★★★	★★	NR	NR
Assembles Low Amounts of DNA with High Efficiency	★★★	★★	★★	★★
Accommodates Flexible Overlap Lengths	★★★	★★★	★	★★
Applications				
2 Fragment Assembly (Simple cloning)	★★★	★★★	★★★	★★★
3-6 Fragment Assembly (one pot)	★★★	★★★	★★★	★★★
7-11 Fragment Assembly (one pot)	★★★	★★	★★★	★★★
12-50+ Fragment Assembly (one pot) [1]	★	★	★★★	NR
Template Construction for *In vitro* Transcription	★★★	★★★	★★★	★★★
Synthetic Whole Genome Assembly	★★★	★	★★★	★
Multiple Site-directed Mutagenesis	★★★	★★	★★	★★
Library Generation	★★★	★★★	★★★	★★
Metabolic Pathway Engineering	★★★	★★	★★★	★★★
TALENs	★★	★★	★★★	★★
Short Hairpin RNA (shRNA) Cloning	★★★	★★	★	★
gRNA Library Generation	★★★	★★	★	★
Large Fragment (> 10 kb) Assembly	★★★	★★★	★★★	★★
Small Fragment (< 100 bp) Assembly	★★★	★	★★★	★★★
Use in Successive Rounds of Restriction Enzyme Assembly	★★★	★	NR	★

[1] Please visit **neb.com/GoldenGate** for more information

KEY	
★★★	Optimal, recommended product for selected application
★★	Works well for selected application
★	Will perform selected application, but is not recommended
N/A	Not applicable to this application
NR	Not recommended

rCutSmart Recommended Buffer · Cloned at NEB · Recombinant Enzyme · rAlbumin Recombinant Albumin · SAM Requires SAM · 37° Incubation Temperature · 65° Heat Inactivation

NEBridge® Golden Gate Assembly Kits

NEBridge Golden Gate Assembly Kit (BsaI-HF v2)
#E1601S 20 reactions
#E1601L 100 reactions

NEBridge Golden Gate Assembly Kit (BsmBI-v2)
#E1602S 20 reactions
#E1602L 100 reactions

NEBridge Ligase Master Mix
#M1100S 50 reactions
#M1100L 250 reactions

- *Seamless cloning – no scar remains following assembly*
- *Ordered assembly of multiple fragments (2-50+) in a single reaction**
- *Can also be used for cloning of single inserts and library preparations*
- *Efficient with regions of high GC content and areas of repeats*
- *Compatible with a broad range of fragment sizes (< 100 bps to > 15 kb)*

* NEB has tested 50+ fragments with NEB #E1601/1602 and 25+ fragments with NEB #M1100.

Description: The NEBridge Golden Gate Assembly Kits (BsaI-HFv2 and BsmBI-v2) contain an optimized mix of Type IIS restriction enzyme and T4 DNA Ligase. Together these enzymes can direct the assembly of multiple inserts using the Golden Gate method. The kits include pGGAselect destination plasmid, which provides a backbone for the assembly, features convenient restriction enzyme sites for subcloning, and has T7/SP6 promoter sequences to enable *in vitro* transcription.

NEBridge Ligase Master Mix performs high efficiency and high-fidelity Golden Gate Assemblies with a broad assortment of Type IIS restriction enzymes which can be ordered separately.

Golden Gate Assembly is a method for efficient and seamless assembly of DNA fragments using Type IIS restriction enzymes and T4 DNA Ligase. Type IIS restriction enzymes bind to their recognition sites but cut the DNA downstream from that site at a positional, not sequence-specific, cut site. Thus, a single Type IIS restriction enzyme can generate DNA fragments with unique overhangs (see Figure below). Ordered assembly of digested fragments proceeds through annealing of complementary overhangs on adjacent fragments. The final assembly product no longer contain Type IIS restriction enzyme recognition sites, so no further digestion is possible, allowing the assembly product to accumulate over time.

While particularly useful for multi-fragment assemblies, the Golden Gate method can also be used for cloning single inserts and inserts from diverse populations to create libraries. Golden Gate is also useful for assembling repetitive elements (e.g., gene circuits and CRISPR guide arrays).

Advances in Ligase Fidelity: Research at NEB has led to increased understanding of ligase fidelity, including the development of a comprehensive method for profiling end-joining ligation fidelity in order to predict which overhangs have improved fidelity. This research allows careful choice of overhang sets, which is especially important for achieving complex Golden Gate Assemblies. To learn more, visit www.neb.com/goldengate.

NEBridge Golden Gate Assembly Kit (BsaI-HFv2) Includes:
- NEBridge Golden Gate Assembly Mix
- T4 DNA Ligase Reaction Buffer (10X)
- pGGAselect Destination Plasmid

NEBridge Golden Gate Assembly Kit (BsmBI-v2) Includes:
- NEBridge Golden Gate Assembly Mix
- T4 DNA Ligase Reaction Buffer (10X)
- pGGAselect Destination Plasmid

NEBridge Ligase Master Mix Includes:
- NEBridge Ligase Master Mix (3X)
- Use with your choice of NEB Type IIS restriction enzyme

* Note: Assemblies up to 24 fragments have been routinely achieved with both precloned and amplicon insert test systems. Assemblies of 35+ fragments have only used amplicon inserts to date.

NEBridge® Golden Gate Assembly Tool

Get started designing your experiments and primers at **GoldenGate.neb.com**

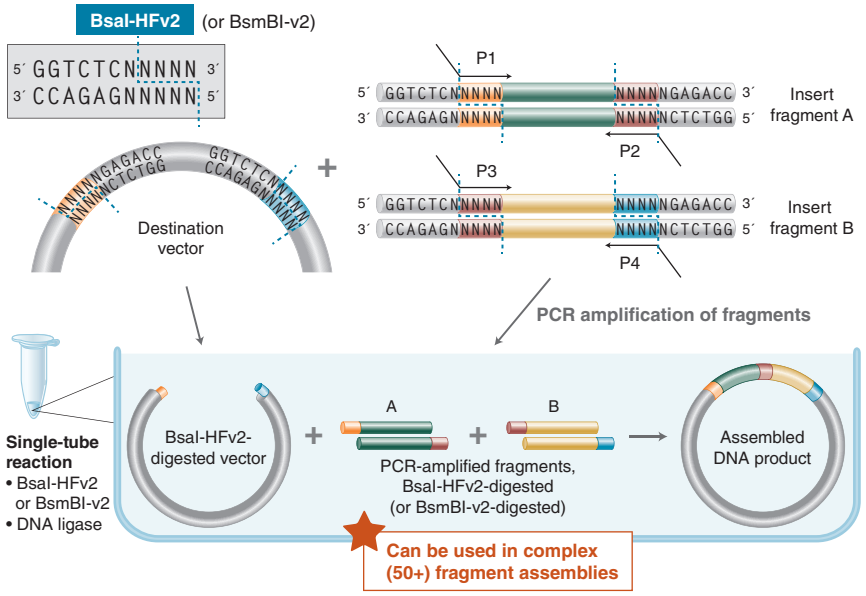
NEBridge® Ligase Fidelity Tools for Golden Gate Assembly

Try our suite of free, online tools at **ligasefidelity.neb.com**

NEBridge Ligase Fidelity Viewer®
Evaluate existing assemblies and visualize overhang ligation preferences

NEBridge GetSet® Tool
Predict high-fidelity overhang sets for new assemblies and expand existing assemblies

NEBridge SplitSet® Tool
Split a DNA sequence into multiple fragments for scarless high-fidelity assembly



In its simplest form, Golden Gate Assembly requires a Type IIS recognition site, in this case, BsaI-HFv2 (GGTCTC), or BsmBI-v2 (CGTCTC) added to both ends of a dsDNA fragment. *After digestion, these sites are left behind, with each fragment bearing the designed 4-base overhangs that direct the assembly.*

Type IIS Enzymes used in Golden Gate:

- BbsI (NEB #R0539)
- BbsI-HF® (NEB #R3539)
- BsaI-HFv2 (NEB #R3733)
- BsmBI-v2 (NEB #R0739)
- BspQI (NEB #R0712)
- BtgZI (NEB #R0703)
- Esp3I (NEB #R0734)
- PaqCI® (NEB #R0745)
- SapI (NEB #R0569)

How does Golden Gate Assembly work?

NEB® PCR Cloning Kit (with or without competent cells)

NEB PCR Cloning Kit
#E1202S 20 reactions

NEB PCR Cloning Kit (Without Competent Cells)
#E1203S 20 reactions

- *In vitro transcription with both SP6 & T7 promoters*
- *Easy cloning of all PCR products, including blunt and TA ends*
- *Fast cloning with 5-minute ligation step*
- *Simplified screening with low/no colony background and no blue/white selection*
- *Save time by eliminating purification steps*
- *More flanking restriction sites available for easy subcloning, including choice of two single digest options*
- *BsaI site removed to allow cloning of Golden Gate modules*

Description: The NEB PCR Cloning Kit contains optimized Cloning Master Mixes with a proprietary ligation enhancer and a linearized vector that uses a novel mechanism for background colony suppression to give a low background. It allows simple and quick cloning of any PCR amplicon, whether the amplification reactions are performed with proofreading DNA polymerases, such as Q5® or Phusion® which produce blunt ends; or nonproofreading DNA polymerases, such as *Taq* or *Taq* mixes (One*Taq*, LongAmp *Taq*) which produce single-base overhangs. This is possible due to "invisible" end polishing components in the master mix that are active during the ligation step. The kit also allows direct cloning from amplification reactions without purification, and works well whether or not the primers used in the PCR possess 5´-phosphate groups.

- Provided analysis primers allow for downstream colony PCR screening or sequencing
- Ready-to-use kit components include 1 kb control amplicon, linearized cloning vector and single-use competent *E. coli* (NEB #E1202 only)
- Longer shelf life (12 months), as compared to some commercially available products

Kit Includes:

- Linearized pMiniT™ 2.0 Vector
- Cloning Mix 1
- Cloning Mix 2
- Amplicon Cloning Control (1 kb)
- Cloning Analysis Forward Primer
- Cloning Analysis Reverse Primer
- NEB 10-beta Competent *E. coli* (Cloning Efficiency) (NEB #E1202 only)
- NEB 10-beta/Stable Outgrowth Medium (NEB #E1202 only)
- pUC19 Control DNA

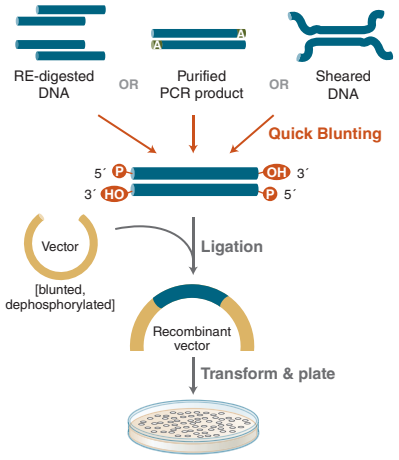
Phusion® is a registered trademark of Thermo Fisher Scientific

Quick Blunting™ Kit

#E1201S 20 reactions
#E1201L 100 reactions

- *Restriction enzyme-digested DNA is blunted in less than 30 minutes*
- *Reactions are performed at room temperature in a ready-to-use mix*
- *Suitable for restriction enzyme-digested DNA, sheared or nebulized DNA or PCR product*

Description: The Quick Blunting Kit is used to convert DNA with incompatible 5´or 3´overhangs to 5´phosphorylated, blunt-ended DNA for efficient blunt-end ligation into DNA cloning vectors. DNA is blunted using T4 DNA Polymerase (NEB #M0203) which has both 3´→ 5´ exonuclease activity and 5´→ 3´ polymerase activity. T4 Polynucleotide Kinase (NEB #M0201) is included in the enzyme mix for phosphorylation of the 5´ends of blunt-ended DNA for subsequent ligation into a cloning vector. This kit is optimized for blunting up to 5 µg of DNA in a single reaction.



How does the NEB PCR Cloning Kit work?

Applications:

- Prepare sheared, nebulized or restriction enzyme digested DNA for blunt-ended ligation into a plasmid, cosmid, fosmid or BAC vector
- Prepare PCR products for efficient blunt-end cloning

Kit Includes:

- Blunting Enzyme Mix
- 10X Blunting Buffer
- Deoxynucleotide Solution Mix (1 mM)

Note: PCR generated DNA must be purified before blunting by using a commercial purification kit, such as Monarch® PCR & DNA Cleanup Kit (NEB #T1030), phenol extraction/ethanol precipitation, or gel electrophoresis. Restriction enzyme digested DNA can be blunted directly without purification.

rCutSmart r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | rAlbumin Recombinant Albumin | SAM Requires SAM | 37° Incubation Temperature | 65° Heat Inactivation

Q5® Site-Directed Mutagenesis Kit (with or without competent cells)

Q5 Site-Directed Mutagenesis Kit
#E0554S 10 reactions

Q5 Site-Directed Mutagenesis Kit
(Without Competent Cells)
#E0552S 10 reactions

KLD Enzyme Mix
#M0554S 25 reactions

- *Robust exponential amplification generates high yields of desired mutations from a wide range of templates.*
- *Low error rate of Q5 High-Fidelity DNA Polymerase reduces screening time.*
- *Room temperature reaction setup*
- *Use of standard primers eliminates need for phosphorylated or purified oligos*
- *Easy-to-use master mix format*

NEBaseChanger®
For help with primer design, visit
NEBaseChanger.neb.com

Description: The Q5 Site-Directed Mutagenesis Kit allows rapid site-specific mutagenesis of double-stranded plasmid DNA in less than 2 hours. The kit utilizes the robust Q5 Hot Start High-Fidelity DNA Polymerase along with custom mutagenic primers to create substitutions, deletions and insertions in a wide variety of plasmids. Transformation into high-efficiency NEB 5-alpha Competent *E. coli*, provided with (NEB #E0554), ensures robust results with plasmids up to 14 kb in length.

Applications:

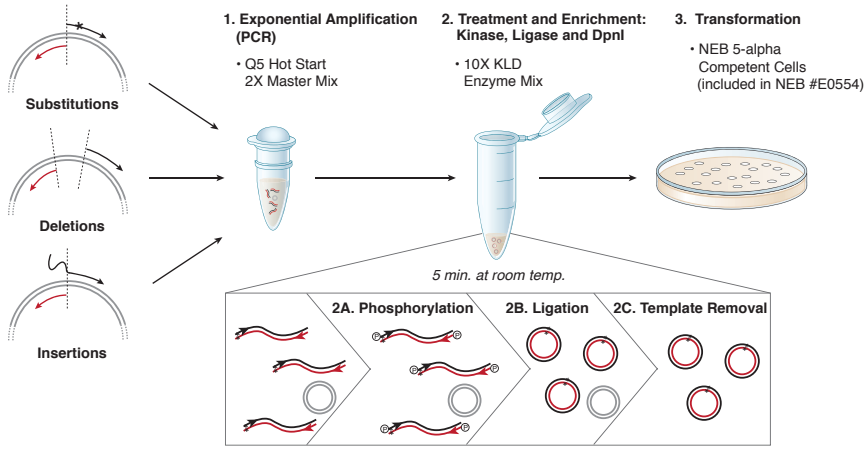
- Generation of mutations, insertions or deletions in plasmid DNA

Q5 Site-Directed Mutagenesis Kit Includes:

- Q5 Hot Start High-Fidelity Master Mix (2X)
- KLD Enzyme Mix (10X)
- Control SDM Plasmid
- Control SDM Primer Mix
- pUC19 Vector (NEB #E0554 only)
- SOC Outgrowth Medium (NEB #E0554 only)
- NEB 5-alpha Competent *E. coli* (High Efficiency) (NEB #E0554 only)

KLD Enzyme Mix Includes:

- KLD Enzyme Mix (10X)
- KLD Reaction Buffer (2X)



Q5 Site-Directed Mutagenesis Kit Overview. *The first step is an exponential amplification using Q5 Hot Start High-Fidelity DNA Polymerase. The second step is a unique enzyme mix containing a kinase, ligase and DpnI. Together, these enzymes allow for rapid circularization of the PCR product and removal of the template DNA. The last step is a high-efficiency transformation into chemically competent cells.*

David (left) and Maura (right) are members of NEB's Information Technologies Department. David joined NEB in 2019 as Senior Network Engineer and also runs NEB's Maple Syrup Production Club. Maura joined NEB in 2022 as HelpDesk Service Supervisor and is also part of the Maple Syrup Production Club.

Learn more about the benefits of the Q5 SDM Kit.

DNA Ligase Selection Chart

NEB offers a variety of ligases for DNA research. Many of these enzymes are recombinant, and all offer the quality and value you have come to expect from our products. While more than one ligase may work for your application, the following selection chart presents our recommendations for optimal performance.

Visit **NEBStickTogether.com** for more information on DNA Ligases.

	Blunt/ TA Ligase Master Mix	Instant Sticky-end Master Mix	Electro-Ligase®	T4 DNA Ligase	Hi-T4™ DNA Ligase	Immo-bilized T4 DNA Ligase	Salt-T4® DNA Ligase	Quick Ligation™ Kit	NEBridge Ligase Master Mix	T3 DNA Ligase	T7 DNA Ligase	HiFi *Taq* DNA Ligase	*Taq* DNA Ligase	9°N™ DNA Ligase	NEBNext Quick Ligation Module	SplintR® Ligase	*E. coli* DNA Ligase
DNA Applications																	
Ligation of sticky ends	★★	★★★	★★	★★	★★	★★★	★★	★★★	★	★★	★★	★	★	★			★
Ligation of blunt ends	★★★	★	★★	★★	★★	★	★★	★★★	★	★★							
T/A cloning	★★★	★	★★	★★	★★		★★	★★		★	★						
Electroporation			★★★	★★	★★												
Golden Gate Assembly				★★★	★★*				★★★		★						
Ligation of sticky ends only											★★★						
Repair of nicks in dsDNA	★★	★★	★★	★★★	★★★		★★★	★★		★★	★★	★★	★★	★★		★★	★★
High-complexity library cloning	★★	★★	★★	★★★				★★									
Adapter Ligation	★★★	★★	★★	★		▲		★★		★					▲		
Ligation-Dependent DNA Sequence & SNP Detection (LCR, LDR & related methods)												★★★	★★	★★			
Ligation-Dependent RNA Sequence & SNP Detection				★												★★★	
Ligation of adjacent ssDNAs on an RNA splint																★★★	
NGS Applications																	
NGS Library Prep dsDNA-dsDNA (ligation)	▲			▲						▲					▲		
Features																	
Salt tolerance (>2X that for T4 DNA Ligase)							●			●							
Ligation in 15 min. or less	●	●		●	●		●	●		●	●	●	●	●	●	●	
Master Mix Formulation	●	●							●						●		
Thermostable												●	●	●			
Thermotolerant					●							●	●	●			
Recombinant	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Reusable/ Removable						●											

* Sorida M., Bonasio, R., (2023) *Cell Reports Methods*, 3, 100564, https://doi.org/10.1016/j.crmeth.2023.100564.

KEY	
★★★	Optimal, recommended ligase for selected application
★★	Works well for selected application
★	Will perform selected application, but is not recommended
▲	Please consult the specific NGS protocol to determine the optimal enzyme for your needs

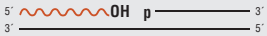
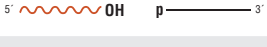
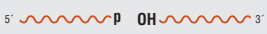
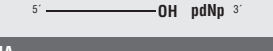
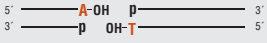
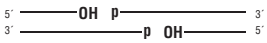
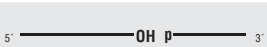
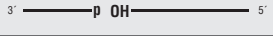
Find an overview of ligation.

rCutSmart r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · rAlbumin Recombinant Albumin · SAM Requires SAM · 37° Incubation Temperature · 65 Heat Inactivation

Substrate-based Ligase Selection Chart

This chart provides our recommendation for a choice of ligase to use in a reaction, based upon the type of substrate present. DNA, RNA and hybrid substrates are represented and require specific enzymes to achieve the highest-efficiency ligation.

DNA
RNA

	Recommended Ligase	Comments
Nicked DNA/RNA		
	T4 RNA Ligase 2	
	T4 RNA Ligase 2	
	T4 RNA Ligase 2	
	T4 DNA Ligase Immobilized T4 DNA Ligase	
	N/A	No ligase optimized for this activity
	T3 DNA Ligase	
	SplintR Ligase	100-1,000-fold higher efficiency than T4 DNA Ligase
	T4 DNA Ligase Immobilized T4 DNA Ligase	For high temperatures, we recommend *Taq* DNA Ligase. For highest fidelity, we recommend HiFi *Taq* DNA Ligase.
ssDNA/RNA		
	N/A	See CircLigase™
	N/A	No ligase optimized for this activity
	T4 RNA Ligase 1	Supplement with ATP
	T4 RNA Ligase 1	Supplement with ATP
	T4 RNA Ligase 2 Truncated KQ	
	T4 RNA Ligase 2 Truncated KQ	
	Thermostable 5´ App DNA/RNA Ligase	We recommend a Proteinase K cleanup
	Thermostable 5´ App DNA/RNA Ligase	We recommend a Proteinase K cleanup
	RtcB Ligase	Supplement with GTP and Mn^{2+}
	RtcB Ligase	Supplement with GTP and Mn^{2+}
	T4 RNA Ligase 1	
	T4 RNA Ligase 1	Reported to work, but ligates inefficiently. Consider pdCp.
	T4 RNA Ligase 1	
	T4 RNA Ligase 1	
dsDNA		
	Blunt T/A Ligase Master Mix	
	Blunt T/A Ligase Master Mix	
	Quick Ligation Kit or Instant Sticky-end Ligase Master Mix	For ligating ends under high salt conditions, we recommend Salt-T4 DNA Ligase. For ligation at temperatures up to 50°C, we recommend Hi-T4 DNA Ligase. For ligation of cohesive ends ONLY, we recommend T7 DNA Ligase.
	Quick Ligation Kit or Instant Sticky-end Ligase Master Mix	For ligating ends under high salt conditions, we recommend Salt-T4 DNA Ligase. For ligation at temperatures up to 50°C, we recommend Hi-T4 DNA Ligase. For ligation of cohesive ends ONLY, we recommend T7 DNA Ligase.

CIRCLIGASE™ is a trademark of EpiCentre Technologies Corp.

T4 DNA Ligase Products

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5´ phosphate and 3´ hydroxyl termini in duplex DNA or RNA. This enzyme will join blunt-end and cohesive-end termini, as well as repair single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids. T4 DNA Ligase is available in a variety of formulations and variants. The table below lists products available from NEB.

Product	NEB #	Features	Reaction Conditions	Size
T4 DNA Ligase	Regular concentration: M0202S M0202L High (5X) concentration: M0202T M0202M	• Standalone enzyme ideal for a variety of ligation reactions • Ligation can be performed in supplied buffer, or in any of the four restriction endonuclease NEBuffers, or in T4 Polynucleotide Kinase Buffer if supplemented with 1 mM ATP	1X T4 DNA Ligase Reaction Buffer. Incubate at 16°C. Heat inactivate at 65°C for 10 minutes.	Regular concentration: S: 20,000 units L: 100,000 units High (5X) concentration: T: 20,000 units M: 100,000 units
Master Mixes				
Instant Sticky-end Ligase Master Mix	M0370S M0370L	• Ready-to-use 2X solution of T4 DNA Ligase and a proprietary ligation enhancer in an optimized reaction buffer • Specifically formulated to rapidly ligate cohesive-end (2-4 bp) substrates and improve transformation	1X Instant Sticky-end Ligase Master Mix with DNA substrates in a 10 µl reaction volume	50 reactions 250 reactions
Blunt/TA Ligase Master Mix	M0367S M0367L	• Ready-to-use 2X solution of T4 DNA Ligase and a proprietary ligation enhancer in an optimized reaction buffer • Specifically formulated to improve ligation and transformation of both-blunt-end and single-base overhang substrates	1X Blunt/TA Ligase Master Mix with DNA substrates in a 10 µl reaction volume	50 reactions 250 reactions
NEBridge Ligase Master Mix	M1100S M1100L	• Ideal for high-efficiency and high-fidelity Golden Gate Assembly with a broad assortment of Type IIS restriction enzymes • Available in 3X master mix format	1X NEBridge Ligase Master Mix, Type IIS restriction enzyme and DNA fragments in a 15 or 30 µl reaction volume	50 reactions 250 reactions
Formulations				
Quick Ligation Kit	M2200S M2200L	• Ligation of cohesive- or blunt-end DNA fragments in 5 minutes at room temperature (25°C)	1X Quick Ligation Reaction Buffer. Incubate at room temperature (25°C).	30 reactions 150 reactions
ElectroLigase	M0369S	• Combines T4 DNA Ligase and an optimized, ready-to-use 2X reaction buffer containing a proprietary ligation enhancer and no PEG • Promotes robust ligation of all types of DNA ends (blunt, sticky, TA) and is suitable for electroporation, without desalting or purification	1X ElectroLigase Reaction Buffer with DNA substrates and 1 µl ElectroLigase in an 11 µl reaction volume incubated at 25°C	50 reactions
Immobilized T4 DNA Ligase	M0569S	• Enzyme is covalently linked to a magnetic bead, and can be removed from a reaction and reused • Enables ligated product to be used directly with no heat inactivation step	1X T4 DNA Ligase Reaction Buffer. Incubate at 25°C. Place on magnet for 3 minutes to remove.	1 mg
Variants				
Hi-T4™ DNA Ligase	M2622S M2622L	• Enables ligation with improved thermostability • Active in temperatures up to 50°C	1X T4 DNA Ligase Reaction Buffer. Incubate at 25°C. Heat inactivate at 65°C for 10 minutes.	20,000 units 100,000 units
Salt-T4® DNA Ligase	M0467S M0467L	• Enables ligation with improved salt tolerance • Active in reactions as high as 300 mM salt with no loss in activity	1X T4 DNA Ligase Reaction Buffer. Incubate at 25–50°C. Heat inactivate at 65°C for 10 minutes.	20,000 units 100,000 units

T4 DNA Ligase (NEB #M0202) is available as a **GMP-grade reagent**.
See page 6 for details.

NEBioCalculator®

For help with molar ratio calculations, visit
NEBioCalculator.neb.com

rCutSmart NEB r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · rAlbumin Recombinant Albumin · SAM Requires SAM · 37° Incubation Temperature · 65° Heat Inactivation

T3 DNA Ligase

#M0317S 100,000 units
#M0317L 750,000 units

- *Ligation of sticky or blunt ends*
- *Increased salt tolerance*
- *Repair of nicks in dsDNA*

Description: T3 DNA Ligase is an ATP-dependent ds-DNA ligase from bacteriophage T3. Cohesive ends, blunt ends, and nick sealing can all be efficiently catalyzed by T3 DNA Ligase. Blunt end ligation is enhanced by the addition of PEG 6000 to the reaction. T3 DNA Ligase exhibits a higher tolerance (2-fold) for NaCl in the reaction compared to T4 DNA Ligase, making the enzyme a versatile choice for *in vitro* molecular biology protocols requiring DNA ligase activity.

Reaction Conditions: StickTogether DNA Ligase Buffer, 25°C.

Unit Definition: One unit is defined as the amount of enzyme required to give 50% ligation of 100 ng HindIII fragments of λ DNA in a total reaction volume of 20 µl in 1 minute at 25°C in 1X StickTogether DNA Ligase Buffer.

Concentration: 3,000,000 units/ml

Note: ATP is an essential cofactor for the reaction. This contrasts with *E. coli* DNA Ligase which requires NAD. T3 DNA Ligase is also active in buffers without PEG 6000, such as our T4 DNA Ligase Buffer and NEBuffers, for applications in which PEG 6000 is detrimental. Please remember to supplement the reaction with 1 mM ATP (final concentration). In these buffers T3 DNA Ligase exhibits an approximately 10-fold reduction in activity. In applications where a high concentration of NaCl needs to be maintained, we suggest using a reaction buffer without PEG 6000.

T7 DNA Ligase

#M0318S 100,000 units
#M0318L 750,000 units

- *Ligation of sticky ends only*
- *Repair of nicks in dsDNA*

Description: T7 DNA Ligase is an ATP-dependent ds-DNA ligase from bacteriophage T7. It will catalyze the formation of a phosphodiester bond between adjacent 5´ phosphate and 3´ hydroxyl groups of duplex DNA. Cohesive end ligation and nick sealing can be efficiently catalyzed by T7 DNA Ligase. However, unlike T4 and T3 DNA Ligases, blunt end ligation is not efficiently catalyzed by T7 DNA Ligase, making it a good choice for applications in which blunt and sticky ends of DNA are present but only the sticky ends are to be joined.

Reaction Conditions: StickTogether DNA Ligase Buffer, 25°C.

Unit Definition: One unit is defined as the amount of enzyme required to give 50% ligation of 100 ng HindIII fragments of λ DNA in a total reaction volume of 20 µl in 30 minutes at 25°C in 1X StickTogether DNA Ligase Buffer.

Concentration: 3,000,000 units/ml

Note: ATP is an essential cofactor for the reaction. T7 DNA Ligase is also active in buffers without PEG 6000, such as our T4 DNA Ligase Buffer and NEBuffer r1.1–r4.1, for applications in which PEG 6000 is detrimental. Please remember to supplement the reaction with 1 mM ATP (final concentration). Using these buffers, the activity of T7 DNA Ligase is reduced approximately 10-fold.

E. coli DNA Ligase

#M0205S 200 units
#M0205L 1,000 units

- *Selective ligation of nicks in dsDNA without significant joining of dsDNA fragments regardless of end type*
- *cDNA synthesis*

Description: *E. coli* DNA Ligase catalyzes the formation of a phosphodiester bond between the 5´-phosphate and 3´-hydroxyl of two adjacent DNA strands in duplex DNA with cohesive ends. It is not appreciably active on blunt-ended substrates. *E. coli* DNA Ligase uses NAD as a cofactor and can be heat-inactivated. *E. coli* DNA Ligase is active at a range of temperatures (4–37°C).

Reaction Conditions: *E. coli* DNA Ligase Reaction Buffer, 16°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of λ DNA (5' DNA termini concentration of 0.12 µM, 300 µg/ml) in a total reaction volume of 20 µl in 30 minutes at 16°C in 1X *E. coli* DNA Ligase Reaction Buffer.

Concentration: 10,000 units/ml

Note: Requires NAD^+ (nicotinamide adenine dinucleotide) as a cofactor, in contrast to other ligases which use rATP. Ligation of blunt-ended fragments is extremely inefficient. For ligation of blunt-ended fragments we recommend Blunt/TA Ligase Master Mix (NEB #M0367).

HiFi *Taq* DNA Ligase

NEBU RR Mo

#M0647S 50 reactions

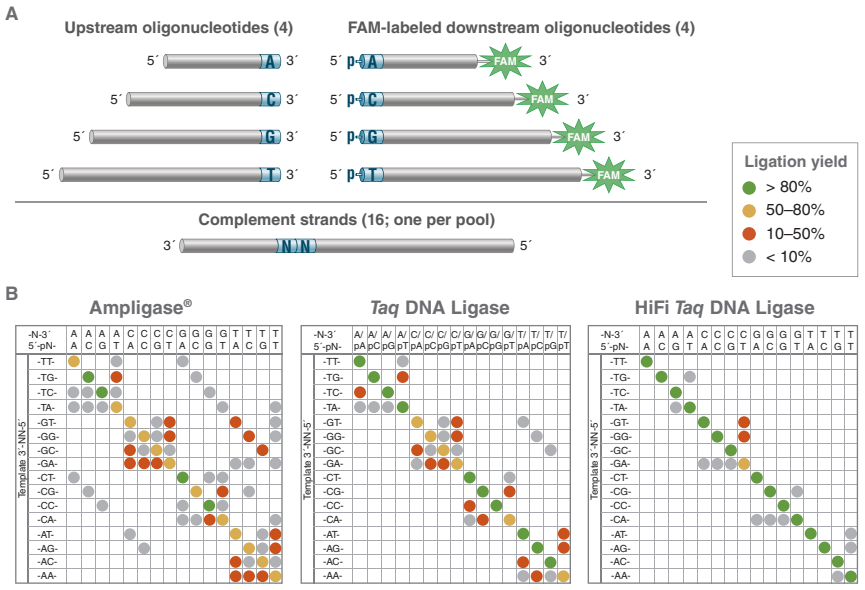
- *High fidelity, thermostable*
- *Repair of nicks in dsDNA*
- *Allele-specific gene detection using ligase-dependent methods, including the Ligase Chain Reaction (LCR) and Ligase Detection Reaction (LDR)*
- *Ligation of padlock probes*

Thermostable Ligase
Reaction Temperature Calculator

For help with calculating ligation temperature, visit **LigaseCalc.neb.com**

Description: An optimized blend of a thermostable DNA Ligase and a proprietary additive, HiFi *Taq* DNA Ligase efficiently seals nicks in DNA with unmatched high fidelity. The formation of a phosphodiester bond between juxtaposed 5´ phosphate and 3´ hydroxyl termini of two adjacent oligonucleotides that are hybridized to a complementary target DNA is enhanced in the improved reaction buffer and mismatch ligation is dramatically reduced. The improved formulation allows higher resolution discrimination between ligation donors and acceptors, enabling precise detection of SNPs and other allele variants. HiFi *Taq* DNA Ligase is active at elevated temperatures (37–75°C).

Reaction Conditions: HiFi *Taq* DNA Ligase Reaction Buffer.



HiFi Taq DNA Ligase displays increased fidelity. *(A) Schematic of multiplexed substrate pools. Each substrate pool contained a single splint with a defined NN at the ligation junction (e.g., AA, AC, AG…) along with all four upstream probes and all four FAM-labeled downstream probes. Each probe that encodes the base at the ligation junction is of unique length allowing for separation and analysis by capillary electrophoresis. A total of 16 substrate pools were prepared, one for each unique splint. (B) Comparison of the ligation fidelity of Ampligase (Epicentre), Taq DNA Ligase and HiFi Taq DNA Ligase. Fidelity measurements were performed using 1 ?l of ligase in a 50 ?l reaction mixture in the supplied buffers at 1X concentration. Reactions were incubated 30 min at 55°C, using multiplexed substrate pools as outlined in (A). Rows represent a single template sequence, while columns indicate a particular ligation product resulting from a specific pair of probes ligating with the indicated bases at the ligation junction. A dot indicates detection of a product (see legend above). The diagonal from the top left to the bottom right represents Watson-Crick ligation products; all other spaces indicate mismatch ligation products. While Taq DNA Ligase and Ampligase perform similarly under these conditions, with a range of mismatch products detectable, HiFi Taq DNA Ligase shows dramatically fewer mismatch products while maintaining high yields.*

Taq DNA Ligase

NEBU RR 45° Mo

#M0208S 2,000 units
#M0208L 10,000 units

- *Thermostable*
- *Repair of nicks in dsDNA*
- *Used in Gibson Assembly method*
- *Allele-specific gene detection using Ligase Detection Reaction and Ligase Chain Reaction*

Description: *Taq* DNA Ligase is a thermostable ligase that catalyzes the formation of a phosphodiester bond between the 5´-phosphate and 3´ -hydroxyl termini of two adjacent DNA strands. The strands to be ligated need to be hybridized and accurately paired, with no gap, to a complementary DNA strand; allowing resolution of single nucleotide variants. *Taq* DNA Ligase uses NAD as a cofactor and is active at elevated temperatures (37–75°C).

Reaction Conditions: *Taq* DNA Ligase Reaction Buffer, 45°C

Unit Definition: (Cohesive End Unit)
One unit is defined as the amount of enzyme required to give 50% ligation of the 12-base pair cohesive ends of 1 µg of BstEII-digested λ DNA in a total reaction volume of 50 µl in 15 minutes at 45°C.

Concentration: 40,000 units/ml

Note: 1X *Taq* DNA Ligase Reaction Buffer requires NAD^+ as a cofactor. NAD^+ is supplied in the 10X *Taq* DNA Ligase Reaction Buffer; the buffer should be stored at -80°C to extend the half life of the NAD^+ cofactor. *Taq* DNA ligase will not ligate short 4-base overlaps (typical of restriction enzyme digests), while it efficiently ligates 12-base pair overhangs.

rCutSmart NEB r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | rAlbumin Recombinant Albumin | SAM Requires SAM | 37° Incubation Temperature | 65° Heat Inactivation

9°N™ DNA Ligase

#M0238S 2,500 units

- *Repair of nicks in DNA while incubating at high temperatures*
- *Thermostable*
- *Allele-specific gene detection using Ligase Detection Reaction and Ligase Chain Reaction*

Description: 9°N DNA Ligase is a thermostable ligase that catalyzes the formation of a phosphodiester bond between the 5´-phosphate and 3´-hydroxyl of two adjacent DNA strands that are hybridized and accurately paired, with no gap, to a complementary DNA strand. 9°N DNA Ligase uses ATP as a cofactor and it is active at elevated temperatures (45–70°C).

Reaction Conditions: 9°N DNA Ligase Reaction Buffer, 45°C

Unit Definition: (Cohesive End Unit) One unit is defined as the amount of enzyme required to give 50% ligation of the 12-base pair cohesive ends of 1 µg of BstEII-digested λ DNA in a total reaction volume of 50 µl in 15 minutes at 45°C. A cohesive end unit is equivalent to the nick-closing unit (1).

Concentration: 40,000 units/ml

Note: 9°N will not ligate short 4-base overlaps (typical of restriction enzyme digests), while it efficiently ligates 12-base pair overlaps.

SplintR® Ligase

#M0375S 1,250 units
#M0375L 6,250 units

- *Ligation of adjacent, single-stranded DNA splinted by a complimentary RNA*
- *Characterization of miRNAs and mRNAs, including SNPs*

Description: SplintR Ligase, also known as PBCV-1 DNA Ligase or *Chlorella* virus DNA Ligase, efficiently catalyzes the ligation of adjacent, single-stranded DNA oligonucleotides splinted by a complementary RNA strand. This previously unreported activity may enable novel approaches for characterization of miRNAs and mRNAs, including SNPs. SplintR is ideally suited for many target enrichment workflows with applications in next-generation sequencing and molecular diagnostics. The robust activity of the enzyme and its affinity for RNA-splinted DNA substrates (apparent Km = 1 nM) enable sub-nanomolar detection of unique RNA species within a complex mixture, making SplintR ligase a superior choice for demanding RNA detection technologies.

Reaction Conditions: SplintR Ligase Reaction Buffer, 25°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme needed to ligate (to 50% completion) 2 picomoles of a tripartite FAM-labeled DNA:RNA hybrid substrate in a 20 µl reaction at 25°C in 15 minutes in 1X SplintR Ligase Reaction Buffer.

Concentration: 25,000 units/ml

Note: If dilution of enzyme for storage is needed, we recommend using Diluent A (NEB #B8001).

T4 Polynucleotide Kinase & T4 Polynucleotide Kinase (3´ phosphatase minus)

T4 Polynucleotide Kinase
#M0201S 500 units
#M0201L 2,500 units

T4 Polynucleotide Kinase
(3' phosphatase minus)
#M0236S 200 units
#M0236L 1,000 units

- *5´ phosphorylation of DNA/RNA for subsequent ligation*
- *End labeling DNA or RNA for probes and DNA sequencing*
- *Removal of 3´ phosphoryl groups with T4 Polynucleotide Kinase (NEB #M0201)*
- *T4 PNK (3´ phosphatase minus) (NEB #M0236) can be used for the 5´ phosphorylation of 3´ phosphorylated mononucleotide to generate a substrate (pNp) that can be added to the 3´ end of DNA or RNA*
- *5´ end labeling of 3´ phosphorylated oligos with T4 PNK (3´ phosphatase minus) (NEB #M0236)*

Description: T4 Polynucleotide Kinase catalyzes the transfer and exchange of P_i from the γ position of ATP to the 5´ hydroxyl terminus of polynucleotides (double- and single-stranded DNA and RNA), as well as nucleoside 3´ monophosphates. The T4 Polynucleotide Kinase (NEB #M0201) also catalyzes the removal of 3´ phosphoryl groups from 3´ phosphoryl polynucleotides, deoxynucleoside 3´ monophosphates and deoxynucleoside 3´ diphosphates. The modified version (NEB #M0236) exhibits full kinase activity with no 3´ phosphatase activity.

Reaction Conditions: 1X T4 Polynucleotide Kinase Reaction Buffer. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Notes: Fresh buffer is required for optimal activity (in older buffers, loss of DTT due to oxidation lowers activity).

CTP, GTP, TTP, UTP, dATP or dTTP can be substituted for ATP as a phosphate donor.

Protocols for phosphorylation (radioactive and non-radioactive) of DNA & RNA can be found at www.neb.com.

The efficiencies of blunt and recessed 5´ end phosphorylation can be improved by heating to 70°C for 5 minutes, then chilling on ice prior to kinase addition and by adding PEG-8,000 to 5% (w/v).

T4 Polynucleotide Kinase requires ATP for activity, but the supplied reaction buffer does not contain ATP to allow for high specific activity radiolabeling reactions.

Often, a kinase reaction is followed by a ligation reaction. In such cases, the T4 PNK reaction is performed in ligase buffer at 37°C for 30 minutes. The product of this reaction can be used directly in the ligation reaction without a buffer change or heat inactivation UNLESS there is a need to keep other DNA fragments dephosphorylated during ligation. When this is desirable, PNK should be heat inactivated prior to ligation.

Unit Definition: One Richardson unit is defined as the amount of enzyme catalyzing the incorporation of 1 nmol of acid-insoluble [^{32}P] in 30 minutes at 37°C.

Concentration: 10,000 units/ml

5-hydroxymethyluridine DNA Kinase

NEBU 37° 80°

#M0659S 1,000 units

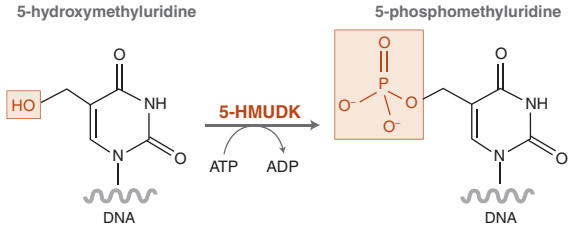
This is an **Enzyme for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. To view the full list, visit **www.neb.com/EnzymesforInnovation.**

Description: 5-hydroxymethyluridine DNA Kinase (5-HMUDK) transfers the gamma phosphate from ATP to the hydroxymethyl moiety of 5-hydroxymethyluridine in polymeric DNA.

Reaction Conditions: T4 DNA Ligase Reaction Buffer, 37°C. Heat inactivation: 80°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg of *Bacillus subtilis* bacteriophage SP8 genomic DNA in 30 minutes at 37°C in a total reaction volume of 20 µl against subsequent cleavage by NcoI-HF restriction endonuclease.

Concentration: 20,000 units/ml



Phosphatase Selection Chart

	Quick CIP* NEB #M0525	Shrimp Alkaline Phosphatase (rSAP) NEB #M0371	Antarctic Phosphatase NEB #M0289
FEATURES			
100% heat inactivation	2 minutes at 80°C	5 minutes at 65°C	2 minutes at 80°C
High specific activity	•	•	
Improved stability	•	•	
Works directly in NEBuffers	•	•	•
Requires additive			• (Zn^{2+})
Quick Protocol (10 minutes)	•		

* Note: NEB recommends Quick CIP for most applications.

Quick CIP

rCutSmart RR 37° 80°

#M0525S 1,000 units
#M0525L 5,000 units

- *Dephosphorylation of 5´ and 3´ ends of DNA and RNA*
- *Dephosphorylation of cloning vector DNA to prevent recircularization during ligation*
- *Removal of dNTPs in PCR reactions prior to sequencing or SNP analysis*
- *Dephosphorylation of DNA prior to end-labeling using T4 Polynucleotide Kinase*

Description: Quick CIP is a heat-labile recombinant version of calf intestinal alkaline phosphatase (CIP). Quick CIP nonspecifically catalyzes the dephosphorylation of 5´ and 3´ ends of DNA and RNA phosphomonoesters. It also hydrolyses ribo- and deoxyribonucleoside triphosphates (NTPs and dNTPs). Quick CIP is useful in many applications that require the dephosphorylation of DNA or RNA ends. In cloning, dephosphorylation prevents re-ligation of linearized plasmid DNA. The enzyme can quickly dephosphorylate 5´ protruding, 5´ recessed, and blunt ends in just 10 minutes. Quick CIP may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing or SNP analysis.

Quick CIP is completely and irreversibly inactivated by heating it at 80°C for 2 minutes, unlike wild type CIP, which is not heat-inactivatable. This makes removal of Quick CIP prior to ligation or end-labeling unnecessary.

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 2 minutes.

Unit Definition: One unit is defined as the amount of enzyme that hydrolyzes 1 µmol of p-Nitrophenyl Phosphate, PNPP (NEB #P0757) in a total reaction volume of 1 ml in 1 minute at 37°C.

Concentration: 5,000 units/ml

rCutSmart NEB r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · rAlbumin Recombinant Albumin · SAM Requires SAM · 37° Incubation Temperature · 65° Heat Inactivation

Shrimp Alkaline Phosphatase (rSAP)

rCutSmart RR 37° 65°

#M0371S 500 units
#M0371L 2,500 units

- *Dephosphorylation of DNA and RNA*
- *Dephosphorylation of cloning vector DNA to prevent recircularization during ligation*
- *Removal of dNTPs and pyrophosphate from PCR reactions prior to sequencing or SNP analysis*
- *Dephosphorylation of DNA prior to 5´ end-labeling using T4 Polynucleotide Kinase*

Description: Shrimp Alkaline Phosphatase (rSAP) is a heat labile alkaline phosphatase purified from a recombinant source. rSAP is identical to the native enzyme, and contains no affinity tags or other modifications. rSAP nonspecifically catalyzes the dephosphorylation of 5´ and 3´ ends of DNA and RNA phosphomonoesters. Also, rSAP hydrolyzes ribo-, as well as deoxyribonucleoside triphosphates (NTPs and dNTPs). rSAP is useful in many applications that require the dephosphorylation of DNA or RNA ends. In cloning, dephosphorylation prevents re-ligation of linearized plasmid DNA. rSAP may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing or SNP analysis. rSAP is completely and irreversibly inactivated by heating at 65°C for 5 minutes, thereby making removal of rSAP prior to ligation or end-labeling unnecessary.

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 5 minutes.

Unit Definition: One unit is defined as the amount of enzyme that hydrolyzes 1 µmol of *p*-Nitrophenyl Phosphate, PNPP (NEB #P0757) in a total reaction volume of 1 ml in 1 minute at 37°C.

Concentration: 1,000 units/ml

Antarctic Phosphatase

NEB RR 37° 80°

#M0289S 1,000 units
#M0289L 5,000 units

- *Dephosphorylation of DNA and RNA*
- *Dephosphorylation of cloning vector DNA to prevent recircularization during ligation*
- *Removal of dNTPs and pyrophosphate from PCR reactions prior to sequencing or SNP analysis*
- *Dephosphorylation of DNA prior to 5´ end-labeling using T4 Polynucleotide Kinase*

Description: Antarctic Phosphatase catalyzes the dephosphorylation of 5´ and 3´ ends of DNA and RNA phosphomonoesters. Antarctic Phosphatase also hydrolyzes ribo-, as well as deoxyribonucleoside triphosphates (NTPs and dNTPs). Antarctic Phosphatase is useful in many applications that require the dephosphorylation of DNA or RNA ends. In cloning, dephosphorylation prevents religation of linearized plasmid DNA. The enzyme acts on 5´ protruding, 5´ recessed and blunt ends. Antarctic Phosphatase may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing. The enzyme is completely and irreversibly inactivated by heating at 80°C for 2 minutes, thereby making removal of Antarctic Phosphatase prior to ligation or end-labeling unnecessary.

Reaction Conditions: Antarctic Phosphatase Reaction Buffer, 37°C. Heat inactivation: 80°C for 2 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will dephosphorylate 1 µg of pUC19 vector DNA cut with a restriction enzyme generating 5´ recessed ends in 30 minutes at 37°C. Dephosphorylation is defined as > 95% inhibition of recircularization in a self-ligation reaction and is measured by transformation into *E. coli*.

Concentration: 5,000 units/ml

Pyrophosphatases

RR Mg

Pyrophosphatase, Inorganic (*E. coli*)
#M0361S 10 units
#M0361L 50 units

Pyrophosphatase, Inorganic (yeast)
#M2403S 10 units
#M2403L 50 units

Thermostable Inorganic Pyrophosphatase
#M0296S 250 units
#M0296L 1,250 units

NudC Pyrophosphatase
#M0607S 250 pmol

- *Increasing RNA yield in transcription reactions*
- *Enhancing DNA replication*

NudC Pyrophosphatase is an Enzyme for Innovation (EFI). EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. To view the full list, visit **www.neb.com/EnzymesforInnovation.**

Description: Inorganic pyrophosphatase (PPase) catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate.

$P_2O_7^{-4} + H_2O \rightarrow 2HPO_4^{-2}$

Source: Pyrophosphatase, Inorganic (*E. coli*) is prepared from a clone of the *E. coli* inorganic pyrophosphatase gene.

Pyrophosphatase, Inorganic (yeast) is an *E. coli* strain containing a genetic fusion of the *Saccharomyces cerevisiae ppa* gene and the gene coding for *Mycobacterium xenopi* GyrA intein. Developed by BioHelix Corporation, now a wholly owned subsidiary of Quidel Corporation, and produced at New England Biolabs.

Thermostable Inorganic Pyrophosphatase is an *E. coli* strain carrying a plasmid encoding a pyrophosphatase from the extreme thermophile *Thermococcus litoralis*.

NudC is a NUDIX pyrophosphatase that efficiently hydrolyzes NAD^{+-} and NADH-capped RNA, generating a ligatible 5´ monophosphate on the RNA (NAD^+ decapping or deNADding). Deletion of the nudC gene has been shown to increase the fraction of NAD^{+-} capped RNA in *E. coli*.

Unit Definition: One unit is defined as the amount of enzyme that will generate 1 µmol of phosphate per minute from inorganic pyrophosphate under standard reaction conditions.

1 µM of NudC hydrolyzes 200 µM or more NAD^+ into NMN^+ and AMP in 1X NEBuffer r3.1 and 5 mM DTT at 37°C for 30 min.

Concentration: Pyrophosphatase, Inorganic (*E. coli*) and Pyrophosphatase, Inorganic (yeast): 100 units/ml. Thermostable Inorganic Pyrophosphatase: 2,000 units/ml. NudC Pyrophosphatase: 10 µM

Find an overview of dephosphorylation.

Apyrase

#M0398S 10 units
#M0398L 50 units

- *Highly efficient degradation of ATP to ADP and ADP to AMP*
- *Removal of deoxynucleotides in DNA pyrosequencing between cycles*
- *Conversion of 5´ triphosphorylated RNA to ligatable monophosphorylated*
- *Conversion of 5´ triphosphorylated RNA to 5´ exonuclease XRN-1 (NEB #M0338) sensitive monophosphorylated RNA*

Description: Apyrase (recombinant, *E. coli*) is a highly active ATP-diphosphohydrolase that catalyses the sequential hydrolysis of ATP to ADP and ADP to AMP releasing inorganic phosphate. It is a recombinant version of one of several isoforms of apyrase. It can also hydrolyse 5´ tri- and diphosphate ribonucleosides and deoxyribonuclesides to their respective 5´ monophosphates. Apyrase can catalyze the conversion of 5´ triphosphorylated RNA to 5´ monophosphorylated RNA by sequential removal of γ and β phosphates.

Reaction Conditions: Apyrase Reaction Buffer, 30°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that catalyses the release of 1 µmol of inorganic phosphate from ATP (1 mM, NEB #P0756) in 1X Apyrase Reaction Buffer in 1 minute at 30°C in a total reaction of 50 µl.

Concentration: 500 units/ml

Note: Apyrase has a higher ratio of activity for ATP:ADP (14:1). Apyrase is a calcium-activated enzyme. It is approximately 50% active when Mg^{2+} substitutes Ca^{2+} in Apyrase Reaction Buffer. As a metal-dependent enzyme, Apyrase can be inhibited by EGTA and EDTA. The activity of Apyrase is approximately 30% in NEBuffers r1.1, r2.1, r3.1 and rCutSmart™ Buffer. Apyrase does not remove 5´ caps from eukaryotic mRNA.

Tte UvrD Helicase

#M1202S 1 µg

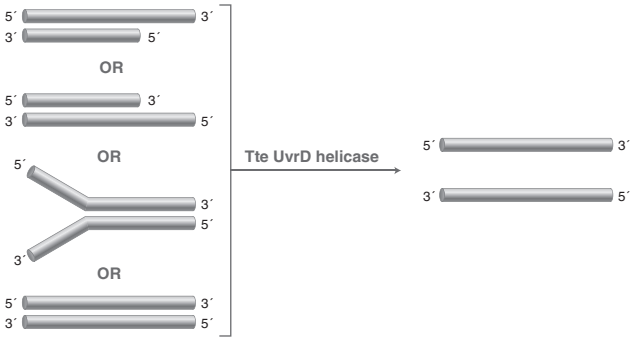
- *Unwinds double-stranded DNA*
- *Thermostable to 65°C*
- *Reduces non-specific product formation in isothermal amplification (e.g., LAMP)*

This is an **Enzyme for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. To view the full list, visit **www.neb.com/EnzymesforInnovation.**

Description: *Tte* UvrD Helicase is a repair helicase from the thermophilic organism *Thermoanaerobacter tengcongensis*. It is capable of unwinding double-stranded DNA without a requirement for a specific flap or overhang structure. *Tte* UvrD Helicase is active on a wide range of DNA substrates and, along with its thermostability (active to 70°C), *Tte* UvrD Helicase has been demonstrated to be a useful additive for improving specificity of isothermal amplification reactions.

Reaction Conditions: Isothermal Amplification Buffer Pack, 65°C. Heat inactivation: 80°C for 5 minutes.

Concentration: 20 µg/ml



Lisa joined NEB in 2006 and is a member of our Research Department. Lisa also manages our Summer Student Internship Program. Learn more about Lisa's role at NEB in her video reel on Instagram.

#NEBiographies

rCutSmart NEB r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | rAlbumin Recombinant Albumin | SAM Requires SAM | 37° Incubation Temperature | 65° Heat Inactivation

Exonucleases and Non-specific Endonucleases: Properties

Enzyme	Polarity	Activity on ssDNA		Activity on dsDNA[1]					Partial Digestion to Generate ss Extension[2]	Products Produced[3]	Inhibition by Phosphoro-thioate[4]	Notes
		Linear	Circular	Linear 5´ Ext	Linear 3´ Ext	Linear Blunt	Nicked (Circular/ Linear)	Circular (Supercoiled)				
Exonuclease I (*E. coli*)	3´→5´	+	—	—	— [15]	— [5]	—	—	No	dNMP, dinucleotide [6]	Yes	15, 5, 6
Thermolabile Exonuclease I	3´→5´	+	—	—	— [15]	— [5]	—	—	No	dNMP, dinucleotide [6]	Yes	15, 5, 6
***Msz* Exonuclease I**	3´→5´	+	—	—	— [15]	— [5]	—	—	No	dNMP, dinucleotide [6]	Yes	15, 5, 6
Exonuclease T	3´→5´	+	—	—	— [7]	— [5]	—	—	No	dNMP,dinucleotide, short oligo	Yes	5, 7
Exonuclease VII	both	+	+ [8]	— [17]	— [17]	—	—	—	No	short oligos	No	8
$RecJ_f$	5´→3´	+	—	— [15]	—	— [5]	—	—	No	dNMP, ssDNA	Yes	5, 15
Mung Bean Nuclease	Endonuclease	+	+	—	—	—	—	—	No	dNMP, ssDNA	No	
Nuclease P1	Endonuclease	+	+	—	—	—	—	—	No	5' mononucleotides	No	
Exonuclease III (*E. coli*)	3´→5´	+/-[16]	—	+	+/- [14]	+	+	—	5´	dNMP, ssDNA	Yes	14
T7 Exonuclease	5´→3´	—	—	+/-	+	+	+	—	3´	dNMP, dinucleotide, ssDNA [9]	Yes	9
Exonuclease V (RecBCD)	both	+	+	+	+	+	—	—	Yes	Short oligos	No	
Exonuclease VIII, truncated	5´→3´	+/- [10]	—	+	+	+	—	—	3´	dNMP, ssDNA	No	10
Lambda Exonuclease	5´→3´	+/- [10]	—	+/- [11]	+	+	+/- [11]	—	3´	dNMP, dinucleotide, ssDNA,	Yes	10, 11
T5 Exonuclease	5´→3´	+	+	+	+	+	+	—	3´	dNMP to 6 mer	No	
DNase I (RNase-free)	Endonuclease	+	+	+	+	+	+	+	NA	dinucleotides, trinucleotides, oligonucleotides, ssDNA, dsDNA,	No	
DNase I-XT	Endonuclease	+	+	+	+	+	+	+	NA	dinucleotides, trinucleotides, oligonucleotides, ssDNA, dsDNA	No	
Duplex DNase	Endonuclease	—	—	+	+	+	+	+	NA	dinucleotides, trinucleotides, oligonucleotides, ssDNA, dsDNA	No	
Micrococcal Nuclease	Endonuclease	+	+	+	+	+	+	+	NA	diphosphonucleotides, ssDNA, dsDNA 3´-monophospho-nucleotides [13]	No	13

Footnotes

(1) The ability to act on short extensions, blunt ends and nicks distinguishes these enzymes; some of these ends are conveniently generated by restriction digestion. The 5´ and 3´ extensions tested were 4 nt in length

(2) Partial digestion of dsDNA by Lambda Exonuclease, T7 Exonuclease and Exonuclease III will produce dsDNA products with ss extensions. Complete digestion produces ssDNA as products.

(3) Complete hydrolysis of the preferred substrate will generate the listed products

(4) To inhibit exonucleases, use of at least 5 phosphorothioate (pt) bonds in a row is recommended. These bonds must be placed at the end of the DNA corresponding to the Polarity of the enzyme; 5´ end for 5´→ 3´ nucleases, the 3´ end for 3´→ 5´ nucleases, and at both ends if the nucleases cannot initiate at both ends. Endonucleases cannot be inhibited by pt bonds unless the entire sequence has pt bonds between all nucleotides.

(5) Depending upon the DNA sequence and amount of exonuclease, $RecJ_f$, Thermolabile Exonuclease I, Exonuclease I, *Msz* Exonuclease I, and Exonuclease T may remove a few nucleotides from blunt termini.

(6) Thermolabile Exonuclease I, Exonuclease I, and *Msz* Exonuclease I release dNMP from ssDNA, except from the last hydrolytic step where a dinucleotide is produced.

(7) Exonuclease T can be used to make 3´ extensions blunt, however, the yield is low.

(8) Exonuclease VII will not be able to digest circular ssDNA when EDTA is present in the reaction. In the absence of Mg^{++} the enzyme will act as a pure exonuclease.

(9) It has been reported that the initial first product hydrolyzed from dsDNA by T7 Exonuclease is a dinucleotide. Subsequent hydrolytic cleavage releases dNMP.

(10) Lambda Exonuclease and Exonuclease VIII, truncated only cut ssDNA if the 5´ contains a phosphate

(11) Lambda Exonuclease has a strong preference for initiating on dsDNA containing a 5´ phosphate. Thus if linear dsDNA has a 5´ phosphate at one end and lacks a 5´ phosphate on the other end, then Lambda Exonuclease will preferentially degrade the DNA that contains the phosphorylated end.

(12) BAL-31 Nuclease has been reported as having both ss endonuclease activity as well as 3´ to 5´ exonuclease activity. Thus any linear DNA is substrate for this enzyme.

(13) Products of Micrococcal Nuclease degradation have 3´ phosphates. Also cuts RNA whereas DNase I does not.

(14) Exonuclease III will be inhibited by overhangs >4 nucleotides

(15) $RecJ_f$ is not suitable for making 5´ extensions blunt. Thermolabile Exonuclease I, Exonuclease I, and *Msz* Exonuclease I are not suitable for making 3´ extensions blunt. These enzymes require longer length ssDNA extensions to initiate than those generated by restriction enzymes.

(16) Exonuclease III exhibits 5-10X less activity on linear ssDNA versus linear dsDNA

(17) For information on removing ssDNA extensions from dsDNA see the Blunting Selection chart

Table Legend

+	Activity, preferred substrate
—	No significant activity
+/-	Activity greatly reduced relative to preferred substrate
NA	Not applicable
ss	Single-stranded
ds	Double-stranded
ext	Extension
dNMP	Deoxyribonucleoside monophosphate

Exonucleases and Endonucleases: Common Applications

Application	Recommended Enzyme(s)	Notes
Removal of 3´ overhangs	• Quick Blunting™ Kit	
5´ overhang Fill in Treatment	• Quick Blunting™ Kit	
Removal of single-stranded primers for nested PCR reactions	• Thermolabile Exonuclease I	
Removal of primers post PCR prior to DNA sequencing or SNP detection	• Exonuclease I • Thermolabile Exonuclease I • Exonuclease VII • *Msz* Exonuclease I	• Quick Heat inactivation versus Exonuclease I for 3´ chemically modified primers • Quick Heat inactivation versus Exonuclease I • Removal of primers with our without 3´ or 5´ phosphorothioate bonds • Quick Heat inactivation versus Exonuclease I for 3´chemically modified primers
Mapping positions of introns in genomic DNA	• Exonuclease VII	
Removal of primers with or without 3´ or 5´ terminal phosphorothioate bonds	• Exonuclease VII	
Generating ssDNA from linear dsDNA: **If 5´ → 3´ polarity required** **If 3´ → 5´ polarity required**	• Lambda Exonuclease • Exonuclease III	• Strand targeted for removal requires one 5´ end with phosphate • Strand targeted for removal requires a 5´ overhang, a blunt end, or a 3´ overhang (with less than 4 bases)
Preparation of nested deletions in double-stranded DNA	• Exonuclease III (*E. coli*) plus Exonuclease VII	
Site-directed mutagenesis	• Exonuclease III (*E. coli*) • T7 Exonuclease	• Removes nicked-strand DNA from 3´ to 5´ • Removes nicked-strand DNA from 5´ to 3´
Nick-site extension	• T7 Exonuclease	
Degradation of denatured DNA from alkaline-based plasmid purification methods for improving DNA cloning	• T5 Exonuclease	
Removal of chromosomal/linear DNA in plasmid preparations	• T5 Exonuclease • Exonuclease V (RecBCD)	• Degrades linear ss + dsDNA, nicked DNA • Degrades linear ss + dsDNA: PREFERRED as Exo V will save nicked plasmids resulting in higher yields especially for low-copy number plasmid prep
Removal of unligated products (linear dsDNA) from ligated circular double-stranded DNA	• T5 Exonuclease • Exonuclease V (RecBCD)	• Only the un-nicked form of ligated circular double-stranded remains • Both nicked and unnicked-form of ligated circular double-stranded DNA remains
Removal of residual gDNA after purification of low copy plasmid	• Exonuclease V (RecBCD)	
Removal of contaminated DNA from RNA samples	• DNase I • DNase I-XT • Duplex DNase	
Removal of gDNA prior to RT-qPCR	• Duplex DNase	
Degradation of DNA strand in DNA:RNA hybrid duplex	• Duplex DNase	
Removal of template DNA from IVT reactions	• DNase I-XT • DNase I (RNase-free)*	
Conversion of single-stranded DNA or RNA to 5´-mononucleotides	• Nuclease P1	
Analysis of base composition, potential damage and modification of nucleic acids	• Nuclease P1	
Preparation of double-stranded DNA fragments with 5´-OH and 3´-phosphate	• Micrococcal Nuclease	
Degradation of nucleic acids (both DNA and RNA) in crude cell-free extracts	• Micrococcal Nuclease	
Preparation of rabbit reticulocyte	• Micrococcal Nuclease	
Chromatin Immunoprecipitation (ChIP) analysis	• Micrococcal Nuclease	

* **GMP-grade reagent** available. See page 6 for details.

What are exonucleases and their applications?

rCutSmart Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · rAlbumin Recombinant Albumin · SAM Requires SAM · 37° Incubation Temperature · 65° Heat Inactivation

DNase I (RNase-Free)

NEW

DNase I-XT

DNase I (RNase-free)
#M0303S 1,000 units
#M0303L 5,000 units

DNase I-XT
#M0570S 1,000 units
#M0570L 5,000 units

- *Removal of contaminating genomic DNA from RNA samples*
- *Degradation of DNA templates in transcription reactions*

DNase I (RNase-Free) (NEB #M0303) is available as a **GMP-grade reagent**. See page 6 for details.

Description: DNase I is a DNA-specific endonuclease that degrades ds- and ss-DNA to release short oligos with 5´ phosphorylated and 3´-hydroxylated ends.

DNase I-XT is a salt tolerant enzyme that exhibits optimal activity between 50-100 mM salt and retains 65% and ~40% activity in 200 and 300 mM salt, respectively. Increased salt tolerance makes it ideal for DNA template removal from an *in vitro* transcription (IVT) reaction.

Both enzymes are RNase-free, allowing for complete removal of DNA from RNA preps, while maintaining RNA integrity.

Reaction Conditions: 1X DNase I or DNase I-XT Reaction Buffer, 37°C. DNase I can be heat inactivated at 75°C for 10 minutes, while DNase I-XT cannot.

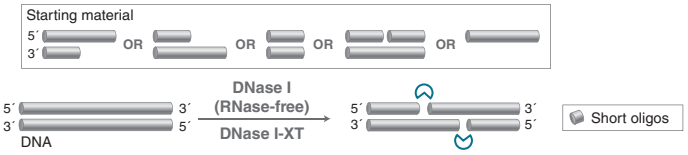
Reagents Supplied:

- DNase I Reaction Buffer (NEB #B0303)
- DNase I-XT Reaction Buffer (NEB #B0570)

Unit Definition: DNase I – One unit is defined as the amount of enzyme which will completely degrade 1 µg of pBR322 DNA in a total reaction volume of 50 µl in 10 minutes at 37°C. DNase I-XT – One unit is defined as the amount of enzyme required to release 210 pmol of FAM from a 35-mer FAM-BHQ1 labeled hairpin oligonucleotide in 1 minute at 30°C in a 50 µl reaction volume.

Concentration: 2,000 units/ml

Notes: DNase I-XT is supplied with an optimized reaction buffer. Use with supplied buffer, and not DNase I Reaction Buffer. Likewise, due to the different salt concentration, do not use DNase I-XT Buffer with DNase I. When using DNase I, EDTA should be added to a final concentration of 5 mM to protect RNA from being degraded during enzyme inactivation.



NEW

Duplex DNase

#M7635S 150 units
#M7635L 750 units

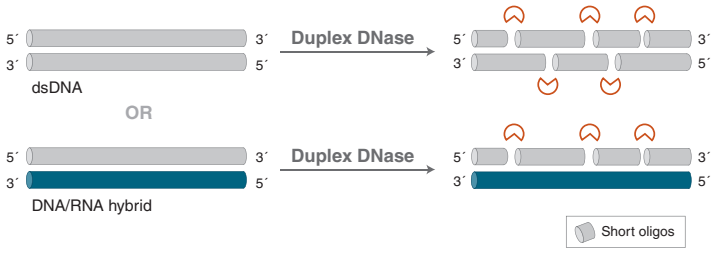
- *DNA-specific endonuclease*
- *Specifically degrades dsDNA in the presence of ssDNA*
- *Cleaves DNA strand of DNA:RNA hybrid duplex*
- *Products are short oligos or ssRNA (if cleaving the DNA strand of DNA:RNA hybrid)*

Description: Duplex DNase is an engineered double-strand-specific DNA endonuclease that preferentially degrades double-stranded DNA (dsDNA) over single-stranded DNA (ssDNA) or RNA. It will also cleave the DNA strand of a DNA/RNA hybrid duplex.

Reaction Conditions: NEBuffer r1.1. Heat inactivation: 75°C for 10 minutes in the presence of 1 mM DTT. If the sample contains RNA, we recommend adding EDTA (10 mM final concentration) and DTT (1 mM final concentration), prior to heat inactivation. RNA may degrade at temperatures >65°C in the presence of divalent metals such as Mg^{2+}.

Unit Definition: One unit is defined as the amount of enzyme required to release 50 pmol of FAM from a 35mer FAM-BHQ1 labeled hairpin oligonucleotide in 1 minute at 30°C in a 50 µl reaction volume.

Concentration: 2,000 units/ml



NEW

NEBExpress® Salt Active Nuclease

#M0764S 0.5 ml

Companion Products:

NEBExpress Ni-NTA Magnetic Beads
#S1423S 1 ml
#S1423S 1 ml

NEBExpress Ni Spin Columns
#S1427S 10 Each
#S1427S 10 Each

NEBExpress® Ni Resin
#S1428S 25 ml

Thermolabile Proteinase K
#P8111S 30 units

- *Degrades ds- and ss-DNA and RNA, linear and circular DNA and RNA, and DNA and RNA hybrids*
- *Ideal in bioprocessing and biomanufacturing workflows for nucleic acid removal from recombinant proteins, enzymes, and viruses and for reducing viscosity in lysates*
- *Up to 2 times more active than other comparable nucleases at 500 mM NaCl/KCl*

Description: NEBExpress Salt Active Nuclease is a proprietary endonuclease (patent-pending) engineered with broad specificity for all forms of DNA and RNA. This salt active nuclease (SAN) has optimal activity in high salt (500 mM NaCl/KCl) and non-specifically cleaves single-stranded, double-stranded, circular and linear DNA, RNA and both strands of DNA/RNA hybrids, to release short oligonucleotides (as short as 5 nucleotides) with 5´-phosphorylated and 3´-hydroxylated ends.

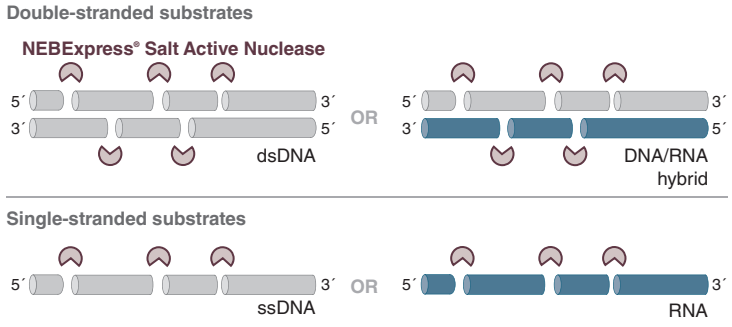
NEBExpress Salt Active Nuclease exhibits greater than 50% activity in salt concentrations from 200-1000 mM, at pH 7.5 to 10 and in presence of 1-50 mM $MgCl_2$, with optimal activity at 500 mM NaCl/KCl. The enzyme functions at a range of temperatures from 4°C through 50°C, and its high isoelectric point (pI) allows for efficient removal by capture on a cation exchange resin.

NEBExpress Salt Active Nuclease is suitable for viscosity reduction and degradation of unwanted DNA and RNA in cell lysates and soluble fractions. The enzyme is compatible with bioprocessing workflows for proteins, viruses, or small molecule purification, and is suitable for nucleic acid digestion in cell lysis steps in viral vector (e.g. adeno-associated (AAV)) production.

Unit Definition: One unit is defined as the amount of enzyme required to release 32 pmol of FAM from FAM-BHQ1 labeled hairpin oligonucleotide in 1 minute at 25°C in a 50 µL reaction in 25 mM Tris-HCl, 500 mM NaCl, 5 mM MgCl2, 0.005% Tween 20 (pH 8.5 @ 25°C).

Concentration: 100,000 units/ml

Note: NEBExpress Salt Active Nuclease requires 1-50 mM $MgCl_2$ or 5-100 mM $MnCl_2$. Phosphate-based buffers (including PBS) and SDS are not recommended, as enzyme activity is drastically reduced. NEBExpress Salt Active Nuclease can be degraded with Proteinase K (NEB #P8107) or Thermolabile Proteinase K (NEB #P8111).



Lambda Exonuclease

#M0262S 1,000 units
#M0262L 5,000 units

- *Generates ssDNA from linear dsDNA for dideoxy sequencing*
- *Preparation of nested deletions in dsDNA*

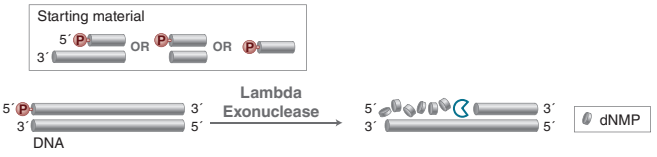
Description: Lambda exonuclease is a highly processive DNA-specific exonuclease that catalyzes the removal of nucleotides from linear or nicked dsDNA in a 5´→ 3´ direction. The preferred substrate is 5´ phosphorylated dsDNA, although it will also degrade ssDNA and non-phosphorylated substrates at a reduced rate.

Reaction Conditions: Lambda Exonuclease Reaction Buffer, 37°C. Heat inactivation: 75°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 10 nmol of acid-soluble deoxyribonucleotide from double-stranded substrate in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X Lambda Exonuclease Reaction Buffer with 1 µg sonicated duplex [^{3}H]-DNA.

Concentration: 5,000 units/ml

Note: Phosphorothiate (PT) bond is resistant to cleavage.



rCutSmart r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · rAlbumin Recombinant Albumin · SAM Requires SAM · 37° Incubation Temperature · 65 Heat Inactivation

Exonuclease I (*E. coli*)

#M0293S 3,000 units
#M0293L 15,000 units

- *Removal of single-stranded primers in PCR reactions prior to Sanger DNA sequencing or SNP analysis*
- *Removal of single-stranded primers for nested PCR reactions*
- *Removal of linear ssDNA, leaving behind dsDNA in the sample*

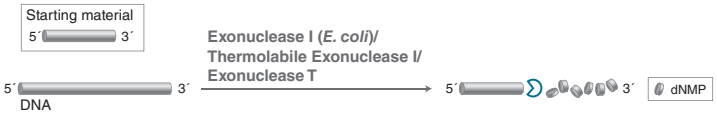
Description: Exonuclease I is a DNA-specific exonuclease that catalyzes the removal of nucleotides from linear single-stranded DNA in the 3´ to 5´ direction.

Reaction Conditions: Exonuclease I Reaction Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will catalyze the release of 10 nmol of acid-soluble nucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X Exonuclease I Reaction Buffer with 0.17 mg/ml single-stranded [^{3}H]-DNA.

Concentration: 20,000 units/ml

Note: Phosphorothiate (PT) bond is resistant to cleavage.



Thermolabile Exonuclease I

#M0568S 3,000 units
#M0568L 15,000 units

- *Removal of single-stranded primers in PCR reactions prior to DNA sequencing or SNP analysis*
- *Removal of single-stranded primers for nested PCR reactions*
- *Removal of linear ssDNA, leaving behind dsDNA in the sample*

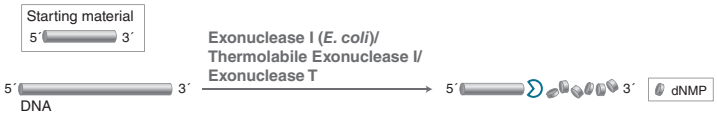
Description: Thermolabile Exonuclease I is a DNA-specific exonuclease that catalyzes the removal of nucleotides from single-stranded DNA in the 3´ to 5´ direction, and can be heat inactivated at 80°C in 1 minute.

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 1 minute.

Unit Definition: One unit of Thermolabile Exonuclease I is defined as the amount of enzyme that will catalyze the release of 2 nmol of acid-soluble nucleotide in a total reaction volume of 100 µl in 6 minutes at 37°C in 50 mM Tris-HCl (pH 7.9), 100 mM NaCl, 10 mM $MgCl_2$ and 100 µg/ml BSA with 0.17 mg/ml single-stranded [3H]-*E. coli* DNA.

Concentration: 20,000 units/ml

Note: Phosphorothiate (PT) bond is resistant to cleavage.



Exonuclease III (*E. coli*)

#M0206S 5,000 units
#M0206L 25,000 units

- *Site-directed mutagenesis*
- *Preparation of ssDNA for dideoxy sequencing*
- *Preparation of nested deletions in dsDNA*

Description: Exonuclease III preferentially degrades linear or nicked dsDNA in the 3´→ 5´ direction. A limited number of nucleotides are removed during each binding event, resulting in coordinated progressive deletions within the population of DNA molecules.

Initiation occurs at the 3´ termini of linear double-stranded DNA with 5´ overhangs or blunt ends and 3´ overhangs containing less than four bases.

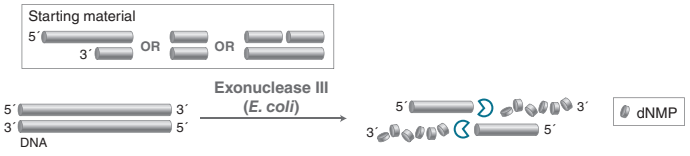
Exonuclease III has also been reported to have RNase H, 3´ phosphatase and AP-endonuclease activities.

Reaction Conditions: NEBuffer 1, 37°C. Heat inactivation: 70°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble total nucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X NEBuffer 1 with 0.15 mM sonicated duplex [^{3}H]-DNA.

Concentration: 100,000 units/ml

Note: Phosphorothioate (PT) bond is resistant to cleavage.



Exonuclease V (RecBCD)

#M0345S 1,000 units
#M0345L 5,000 units

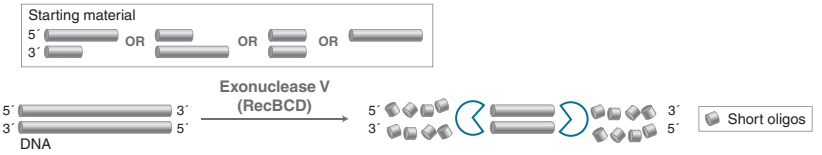
- *Degradation of contaminating linear DNA in plasmid samples*
- *Removal of residual gDNA after purification of low copy plasmid*

Description: Exonuclease V, (RecBCD) is a DNA-Specific exonuclease that also acts as an endonuclease on ssDNA. Activity initiates at both the 5´ and 3´ ends and is processive, generating oligonucleotides. Activity requires ATP and divalent cations. Mg^{2+} is required for the exonuclease activity, while Ca^{2+} inhibits the exonuclease activity and allows dsDNA unwinding (helicase activity).

Reaction Conditions: NEBuffer 4, 37°C. Supplement with 1 mM ATP. Heat inactivation: 70°C for 30 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from double-stranded DNA in 30 minutes at 37°C in a total reaction volume of 50 µl.

Concentration: 10,000 units/ml



Exonuclease VII

#M0379S 200 units
#M0379L 1,000 units

- *Removal of primers with or without 3´ or 5´ terminal phosphorothioate bonds*
- *Mapping positions of introns in genomic DNA*
- *Removal of ssDNA, leaving behind the dsDNA in a sample*

Description: Exonuclease VII, (Exo VII) is a DNA-specific exonuclease that cleaves linear ssDNA in both 5´→3´ and 3´→5´ direction. The preferred substrate is linear ssDNA.

Reaction Conditions: Exonuclease VII Reaction Buffer, 37°C. Heat inactivation: 95°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will catalyze the release of 1 nmol of acid-soluble nucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C.

Concentration: 10,000 units/ml

Exonuclease VIII, truncated

#M0545S 1,000 units

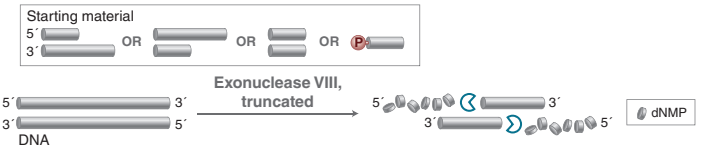
- *Removal of linear dsDNA, leaving behind circular DNA in the sample*

Description: Exonuclease VIII, truncated, is an exonuclease that prefers dsDNA. Exonuclease VIII, truncated initiates nucleotide removal from the 5´ termini of linear double-stranded DNA in the 5´ to 3´ direction. The enzyme does not degrade supercoiled dsDNA and circular ssDNA.

Reaction Conditions: NEBuffer 4, 37°C. Heat inactivation: 70°C for 15 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from double-stranded DNA in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X NEBuffer 4 with 0.15 mM sonicated duplex [^{3}H] DNA.

Concentration: 10,000 units/ml



rCutSmart r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · rAlbumin Recombinant Albumin · SAM Requires SAM · 37° Incubation Temperature · 65° Heat Inactivation

Exonuclease T

#M0265S 250 units
#M0265L 1,250 units

- *Removal of 3´ overhangs of dsDNA to generate blunt-ends (sequence dependent)*
- *Removal of single-stranded primers in PCR reactions prior to DNA sequencing of SNP analysis*
- *Removal of single-stranded primers for nested PCR reactions*
- *Removal of ssDNA, leaving behind dsDNA in the sample*

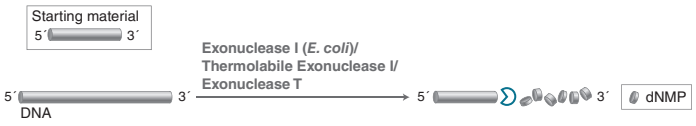
Description: Exonuclease T (Exo T), also known as RNase T, is a single-stranded RNA or DNA specific nuclease that requires a free 3´ terminus and removes nucleotides in the 3´→ 5´ direction. Exo T can be used to generate blunt ends from RNA or DNA having 3´ extensions.

Reaction Conditions: NEBuffer 4, 25°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to release 1 nmol of single dT nucleotides from 50 pmol of Fam-labeled polythymidine substrate in 30 minutes at 25°C.

Concentration: 5,000 units/ml

Note: Exonuclease T has a different activity on RNA vs. DNA. For RNA, 1 unit of Exonuclease T is required to completely digest 1.0 pmol of rA20 under standard reaction conditions as measured by gel electrophoresis.



Micrococcal Nuclease

#M0247S 320,000 gel units

- *Preparation of double-stranded DNA fragments with 5´-OH and 3´-phosphate*
- *Studies of chromatin structure*
- *Degradation of nucleic acids in crude cell-free extracts*
- *Preparation of rabbit reticulocyte*

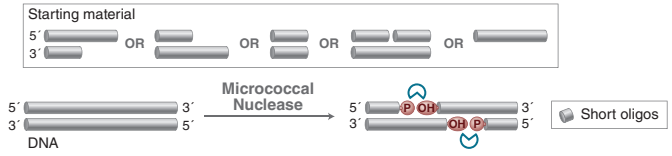
Description: Micrococcal Nuclease is a DNA and RNA endonuclease that degrades ds-and ss-DNA and RNA. Both DNA and RNA are degraded to 3´ phosphomononucleotides and dinucleotides.

Reaction Conditions: Micrococcal Nuclease Reaction Buffer, 37°C. Supplement with 100 µg/ml Recombinant Albumin, Molecular Biology Grade.

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in 15 minutes at 37°C, to the extent that the accumulation of low molecular DNA fragments is <400 base pairs as determined by agarose gel electrophoresis.

Concentration: 2,000,000 gel units/ml

Note: 1-5 mM Ca^{2+} is required for activity. The enzyme is active in the pH range 7-10, with optimal activity at 9.2, as long as salt concentration is less than 100 mM. Enzyme can be inactivated by addition of excess EGTA.



Msz Exonuclease I

#M0527S 1,000 units

- *Removal of single-stranded primers in PCR reactions prior to Sanger Sequencing or SNP analysis*
- *Removal of single-stranded primers for nested PCR reactions*
- *Removal of linear ssDNA, leaving behind dsDNA in the sample*

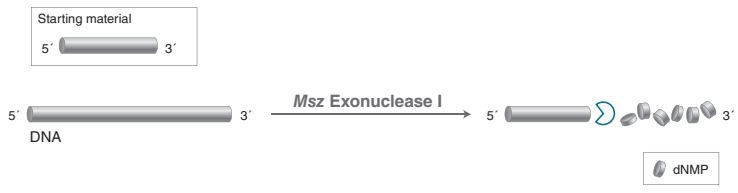
This is an **Enzyme for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. To view the full list, visit **www.neb.com/EnzymesforInnovation.**

Description: *Msz* Exonuclease I is a DNA specific exonuclease that catalyzes the removal of nucleotides from linear single-stranded DNA in the 3´ to 5´ direction, with optimal activity between 45°C and 60°C.

Reaction Conditions: rCutSmart Buffer, 55°C. Heat inactivation: 80°C for 1 minute.

Unit Definition: One unit of *Msz* Exonuclease I is defined as the amount of enzyme that will catalyze the release of 5 nmol of acid-soluble nucleotide in a total reaction volume of 100 µl in 15 minutes at 55°C in 1X rCutSmart Buffer.

Concentration: 10,000 units/ml



Mung Bean Nuclease

NEBuffer 3.0 | 30°

#M0250S 1,500 units
#M0250L 7,500 units

- *Removal of both 3´ and 5´ single-stranded overhangs from dsDNA to create blunt ends*
- *Cleavage of ssDNA and RNA*
- *Cleavage of the single-stranded region in a DNA hairpin*
- *Mapping of RNA transcripts*

Description: Mung Bean Nuclease is a single-strand specific DNA and RNA endonuclease which will degrade single-stranded extensions from the ends of DNA and RNA molecules, leaving blunt, ligatable ends.

Reaction Conditions: Mung Bean Nuclease Reaction Buffer, 30°C.

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of M13mp18 single-stranded DNA to fragments less than 1 kb in length in a total reaction volume of 80 µl in 1X Mung Bean Nuclease Reaction Buffer when incubated for 15 minutes at 37°C.

Concentration: 10,000 units/ml

Note: Do not attempt to heat inactivate, DNA will "breathe" before enzyme inactivates, causing undesirable degradation.

Nuclease P1

NEBuffer 3.0 | 37° | 75°

#M0660S 10,000 units

- *Conversion of ssDNA or RNA to 5´ mononucleotides*
- *Analysis of the base composition of nucleic acids*
- *Studies of the potential damage and modification of DNA*

Description: Nuclease P1 (from *P. citrinum*) is a zinc-dependent ssDNA or RNA specific endonuclease which hydrolyzes 3´→ 5´ phosphodiester bonds in RNA and ssDNA with no base specificity. Nuclease P1 also exhibits 3´-phosphomonoesterase activity.

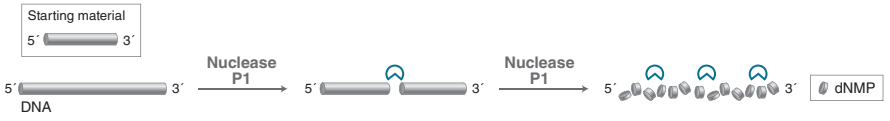
Although a single-strand specific nuclease, Nuclease P1 does display some activity toward double-stranded DNA (dsDNA) in Nuclease P1 Reaction Buffer. If preferentially degrading single-stranded nucleic acids (ssDNA or RNA) in the presence of dsDNA, we recommend using Nuclease P1 in 1X NEBuffer r1.1, to limit activity on dsDNA while maintaining single-strand nuclease activity.

Reaction Conditions: Nuclease P1 Reaction Buffer, 37°C. Heat inactivation: 75°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to liberate 1.0 µg of acid soluble nucleotides from Torula Yeast total RNA per min at 37°C in 1X Nuclease P1 Reaction Buffer.

Concentration: 100,000 units/ml

Note: Substrate specificity for Nuclease P1 is as follows: 3´ AMP > RNA > ssDNA >> dsDNA. The rate of hydrolysis of 2´-AMP is 3,000-fold less than that of 3´-AMP.



$RecJ_f$

NEBuffer 2 | 37° | 65°

#M0264S 1,000 units
#M0264L 5,000 units

- *Degradation of single-stranded DNA from the 5´-end*
- *Removal of 5´ protruding single-stranded termini at the ends of linear dsDNA (blunt ends are not exclusively created)*

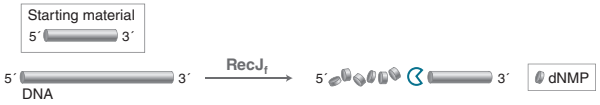
Description: $RecJ_f$ is a ssDNA-specific exonuclease that catalyzes the removal of nucleotides from linear ssDNA in the 5´ → 3´ direction. The preferred substrate is dsDNA with 5´ single-stranded overhangs > 6 nucleotides long.

DNA substrate containing a 22 base 5´ extension results in products that are a mixture of DNA fragments that have blunt-ends, 5´ extensions (1–5 nucleotides) and recessed 5´ ends (1–8 nucleotides). $RecJ_f$ does not require a 5´ phosphate.

Reaction Conditions: NEBuffer 2, 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 0.05 nmol TCA soluble deoxyribonucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X NEBuffer 2 with 1.5 µg sonicated single-stranded [^{3}H]-labeled *E. coli* DNA.

Concentration: 30,000 units/ml



rCutSmart | NEB r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | rAlbumin Recombinant Albumin | SAM Requires SAM | 37° Incubation Temperature | 65° Heat Inactivation

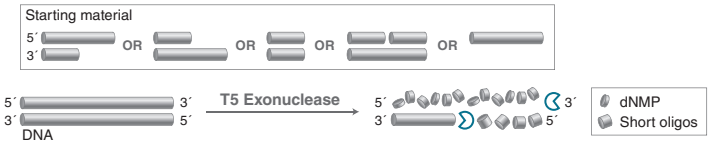
T5 Exonuclease

#M0663S 1,000 units
#M0663L 5,000 units

- *Removal of incomplete ligation products from ligated circular dsDNA*
- *Degradation of denatured DNA from alkaline-based plasmid purification methods for improved DNA cloning*
- *Degradation of contaminating linear and nicked DNA in plasmid samples*

GMP-grade reagent now available.
See page 6 for details.

Description: T5 Exonuclease is a dsDNA-specific exonuclease and ssDNA endonuclease. It initiates at the 5´ termini of linear or nicked dsDNA, and cleaves in the 5´→ 3´ direction. T5 Exonuclease is able to initiate nucleotide removal from the 5´ termini or at gaps and nicks of linear or circular dsDNA. However, the enzyme does not degrade supercoiled dsDNA.



Reaction Conditions: NEBuffer 4, 37°C.

Unit Definition: 1 unit of T5 Exonuclease is defined as the amount of enzyme required to cause the change of 0.00032 A260 nm/min at 37° C in rCutSmart Buffer.

Concentration: 10,000 units/ml

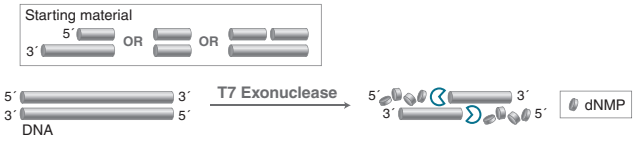
T7 Exonuclease

#M0263S 1,000 units
#M0263L 5,000 units

- *Site-directed mutagenesis*
- *Nick-site extension*

Description: T7 Exonuclease is a dsDNA specific exonuclease that catalyzes removal of nucleotides from linear or nicked dsDNA in the 5´ to 3´ direction. It initiates at the 5´ termini or at gaps and nicks of double-stranded DNA. It will degrade both 5´ phosphorylated or 5´ dephosphorylated DNA. It has also been reported to degrade RNA and DNA from RNA/DNA hybrids in the 5´ to 3´ direction, but it is unable to degrade either ds- or ssRNA.

Reaction Conditions: NEBuffer 4, 25°C.



Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X NEBuffer 4 with 0.15 mM sonicated duplex [^{3}H]-DNA.

Concentration: 10,000 units/ml

Note: Phosphorothiate (PT) bond is resistant to cleavage.

Nucleoside Digestion Mix

#M0649S 50 reactions

- *Convenient one-step protocol*
- *Digests both DNA and RNA to single nucleosides*
- *Low-glycerol formulation significantly reduces glycerol-induced ion suppression during MS analysis*

Description: The Nucleoside Digestion Mix is a mixture of enzymes that provides a convenient one-step method to generate single nucleosides from DNA or RNA. Optimized for quantitative analysis by liquid chromatography-mass spectrometry (LC-MS), this reagent eliminates the need for sequential multi-step, time-consuming digestion protocols. The Nucleoside Digestion Mix digests ssDNA, dsDNA, DNA/RNA hybrids and RNA (except mRNA cap structures) containing epigenetically modified (m5C, hm5C, f5C, ca5C, m4C, m6A, etc.), unnatural, or damaged bases. Moreover, the low-glycerol formulation (< 1%) significantly reduces glycerol-induced ion suppression during mass spectrometry analysis.

Reaction Conditions: Nucleoside Digestion Mix Reaction Buffer, 37°C.

DNA Repair Enzymes and Structure-specific Endonucleases: Properties

NEB carries an array of reliable DNA repair enzymes, for use in multiple applications.

Enzyme	Major Substrate [1,2]	Cleavage Site	Product(s) Produced	Termini Created From Cleavage		Major Activity	Thermostable
				5´-Terminus	3´-Terminus		
APE 1	AP site	1st phosphodiester bond 5´ to AP site	1 nt gap	dR5P	OH	Endonuclease	
Authenticase	Cruciform, mismatches, Holliday junctions, mismatches except G/A	Phosphodiester bond 5´ and/or 3´ to structure	5´ and/or 3´ overhang	P	OH	Endonuclease	
Mismatch Endonuclease I	T:T, G:G and G:T mismatches in dsDNA	3rd phosphodiester bond on the 5´ side of the mismatched base in both strands	5 bp overhang	P	OH	Endonuclease	
T7 Endo I	Cruciforms, mismatches, Holliday junctions, across DNA nicks	Phosphodiester bond 5´ to structure	Nick	P	OH	Endonuclease	
Endo III	AP site, damaged pyrimidines, Tg	N-glycosidic bond, 1st phosphodiester bond 3´ to AP site	1nt gap	P	PA	Glycosylase & AP lyase	
Endo IV	AP site	1st phosphodiester bond 5´ to AP site	1nt gap	dR5P	OH	Endonuclease	
Tth Endo IV	AP site	1st phosphodiester bond 5´ to AP site	1nt gap	dR5P	OH	Endonuclease	Yes
Endo V	dI[4], dU, AP site	2nd phosphodiester bond 3´ to dI	Nick	P	OH	Endonuclease	
T4 PDG	CPD, AP site	N-glycosidic bond, phosphodiester bond 3´ to AP site	AP site, 1nt gap	P[6]		Glycosylase & AP lyase	
Endo VIII	AP site[4]	Phosphodiester bond 3´ and 5´ to AP site	1nt gap	P	P	AP lyase	
Thermostable Endo Q	dU, dI, dX, AP site[4]	1st phosphodiester bond 5´ to modified nucleobase	Nick	P	OH	Endonuclease	Yes
Thermostable FEN1	5´ DNA flap[3]	Phosphodiester bond at base of flap	Nick	P	OH (on flap)	Endonuclease	Yes
Fpg	8-oxoG, oxidized purines	N-glycosidic bond, phosphodiester bond 3´ and 5´ to AP site	AP site, 1nt gap	P	P	Glycosylase & AP lyase	
hAAG	3mA, 7mG, dI, dX	N-glycosidic bond	AP site	N/A	N/A	Glycosylase	
hSMUG1	dU4, 5-hmU, 5-hoU, 5fU	N-glycosidic bond	AP site	N/A	N/A	Glycosylase	
Thermostable OGG	8-oxoG	N-glycosidic bond, phosphodiester bond 3´ and 5´ to AP site	AP site, 1nt gap	P	P	Glycosylase	Yes
RNaseHII	rN in dsDNA	phosphodiester bond 5´ to ribo	Nick	P	OH	Endonuclease	
UDG	dU[4]	N-glycosidic bond	AP site	N/A	N/A	Glycosylase	
Afu UDG	dU[4]	N-glycosidic bond	AP site	N/A	N/A	Glycosylase	Yes
WarmStart Afu UDG	dU[4]	N-glycosidic bond	AP site	N/A	N/A	Glycosylase	Yes
Antarctic Thermolabile UDG[5]	dU[4]	N-glycosidic bond	AP site	N/A	N/A	Glycosylase	
USER Enzyme	dU	N-glycosidic bond; phosphodiester bond 3´ & 5´ to AP site	1 nt gap	P	P	Glycosylase & AP lyase	No
Thermolabile USER II	dU	N-glycosidic bond; phosphodiester bond 3´ & 5´ to AP site	1 nt gap	P	PA	Glycosylase & AP lyase	No
Thermostable USER III	dU	N-glycosidic bond; phosphodiester bond 3´ & 5´ to AP site	1 nt gap	dR5P	OH	Glycosylase & AP lyase	Yes

Table Legend:

3mA	3-methyladenine
5fU	5-formyluridine
5-hmU	5-hydroxymethyluridine
5-hoU	5-hydroxyuridine
7mG	7-methylguanine
8-oxoG	8-oxo-7, 8-dihydroguanine
AP	apurinic/apyrimidinic sites
CPDs	Cyclobutane pyrimidine dimers
dI	deoxyinosine
dR5P	deoxyribose-5´-phosphate
dU	deoxyuridine
dX	deoxyxanthosine
NA	not applicable
OH	Hydroxyl
P	Phosphate
PA	3´ phospho-a, b-unsaturated aldehyde
rN	ribonucleotides
Tg	Thymine Glycol

Footnotes:

(1) Activity is on dsDNA unless noted otherwise.
(2) Minor activies, substrates, and references can be found at www.neb.com.
(3) 5´ flaps of 1-40 nt in length have been confirmed substrates.
(4) Enzyme has robust activity on ssDNA in addition to dsDNA.
(5) Antarctic Thermolabile UDG can be heat inactivated.
(6) CPD still covalently attached.

What are endonucleases and their applications?

DNA Repair Enzymes on Damaged and Non-standard Bases

NEB carries an array of endonucleases and glycosylases for Base-excision repair (BER) for use in multiple applications. The following table indicates the level of repair on either double-stranded or single-stranded DNA oligos for various damaged and non-standard bases.

Double-stranded DNA Oligos (34-mers)

Repair Enzyme	Ap:A	Dht:A	5-Hmu:A	5-Hmu:G	I:T	6-Mea:T	8-Og:C	8-Og:G	U:A	U:G	X:G	Thymine Glycol:A
APE 1	++++	+	-	-	-	-	-	-	-	-	-	-
Endo III	++++	+	-	-	-	-	-	-	-	-	-	-
Endo IV	++++	+	-	-	-	-	-	-	-	-	-	-
Tth Endo IV	++++	+	-	-	-	-	-	+	-	-	-	-
Endo V*	+++	+	+	+	++++	+	++	+	+	++++	-	++
T4 PDG	++++	-	-	-	-	-	-	-	-	-	-	-
Endo VIII	++++	++	-	-	-	-	-	-	-	-	-	++++
Thermostable EndoQ	+++	-	-	-	+++	-	-	-	++++	++++	++++	-
Fpg	+	+	-	-	-	-	++++	++++	-	-	-	+
hAAG	-	-	-	-	++++	-	-	-	-	-	++	-
hSMUG1	N/A	-	+++	+++	-	-	-	-	++++	++++	-	-
Thermostable OGG**	-	-	-	-	-	-	++++	++++	-	-	-	-
UDG	N/A	-	-	-	-	-	-	-	++++	+	-	-
Afu UDG	N/A	-	-	-	-	-	-	-	++++	+	-	-
Antarctic Thermolabile UDG	N/A	-	-	-	-	-	-	-	++++	+	-	-
WarmStart® Afu Uracil-DNA Glycosylase (UDG)	N/A	-	-	-	-	-	-	-	++++	+	-	-

Standard reaction conditions were used to titer the enzymes with the alternate base

* Nicks only, does not remove damage

** Tested on 60-mer

Single-stranded DNA Oligos (34-mers)

Repair Enzyme	Ap	Dht	5-Hmu	I	6-Mea	8-Og	U	X
APE 1	++	-	-	-	-	-	-	-
Endo III	++	-	-	-	-	-	-	-
Endo IV	-	-	-	-	-	-	-	-
Tth Endo IV	-	-	-	-	-	-	-	-
Endo V	+	-	-	++++	-	+	-	-
T4 PDG	-	-	-	-	-	-	-	-
Endo VIII	+++	-	-	-	-	-	-	-
Thermostable EndoQ	++++	-	-	++++	-	-	++++	++++
Fpg	+	+	-	-	-	++++	-	-
hAAG	-	-	-	+	-	-	-	-
hSMUG1	N/A	-	++	-	-	-	+++	-
Thermostable OGG	N/A	N/A	N/A	N/A	N/A	+	N/A	N/A
UDG	N/A	-	-	-	-	-	++++	-
Afu UDG	N/A	-	-	-	-	-	++++	-
Antarctic Thermolabile UDG	N/A	-	-	-	-	-	++++	-
WarmStart Afu Uracil-DNA Glycosylase (UDG)	N/A	-	-	-	-	-	++++	-

Table Legend:

AP apurinic/apyrimidinic site. The AP site is created by treating a uracil containing oligo with UDG.

DHT 5,6-dihydrothymine

5-hmU 5-hydroxymethyluracil

I Inosine

6-MeA 6-methyladenine

8-OG 8-oxoguanine

U uridine

AP:A apurinic/apyrimidinic site base paired with adenine

DHT:A 5,6 dihydrothymine base paired with an adenine

5-hmU:A 5-hydroxymethyluracil base paired with an adenine

5-hmU:G 5-hydroxymethyluracil base paired with a guanine

I:T inosine base paired with a thymine

6-MeA:T 6-methyladenine base paired with a thymine

8-OG:C 8-oxoguanine base paired with a cytosine

8-OG:G 8-oxoguanine base paired with a guanine

NT: not tested

U:A uridine base paired with an adenine

U:G uridine base paired with a guanine

X: xanthine

X:G xanthine base paired with a guanine

Level of Repair:

++++	100%
+++	50%
++	10% –25%
+	<10%
–	no detectable enzyme activity (<0.7%) Some data were based on oligo data and visualization on a gel using ethidium bromide staining. Depending on the reaction conditions and sensitivity of detection, results may vary. Please be aware that star-activity (non-specific cleavage) may occur if enzyme is in excess.

N/A: not applicable

APE 1

#M0282S 1,000 units
#M0282L 5,000 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*
- *Modified nick translation*

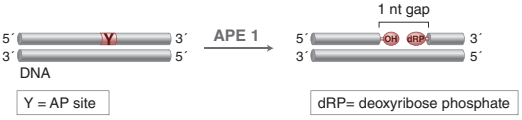
Description: Human apurinic/apyrimidinic (AP) endonuclease, APE 1, also known as HAP 1 or Ref-1, shares homology with *E. coli* Exonuclease III. APE 1 catalyzes the cleavage of DNA phosphodiester backbone at AP sites via hydrolysis leaving a 1 nucleotide gap with 3´-hydroxyl and 5´-deoxyribose phosphate termini. APE 1 has also been reported to have weak DNA 3´-diesterase, 3´ to 5´ exonuclease and RNase H activities.

Reaction Conditions: NEBuffer 4, 37°C.
Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 20 pmol of a 34 mer oligonucleotide duplex containing a single AP site* in a total reaction volume of 10 µl in 1 hour at 37°C.

*An AP site is created by treating 20 pmol of a 34 mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C.

Concentration: 10,000 units/ml



NEW

Authenticase®

#M0689S 25 reactions
#M0689L 125 reactions

Companion Product:

Mismatch Endonuclease I
#M0678S 4,000 units

- *Recognizes single base mismatches: C/C, T/C, A/C, T/G, G/G, T/T and A/A*
- *Error-correction in oligonucleotide synthesis*
- *Mismatch Detection Assay*

Description: Authenticase is a mixture of structure-specific nucleases capable of recognizing and cleaving outside mismatch and indel regions from 1-10 bp on double-stranded DNA. The formulation has limited non-specific activity on homoduplex regions of DNA. Authenticase can be used as an error-correction reagent in oligo-based PCR gene assembly by enzymatically removing mistakes prior to the final renaturation and amplification step (i.e. removes mismatch/indel errors caused by oligonucleotide synthesis). Alternatively, Authenticase can replace T7 Endonuclease I in the mismatch detection assay used to assess the efficiency of genome editing.

Reaction Conditions: Authenticase Reaction Buffer, 42°C.

Mismatch Endonuclease I

#M0678S 4,000 units

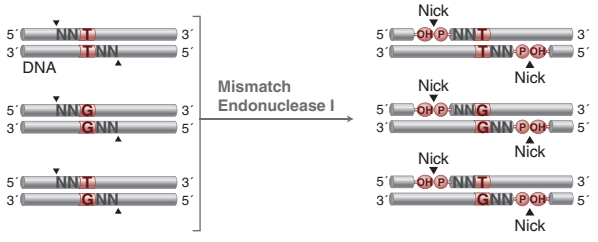
- *Catalyzes the cleavage of some DNA mismatches (T:T, G:G and G:T)*

Description: Mismatch Endonuclease I is a Mg^{2+} dependent DNA endonuclease that specifically cleaves mismatched base pairs (T:T, G:G and T:G mismatches). Mismatch Endonuclease I cleaves the 3rd phosphodiester bond on the 5´ side of the mismatched base in both strands, leaving a 5-base pair overhang. While Mismatch Endonuclease I prefers the cleave T:T, G:G and T:G mismatches, it will also readily cleave T:I, G:I and G:U DNA mismatches.

Reaction Conditions: NEBuffer r2.1, 37°C.
Heat inactivation: 70°C for 5 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 50% of 0.2 pmol of a fluorescently labeled 60mer oligonucleotide duplex containing a single T:T mismatch in 30 minutes at 37°C in a total reaction volume of 20 µl in 1X NEBuffer r2.1.

Concentration: 80,000 units/ml



rCutSmart NEB r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | rAlbumin Recombinant Albumin | SAM Requires SAM | 37° Incubation Temperature | 65° Heat Inactivation

T7 Endonuclease I

#M0302S 250 units
#M0302L 1,250 units

- *Recognition of mismatched DNA*
- *Resolve four-way junction or branched DNA*
- *Detection or cleavage of heteroduplex and nicked DNA*
- *Random cleavage of linear DNA for shotgun cloning*
- *Key enzyme for genome editing mutation detection*
- *Also available: EnGen® Mutation Detection Kit (NEB #E3321)*

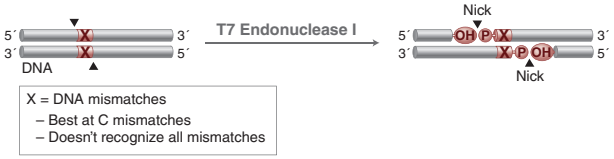
Description: T7 Endonuclease I is a DNA endonuclease that catalyzes the cleavage of DNA mismatches and non-β DNA structures, including Holliday junctions and cruciform, leaving 3´-OH and 5´ phosphate. It is best at C mismatches and does not recognize all DNA mismatches, and to a lesser extent cleaves across a nick in dsDNA.

Reaction Conditions: NEBuffer 2, 37°C.

Unit Definition: One unit is defined as the amount of enzyme required to convert > 90% of 1 µg of supercoiled cruciform pUC(AT) to > 90% linear form in a total reaction volume of 50 µl in 1 hour at 37°C.

Concentration: 10,000 units/ml

Note: It is important to control the amount of enzyme and the reaction time used for cleavage of a particular substrate. Temperatures above 42°C cause an increase in nonspecific nuclease activity and should be avoided.



Endonuclease III (Nth)

#M0268S 1,000 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

Description: Endonuclease III (Nth) protein from *E. coli* acts both as a *N*-glycosylase and an AP-lyase. The *N*-glycosylase activity releases damaged pyrimidines, including thymine glycol and 5, 6-dihydroxythymine, generating an AP site. The AP lyase activity cleaves an AP site via β-elimination, creating a 1-nucleotide gap with 3´-α, β-unsaturated aldehyde and 5´-phosphate termini.

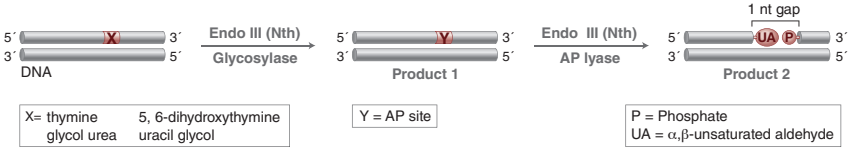
Some of the damaged bases recognized and removed by Endonuclease III (Nth) include urea, 5, 6 dihydroxythymine, thymine glycol, 5-hydroxy-5 methylhydanton, uracil glycol, 6-hydroxy-5, 6-dihdrothimine and methyltartronylurea.

Reaction Conditions: Endonuclease III (Nth) Reaction Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34 mer oligonucleotide duplex containing a single AP site* in a total reaction volume of 10 µl in 1 hour at 37°C in 1X Endonuclease III Reaction Buffer containing 10 pmol of fluorescently labeled oligonucleotide duplex.

* An AP site is created by treating 10 pmol of a 34 mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C.

Concentration: 10,000 units/ml



Endonuclease IV

#M0304S 1,000 units
#M0304L 5,000 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

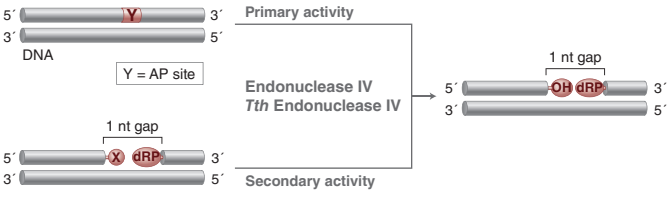
Description: Endonuclease IV can act on several types of oxidative damage in DNA. The enzyme is an apurinic/apyrimidinic (AP) endonuclease that catalyzes the cleavage of DNA phosphodiester backbone at AP sites via hydrolysis leaving a 1 nucleotide gap with 3´-hydroxyl and 5´ deoxyribose phosphate (dRP) termini. The enzyme has 3´-diesterase activity which can remove 3´ phosphate, 3´-α, β-unsaturated aldehyde, phosphoglycoaldehyde, and other 3´ blocking groups.

Reaction Conditions: NEBuffer 3, 37°C. Heat inactivation: 85°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34-mer oligonucleotide duplex containing a single AP site* in a total reaction volume of 10 µl in 1 hour at 37°C.

*An AP site is created by treating 10 pmol of a 34 mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C.

Concentration: 10,000 units/ml



Tth Endonuclease IV

#M0294S 500 units

- *Thermostable*
- *Alkaline elution*
- *Alkaline unwinding*

Description: *Tth* Endonuclease IV is a thermostable apurinic/apyrimidinic (AP) endonuclease that catalyzes the cleavage of DNA phosphodiester backbone at AP sites via hydrolysis, leaving a 1 nucleotide gap with 3´-hydroxyl and 5´ deoxyribose phosphate (dRP) termini. The enzyme also has a 3´-diesterase activity that can remove 3´ phosphate, 3´-α, β-unsaturated aldehyde, phosphoglycoaldehyde, and other 3´ blocking groups.

Applications:

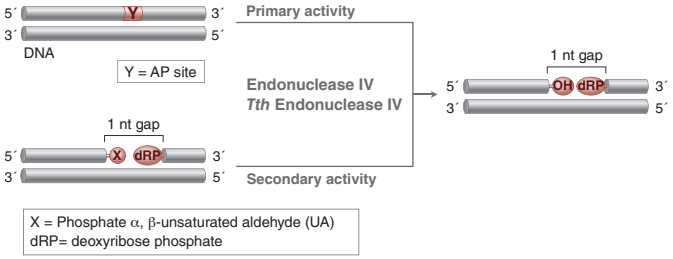
- Alkaline elution (1)
- Alkaline unwinding (2)

Reaction Conditions: ThermoPol Reaction Buffer, 65°C.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 60-mer oligonucleotide duplex containing a single AP site* in a total reaction volume of 10 µl in 1 hour at 65°C.

* An AP site is created by treating 10 pmol of a 60-mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C.

Concentration: 10,000 units/ml



NEW

Thermostable Endonuclease Q

#M0701S 50 units

- *Enzymatic DNA synthesis (cleaving oligos from solid supports)*
- *Cleavage of deaminated DNA*
- *Active on both ssDNA and dsDNA, with a preference for ssDNA*

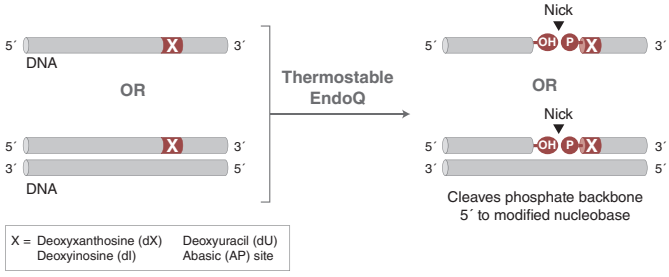
Description: Thermostable Endonuclease Q (EndoQ) is an archaeal DNA endonuclease that cleaves the phosphate backbone of a DNA substrate 5´ to the position of the modified nucleobases of deoxyxanthosine (dX), deoxyinosine (dI), deoxyuracil (dU), and an abasic (AP) site. The result is a 3´-OH and a 5´-phosphate.

Reaction Conditions: NEBuffer r2.1, 65°C.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 60-mer oligonucleotide ssDNA containing a single deoxyxanthosine site* in a total reaction volume of 20 µl after 1 hour at 65°C.

*A deoxyxanthosine site is synthetically prepared with dX at the 24th position of a 5´ FAM-labelled 60-mer ssDNA oligo.

Concentration: 1,000 units/ml



rCutSmart NEB r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | rAlbumin Recombinant Albumin | SAM Requires SAM | 37° Incubation Temperature | 65° Heat Inactivation

Endonuclease V

NEB4 RR 37° 65°

#M0305S 250 units

- *Cleavage of oligonucleotides containing deoxyinosines*
- *Mismatch cleavage*

Description: Endonuclease V is a repair enzyme found in *E. coli* that recognizes deoxyinosine, a deamination product of deoxyadenosine in DNA. Endonuclease V, often called Deoxyinosine 3´ Endonuclease, recognizes DNA containing deoxyinosines (paired or not) on dsDNA, ssDNA with deoxyinosines and, to a lesser degree, DNA containing abasic sites (AP) or urea, base mismatches, insertion/deletion mismatches, hairpin or unpaired loops, flaps and pseudo-Y structures.

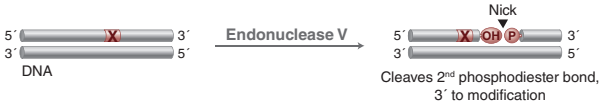
Endonuclease V catalyzes cleavage of the second phosphodiester bond 3´ to the mismatch of deoxyinosine, leaving a nick with 3´-hydroxyl and 5´-phosphate.

Reaction Conditions: NEBuffer 4, 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34 mer oligonucleotide duplex containing a single deoxyinosine site* in a total reaction volume of 10 µl in 1 hour at 37°C.

* A deoxyinosine site is synthetically prepared with a dI in the middle of one strand of a 34 mer oligonucleotide duplex.

Concentration: 10,000 units/ml



X =	Major	Minor		
	Deoxyinosine	AP site	Mismatches	Minor flaps
		Urea	Hairpin	Pseudo-Y

P= Phosphate

T4 PDG (T4 Endonuclease V)

NEBU RR 37° 70 BSA

#M0308S 2,000 units

- *DNA damage studies*
- *Single-cell gel electrophoresis (Comet assay)*

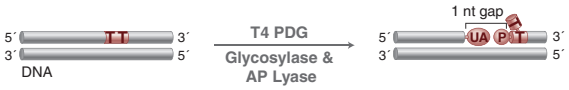
Description: T4 PDG (pyrimidine dimer glycosylase) has both DNA glycosylase and AP-lyase activity. The *N*-glycosylase activity releases cis-syn cyclobutane pyrimidine dimers, including T^T, T^C and C^C, generating an AP site. The AP lyase activity cleaves an AP site via β-elimination, creating a 1 nucleotide DNA gap with 3´-α, β-unsaturated aldehyde and 5´-phosphate termini.

Reaction Conditions: T4 PDG Reaction Buffer, 37°C. Supplement with 100 µg/ml Recombinant Albumin, Molecular Biology Grade.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the conversion of 0.5 µg of UV irradiated supercoiled pUC19 DNA to > 95% nicked plasmid in a total reaction volume of 20 µl in 30 minutes at 37°C. Nicking is assessed by agarose gel electrophoresis. Irradiated plasmid contains an average of 3-5 pyrimidine dimers.

Concentration: 10,000 units/ml

Note: For best results, incubation time should be 30 minutes or less.



TT = cyclobutane pyrimidine dimers

Endonuclease VIII

NEBU RR 37° 75°

#M0299S 1,000 units
#M0299L 5,000 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

Description: Endonuclease VIII acts as both an *N*-glycosylase and an AP-lyase. The *N*-glycosylase activity releases damaged pyrimidines, including thymine glycol and uracil glycol. The AP lyase activity cleaves DNA phosphodiester backbone at AP sites via β and δ-elimination, creating a 1 nucleotide DNA gap with 5´ and 3´ phosphate termini.

Damaged bases recognized and removed by Endonuclease VIII include urea, 5, 6- dihydroxythymine, thymine glycol, 5-hydroxy-5- methylhydanton, uracil glycol, 6-hydroxy-5, 6-dihydrothymine and methyltartronylurea. While Endonuclease VIII is similar to Endonuclease III, Endonuclease VIII has β and δ lyase activity while Endonuclease III has β lyase activity.

Reaction Conditions: Endonuclease VIII Reaction Buffer, 37°C. Heat inactivation: 75°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34 mer oligonucleotide duplex containing a single AP site* in a total reaction volume of 10 µl in 1 hour at 37°C in 1X Endonuclease VIII Reaction Buffer containing 10 pmol of fluorescently labeled oligonucleotide duplex.

* An AP site is created by treating 10 pmol of a 34 mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C.

Concentration: 10,000 units/ml

X=		
	urea	5, 6-dihydroxythymine
	thymine glycol	5-hydroxy-5-methylhydanton
	uracil glycol	6-hydroxy-5,6-dihydrothymine
	methyltartronylurea	

Y = AP site

P = phosphate

Fpg

NEB1 RR 37° 60° BSA

#M0240S 500 units
#M0240L 2,500 units

- *Single-cell electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

Description: Fpg (formamidopyrimidine [fapy]-DNA glycosylase), also known as 8-oxoguanine DNA glycosylase, acts both as an *N*-glycosylase and an AP-lyase. *N*-glycosylase activity releases damaged purines, including 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) and 8-oxo-7,8-dihydroguanine (8-oxoG), generating an AP site. The AP lyase activity cleaves an AP site, via β and δ-elimination, creating a 1 nucleotide DNA gap with 5´ and 3´ phosphate termini.

Some of the damaged bases recognized and removed by Fpg include 7, 8-dihydro-8-oxoguanine (8-oxoguanine), 8-oxoadenine, fapy-guanine, methy-fapy-guanine, fapy-adenine, aflatoxin B1-fapy-guanine, 5-hydroxy-cytosine and 5-hydroxy-uracil.

Reaction Conditions: NEBuffer 1, 37°C. Supplement with 100 µg/ml Purified BSA or rAlbumin. Heat inactivation: 60°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 10 pmol of a 34-mer oligonucleotide duplex containing a single 8-oxoguanine base paired with a cytosine in a total reaction volume of 10 µl in 1 hour at 37°C.

Concentration: 8,000 units/ml

X = 8-oxoguanine damaged purines

Y = AP site

hAAG

NEBU RR 37° 65°

#M0313S 500 units

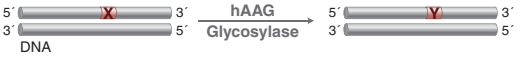
- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

Description: Human Alkyladenine DNA Glycosylase (hAAG) excises alkylated and oxidative DNA damaged sites, including 3-methyladenine, 7-methylguanine, 1,N^6-ethenoadenine and hypoxanthine. hAAG catalyzes the hydrolysis of the *N*-glycosidic bond to release the damaged base. hAAG is also known as methylpurine DNA glycosylase (MPG) or 3-methyladenine-DNA glycosylase (ANPG).

Reaction Conditions: ThermoPol Reaction Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to create an AP site from 1 pmol of a 34-mer oligonucleotide duplex containing a single deoxyinosine site in a total reaction volume of 10 µl in 1 hour at 37°C.

Concentration: 10,000 units/ml



X = 3mA, 7mG, dI, dX

Y = AP site

hSMUG1

NEB1 RR 37° 65° rAlbumin

#M0336S 500 units

- *Oxidative DNA damage studies*
- *Single-cell gel electrophoresis (Comet assay)*

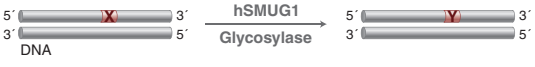
Description: Human single-strand-selective monofunctional uracil-DNA Glycosylase (hSMUG1) excises deoxyuracil and deoxyuracil-derivatives bearing an oxidized group at C5, such as 5-hydroxyuracil, 5-hydroxymethyluracil and 5-formyluracil in ssDNA and dsDNA. Major substrates include uracil, 5-hydroxyuracil, 5-hydroxymethyluracil, and 5-formyluracil.

Reaction Conditions: NEBuffer 1, 37°C. Supplement with 100 µg/ml Recombinant Albumin, Molecular Biology Grade. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to excise 1 pmol of deoxyuracil from a 34 mer oligonucleotide duplex containing a single dU site in a total reaction volume of 10 µl in 1 hour at 37°C.

Concentration: 5,000 units/ml

Note: hSMUG1 has 50% activity on 5-hydroxymethyluracil when compared to uracil. hSMUG1 has 50% activity on ssDNA compared to dsDNA.



X = dU and dU derivatives

Y = AP site

rCutSmart NEB r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | rAlbumin Recombinant Albumin | SAM Requires SAM | 37° Incubation Temperature | 65° Heat Inactivation

Thermostable FEN1

#M0645S 1,600 units

This is an **Enzyme for Innovation (EFI)** graduate. To learn more, visit **www.neb.com/EnzymesforInnovation.**

Description: Thermostable Flap Endonuclease I, FEN1, is a thermostable DNA and RNA endonuclease that catalyzes the cleavage of 5´ DNA flaps from branched dsDNA substrates, creating a 5´ phosphate terminus. FEN1 products can be ligated by DNA ligase to create dsDNA.

Reaction Conditions: ThermoPol Reaction Buffer, 65°C.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 10 pmol of 5´ flap containing oligonucleotide substrate in a total reaction volume of 10 µl for 10 min at 65°C.

Concentration: 32,000 units/ml

NEW

Thermostable OGG

#M0464S 500 units

Companion Product:

Fpg
#M0240S 500 units
#M0240L 2,500 units

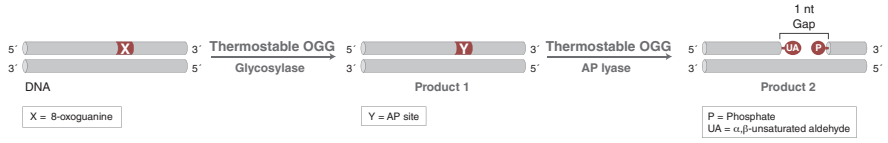
- *Thermostable oxoguanine glycosylase*
- *Bifunctional DNA glycosylase with DNA N-glycosylase and AP lyase activities*

Description: Thermostable OGG is an archaeal 8-oxoguanine (8-oxoG) DNA glycosylase which acts both as a *N*-glycosylase and an AP-lyase. The N-glycosylase activity releases the damaged purine (8-oxoguanine) from double-stranded DNA, generating an apurinic (AP) site. The AP-lyase activity cleaves 3´ to the AP site leaving a 5´ phosphate and a 3´-phospho-α, β-unsaturated aldehyde. Unlike some other DNA glycosylases, Thermostable OGG specifically recognizes and cleaves only 8-oxoG and no other modified bases.

Reaction Conditions: rCutSmart Buffer, 65°C.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 10 pmol of a 60-mer fluorescently labeled oligonucleotide duplex containing a single 8-oxoguanine base paired with a cytosine in a total reaction volume of 10 µl in 1 hour at 65°C.

Concentration: 8,000 units/ml



Edwin joined NEB in 2022 as a Research Scientist I in the Protein Research Mass Spectrometry Facility. He is a member of the Enzymology, RNA and Running Clubs at NEB.

NEW

Uracil DNA Glycosylase (UDG)

NEW

Afu Uracil DNA Glycosylase (UDG)

NEW

Warmstart *Afu* Uracil DNA Glycosylase

Antarctic Thermolabile (UDG)

Uracil-DNA Glycosylase (UDG)
#M0280S 1,000 units
#M0280L 5,000 units

Afu Uracil-DNA Glycosylase (UDG)
#M0279S 200 units

WarmStart *Afu* Uracil-DNA Glycosylase (UDG)
#M1282S 200 units

Antarctic Thermolabile UDG
#M0372S 100 units
#M0372L 500 units

Companion Product:

Uracil Glycosylase Inhibitor (UGI)
#M0281S 200 units
#M0281L 1,000 units

- *Eliminates PCR carry-over contamination*
- *Release of uracil from ss- or ds-DNA*

Description: *E. coli* Uracil-DNA Glycosylase (UDG) catalyses the release of free uracil from uracil-containing DNA. UDG efficiently hydrolyzes uracil from single-stranded or double-stranded DNA, but not from oligomers (6 or fewer bases).

Afu Uracil-DNA Glycosylase (UDG) is a thermostable homolog of the *E. coli* Uracil-DNA Glycosylase (UDG) from *Archaeoglobus fulgidus*.

WarmStart *Afu* Uracil-DNA Glycosylase (UDG) from *Archaeoglobus fulgidus* is formulated with a reversibly-bound aptamer which inhibits its activity at temperatures below 42°C.

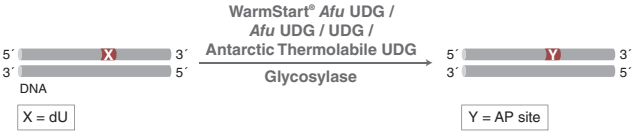
Antarctic Thermolabile UDG is sensitive to heat and can be rapidly and completely inactivated at temperatures above 50°C.

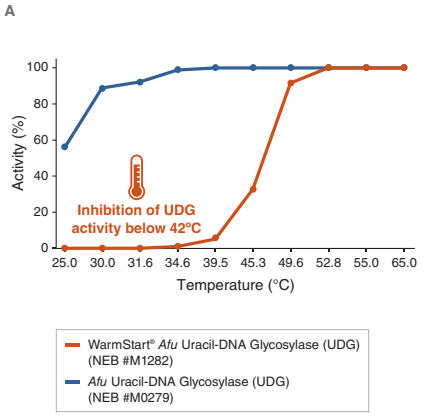
Reaction Conditions: Uracil-DNA Glycosylase (UDG): UDG Reaction Buffer, 37°C. *Afu* Uracil-DNA Glycosylase (UDG) & WarmStart *Afu* Uracil-DNA Glycosylase (UDG): ThermoPol II (Mg-free) Reaction Buffer, 65°C. Antarctic Thermolabile UDG: Standard *Taq* Reaction Buffer, 37°C, heat inactivation: 50°C for 5 minutes.

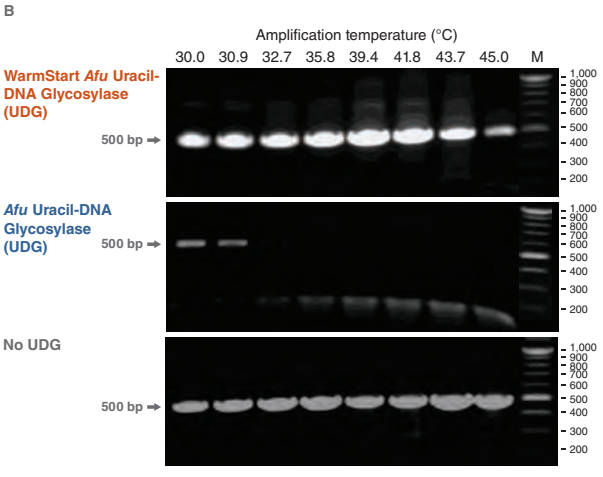
Unit Definition: One unit is defined as the amount of enzyme required to release 60 pmol per minute of a fluorescently-labeled 47-mer single-stranded DNA oligonucleotide containing a single uracil base in 30 minutes. *E. coli* UDG is incubated at 37°C in a total reaction volume of 50 µl in 1X UDG Buffer. *Afu* UDG and WarmStart *Afu* UDG are incubated at 65°C in a total reaction volume of 50 µl in 1X Thermopol II Buffer. Antarctic Thermolabile UDG is incubated at at 30°C in a total reaction volume of 50 µl in 1X Standard Taq Reaction Buffer.

Concentration: *E. coli* UDG: 5,000 units/ml. *Afu* UDG, WarmStart *Afu* UDG: 2,000 units/ml. Antarctic Thermolabile UDG: 1,000 units/ml.

Note: UDG is active over a broad pH range, with an optimum pH 8.0, does not require divalent cation and is inhibited by high ionic strength (>200 mM). *Afu* UDG retains 50% activity in the presence of 150 mM NaCl. *Afu* UDG retains less than 1% activity after boiling for 30 minutes in standard reaction conditions. Under standard reaction conditions, uracil glycosylase inhibitor (UGI) does not inhibit *Afu* UDG.







WarmStart Afu Uracil-DNA Glycosylase (UDG) displays no detectable activity at temperatures below 42ºC. **A.** *Temperature profile comparison of the UDG activity of Afu Uracil-DNA Glycosylase (UDG) (NEB #M0279) and WarmStart Afu Uracil-DNA Glycosylase (UDG) (NEB #M1282) illustrates the WarmStart inhibition of UDG activity below 42ºC. UDG activity was measured by incubating 0.002 U of either Afu Uracil-DNA Glycosylase (UDG) or WarmStart Afu Uracil-DNA Glycosylase (UDG) with 1.8 pmol of a fluorescently labeled 47-mer single-stranded DNA oligonucleotide containing a single uracil base in 30 minutes at various temperatures ranging from 25-65ºC. Following uracil excision, generated abasic sites were chemically cleaved (1M NaOH, 85°C for 10 minutes) before the reactions were analyzed by capillary electrophoresis (CE) fragment analysis on an Applied Biosystems 3730xl Genetic Analyzer (96 capillary array). The resulting activity demonstrates that while the WarmStart version of Afu Uracil-DNA Glycosylase (UDG) exhibits similar activity to Afu Uracil-DNA Glycosylase at 65°C, WarmStart Afu Uracil-DNA Glycosylase (UDG) has no detectable activity at 25°C.* **B.** *Strand Displacement Amplification (SDA) of a 500 bp amplicon of Lambda DNA was performed in the presence of dUTP at a low temperature range (30-45ºC) with WarmStart Afu Uracil-DNA Glycosylase (UDG) (top panel), with Afu Uracil-DNA Glycosylase (UDG) (middle panel) or without UDG (bottom panel). SDA generated a 500 bp band across the entire temperature range in the absence of UDG (bottom) but was completely inhibited by Afu Uracil-DNA Glycosylase (UDG) at temperatures >31ºC (middle). The inhibition of amplicon generation was due to the inherent activity of Afu Uracil-DNA Glycosylase (UDG) at low temperatures, which generates abasic sites in dU-containing amplicons, thus preventing them from being further amplified. The presence of WarmStart Afu Uracil-DNA Glycosylase (UDG) (top), however, has almost no effect on the amplification of dU-containing amplicons, except a slight decrease of product yield around 45°C, indicating that WarmStart Afu Uracil-DNA Glycosylase (UDG) is active at temperatures >42°C.*

PreCR® Repair Mix

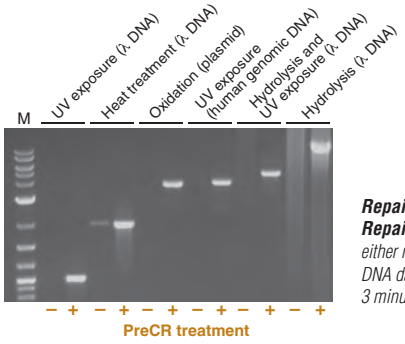
#M0309S 30 reactions
#M0309L 150 reactions

Companion Product:

β-Nicotinamide adenine dinucleotide (NAD+)
#B9007S 0.2 ml

- *Repair DNA prior to its use in DNA-related technologies*
- *Easy-to-use protocols*
- *Does not harm template*

Description: The PreCR Repair Mix is an enzyme cocktail formulated to repair damaged template DNA prior to its use in the polymerase chain reaction (PCR), microarrays, or other DNA technologies. The PreCR Repair Mix is active on a broad range of DNA damage, including those that block PCR (e.g., apurinic/apyrimidinic sites, thymidine dimers, nicks and gaps) and those that are mutagenic (e.g., deaminated cytosine and 8-oxo-guanine). In addition, it will remove a variety of moieties from the 3´ end of DNA leaving a hydroxyl group. The PreCR Repair Mix will not repair all damage that inhibits/interferes with PCR. It can be used in conjunction with any thermophilic polymerase.



Repair of different types of DNA damage with the PreCR Repair Mix. *The gel shows amplification of damaged DNA that was either not treated (–) or treated (+) with the PreCR Repair Mix. Type of DNA damage is shown. Note: heat treated DNA is incubated at 99°C for 3 minutes. Marker (M) is the 1 kb Plus DNA Ladder (NEB #N3200).*

USER® Enzyme

rCutSmart RR 37° No65

Thermolabile USER® II Enzyme

rCutSmart RR 37° 65°

Thermostable USER® III Enzyme

NEBU 65°

USER Enzyme
#M5505S 50 units
#M5505L 250 units

Thermolabile USER II Enzyme
#M5508S 50 units
#M5508L 250 units

Thermostable USER III Enzyme
#M5509S 50 units

Description: USER (Uracil-Specific Excision Reagent) Enzyme generates a single nucleotide gap at the location of a uracil. USER Enzyme is a mixture of Uracil DNA glycosylase (UDG) and the DNA glycosylase-lyase Endonuclease VIII. UDG catalyses the excision of a uracil base, forming an abasic (apyrimidinic) site while leaving the phosphodiester backbone intact. The lyase activity of Endonuclease VIII breaks the phosphodiester backbone at the 3´ and 5´ sides of the abasic site so that base-free deoxyribose is released.

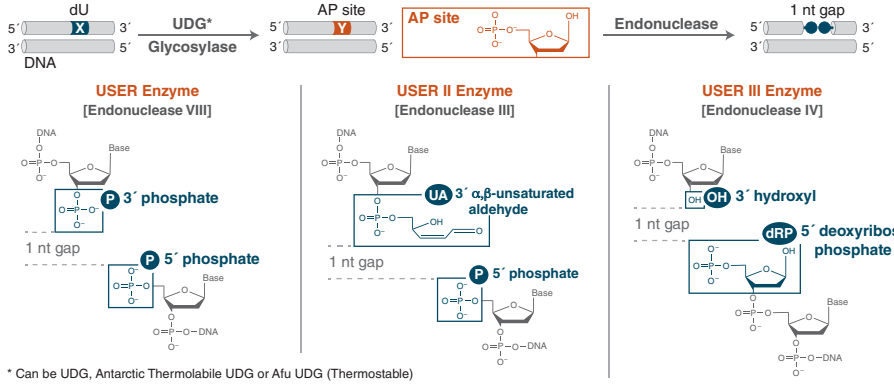
Thermolabile Uracil DNA Glycosylase (UDG) catalyzes the excision of a uracil base, forming an abasic (apyrimidinic) site while leaving the phosphodiester backbone intact. The lyase activity of Endonuclease III breaks the phosphodiester backbone at the 3´ and 5´ sides of the abasic site. In addition to generating a different 3´- terminus than USER Enzyme, Thermolabile USER II Enzyme can also be completely heat inactivated after 10 minutes at 65°C.

Thermostable USER III generates a single nucleotide gap at the location of a uracil. It is a mixture of Afu UDG and DNA glycosylase-lyase Endonuclease IV and is active between 50-75°C, with optimal activity observed at 65°C.

Reaction Conditions: USER & Thermolabile USER II: rCutSmart Reaction Buffer, 37°C. Thermolabile USER II Enzyme can be heat inactivated at 65°C for 10 minutes, while USER cannot. Thermostable USER III: ThermoPol Reaction Buffer, 65°C.

Unit Definition: One unit is defined as the amount of enzyme required to nick 10 pmol of a 34-mer (USER & USER II) or 60-mer (USER III) oligonucleotide duplex containing a single uracil base, in 15 minutes at 37°C in a total reaction volume of 10 µl. Unit assay conditions can be found at www.neb.com.

Concentration: 1,000 units/ml



* Can be UDG, Antarctic Thermolabile UDG or Afu UDG (Thermostable)

USER Enzyme, Thermolabile USER II Enzyme and Thermostable USER III Enzyme generate different functional ends after cleavage of DNA. *The different USER Enzymes generate different 3´ and 5´ termini after cleavage. USER Enzyme (NEB #M5505) contains Endonuclease VIII and leaves a 3´ and 5´ phosphate after cleavage. Thermolabile USER II Enzyme (NEB #M5508) contains Endonuclease III and leaves a 3´-phospho-α, β-unsaturated aldehyde and 5´ phosphate after cleavage. Thermostable USER III Enzyme (NEB #M5509) contains Endonuclease IV and leaves a 3´-hydroxyl and 5´-deoxyribose phosphate.*

Cre Recombinase

NEBU RR 37° 70°

#M0298S 50 units
#M0298L 250 units

for high (15X) concentration
#M0298M 250 units

- *Excision of DNA between two loxP sites*
- *Fusion of DNA molecules containing loxP sites*
- *Inversion of DNA between loxP sites*

Description: Cre Recombinase is a Type I topoisomerase from bacteriophage P1 that catalyzes the site-specific recombination of DNA between *loxP* sites. The enzyme requires no energy cofactors, and Cre-mediated recombination quickly reaches equilibrium between substrate and reaction products. The *loxP* recognition element is a 34 base pair (bp) sequence comprised of two 13 bp inverted repeats flanking an 8 bp spacer region which confers directionality. Recombination products depend on the location and relative orientation of the *loxP* sites. Two DNA species containing single *loxP* sites will be fused. DNA between repeated *loxP* sites will be excised in circular form while DNA between opposing *loxP* sites will be inverted with respect to external sequences.

Reaction Conditions: Cre Recombinase Reaction Buffer, 37°C. Heat inactivation: 70°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme necessary to produce maximal site-specific recombination of 0.25 µg pLox2+ control DNA in 30 minutes at 37°C in a total reaction volume of 50 µl. Maximal recombination is determined by agarose gel analysis and by transformation of reactions followed by selection on ampicillin plates.

Concentration: 1,000 and 15,000 units/ml

TelN Protelomerase

NEBU 30° 75°

#M0651S 250 units

This is an **Enzyme for Innovation (EFI)** graduate. To learn more, visit **www.neb.com/EnzymesforInnovation.**

GMP-grade reagent now available. See page 6 for details.

Description: TelN Protelomerase, from phage N15, cuts dsDNA at a TelN recognition sequence (56 bp) and leaves covalently closed ends at the site of cleavage.

Reaction Conditions: ThermoPol Reaction Buffer, 30°C. Heat inactivation: 75°C for 5 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 0.5 µg of pMiniT-TelN BsaI-linearized control plasmid (313 fmol of TelN recognition sites) in a total reaction volume of 50 µl in 30 minutes at 30°C in 1X ThermoPol Reaction Buffer.

Concentration: 5,000 units/ml

Topoisomerase I (*E. coli*)

rCutSmart RR 37° 65°

#M0301S 100 units
#M0301L 500 units

- *Recognition of mismatched DNA*
- *Catalyzes relaxation of negatively-supercoiled DNA*

Description: Topoisomerase I (*E. coli*) catalyzes the relaxation of negatively-supercoiled DNA. Topoisomerase I has also been implicated in knotting and unknotting DNA, and in linking complementary rings of single-stranded DNA into double-stranded rings. The intact holoenzyme is a 97 kDa protein.

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the relaxation of > 95% of 0.5 µg of pUC19 RF I (negatively supercoiled) DNA in 15 minutes at 37°C in a total reaction volume of 25 µl. DNA supercoiling is assessed by agarose gel electrophoresis in the absence of ethidium bromide.

Concentration: 5,000 units/ml

β-Agarase I

NEBU RR 42° 65°

#M0392S 100 units
#M0392L 500 units

- *Extraction of DNA from agarose gels*
- *DNase and RNase free*

Description: β-Agarase cleaves the agarose subunit, unsubstituted neoagarobiose [3,6-anhydro-α-L-galactopyranosyl-1-3-D-galactose] to neoagaro-oligosaccharides. β-Agarase I digests agarose, releasing trapped DNA and producing carbohydrate molecules which can no longer gel. The remaining carbohydrate molecules and β-Agarase I will not, in general, interfere with subsequent DNA manipulations such as restriction endonuclease digestion, ligation and transformation.

Reaction Conditions: β-Agarase I Reaction Buffer, 42°C. Heat inactivation: 65°C for 15 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to digest 200 µl of molten low melting point or NuSieve agarose to nonprecipitable neoagaro-oligosaccharides in 1 hour at 42°C.

Concentration: 1,000 units/ml

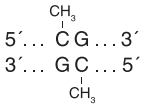
Note: Incubation at 95°C for 2 minutes or incubation at 65°C for 15 minutes inactivates 50 units of β-Agarase I. β-Agarase I retains activity for several hours at 40-45°C and is stabilized by the presence of agarose in the reaction.

CpG Methyltransferase (M.SssI)

NEB2 RR 37° 65° SAM

#M0226S 100 units
#M0226L 500 units

for high (5X) concentration
#M0226M 500 units



- *Blocking restriction enzyme cleavage*
- *Studying of CpG methylation-dependent gene expression*
- *Probing sequence-specific contacts within the major groove of DNA*
- *Altering the physical properties of DNA*
- *Uniform [^{3}H]-labeling of DNA*
- *Decreasing the number of RE cut sites, yielding an apparent increase in specificity*

Description: The CpG Methyltransferase (M.SssI) methylates all cytosine residues (C^5) within the double-stranded dinucleotide recognition sequence 5´...CG...3´.

Reaction Conditions: NEBuffer 2, 37°C. Supplement with 160 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg of λ DNA in a total reaction volume of 20 µl in 1 hour at 37°C against cleavage by BstUI restriction endonuclease.

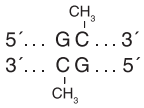
Concentration: 4,000 and 20,000 units/ml

Note: $MgCl_2$ is not required as a cofactor. In the presence of Mg^{2+}, methylation by M.Sss I becomes distributive rather than processive and also exhibits topoisomerase activity.

GpC Methyltransferase (M.CviPI)

NEBU RR 37° 65° SAM

#M0227S 200 units
#M0227L 1,000 units



- *Blocking restriction enzyme cleavage*
- *Altering the physical properties of DNA*
- *Uniform [3H]-labeling of DNA*

Description: The GpC Methyltransferase (M.CviPI) methylates all cytosine residues (C^5) within the double-stranded dinucleotide recognition sequence 5´...GC...3´.

Reaction Conditions: GC Reaction Buffer, 37°C. Supplement with 160 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg of λ DNA in a total reaction volume of 20 µl in 1 hour at 37°C against cleavage by HaeIII restriction endonuclease.

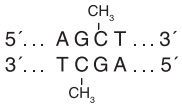
Concentration: 4,000 units/ml

Note: $MgCl_2$ is not required as a cofactor.

AluI Methyltransferase

NEBU RR 37° 65° SAM

#M0220S 100 units



Description: AluI Methyltransferase modifies the cytosine residue (C5) in the sequence to the left.

Reaction Conditions: AluI Methyltransferase Reaction Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 20 minutes.

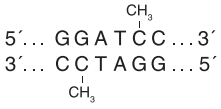
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by AluI restriction endonuclease.

Concentration: 5,000 units/ml

BamHI Methyltransferase

NEBU RR 37° NO SAM

#M0223S 100 units



Description: BamHI Methyltransferase modifies the internal cytosine residue (N^4) in the sequence to the left.

Reaction Conditions: BamHI Methyltransferase Reaction Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM).

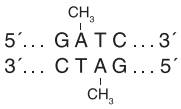
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by BamHI restriction endonuclease.

Concentration: 4,000 units/ml

dam Methyltransferase

NEBU RR 37° 65° SAM

#M0222S 500 units
#M0222L 2,500 units



Description: *dam* Methyltransferase modifies the adenine residue (N^6) in the sequence to the left.

Reaction Conditions: dam Methyltransferase Reaction Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 20 minutes.

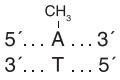
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg (*dam*-) Lambda DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by MboI restriction endonuclease.

Concentration: 8,000 units/ml

EcoGII Methyltransferase

rCutSmart RR 37° 65° SAM

#M0603S 200 units



This is an **Enzyme for Innovation (EFI)** graduate. To learn more, visit **www.neb.com/EnzymesforInnovation.**

Available in **higher concentrations**.

Description: EcoGII Methyltransferase is a non-specific methyltransferase that modifies adenine residues (N^6) in any sequence context.

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with 160 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 100 ng FAM-labeled dsDNA in 30 minutes at 37°C in a total reaction volume of 20 µl against cleavage by MboI restriction endonuclease.

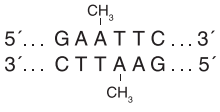
Concentration: 5,000 units/ml

Note: For use of methylation reaction the SAM should be diluted 1:200 in H_2O to a final concentration of 160 µM. EcoGII Methyltransferase is sensitive to salt. Make sure the DNA solution is low in salt concentration or that it makes up only a small percentage of the final reaction volume. If salt is a problem, reduce the salt concentration by drop dialysis.

EcoRI Methyltransferase

rCutSmart RR 37° 65° SAM

#M0211S 10,000 units



Description: EcoRI Methyltransferase modifies the internal adenine residue (N^6) in the sequence to the left.

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by EcoRI restriction endonuclease

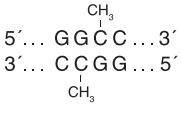
Concentration: 40,000 units/ml

Note: EcoRI Methyltransferase is inhibited by $MgCl_2$. Only 50% activity is retained at a concentration of 4 mM $MgCl_2$.

HaeIII Methyltransferase

NEBU RR 37° 65° SAM

#M0224S 500 units



Description: HaeIII Methyltransferase modifies the internal cytosine residue (C^5) in the sequence to the left.

Reaction Conditions: HaeIII Methyltransferase Reaction Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by HaeIII restriction endonuclease.

Concentration: 10,000 units/ml

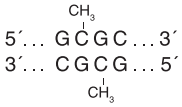
Note: HaeIII Methyltransferase protects DNA against cleavage by NotI.

rCutSmart NEB r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | rAlbumin Recombinant Albumin | SAM Requires SAM | 37° Incubation Temperature | 65° Heat Inactivation

HhaI Methyltransferase

rCutSmart RR 37° No SAM

#M0217S 1,000 units



Description: HhaI Methyltransferase modifies the internal cytosine residue (C^5) in the sequence to the left.

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM).

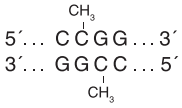
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by HhaI restriction endonuclease.

Concentration: 25,000 units/ml

HpaII Methyltransferase

rCutSmart RR 37° No 65° SAM

#M0214S 100 units



Description: HpaII Methyltransferase recognizes the same sequence as the MspI Methyltransferase, but modifies the internal cytosine residue (C^5) in the sequence to the left.

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 20 minutes.

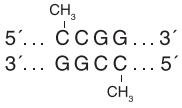
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by HpaII restriction endonuclease.

Concentration: 4,000 units/ml

MspI Methyltransferase

NEBU RR 37° No SAM

#M0215S 100 units



Description: MspI Methyltransferase recognizes the same sequence as the HpaII Methyltransferase, but modifies the external cytosine residue (C^5) in the sequence to the left.

Reaction Conditions: MspI Methylase Reaction Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM).

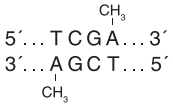
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by MspI restriction endonuclease.

Concentration: 5,000 units/ml

*Taq*I Methyltransferase

rCutSmart RR 65° No SAM

#M0219S 1,000 units



Description: *Taq*I Methyltransferase modifies the adenine residue (N^6) in the sequence to the left.

Reaction Conditions: rCutSmart Buffer, 65°C. Supplement with 80 µM S-adenosylmethionine (SAM).

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 65°C in a total reaction volume of 20 µl against cleavage by *Taq*I restriction endonuclease.

Concentration: 10,000 units/ml

Note: *Taq*I Methyltransferase gives 25% activity at 37°C.

RecA

#M0249S	200 µg
#M0249L	1,000 µg

- *Visualization of DNA structures with electron microscopy*
- *D-loop mutagenesis*
- *Screening libraries using RecA-coated probes*
- *Cleavage of DNA at a single predetermined site*
- *RecA mediated affinity capture for full length cDNA cloning*

Description: *E. coli* RecA is necessary for genetic recombination, reactions involving DNA repair and UV-induced mutagenesis. RecA promotes the autodigestion of the lexA repressor, umuD protein and lambda repressor. Cleavage of LexA derepresses more than 20 genes. *In vitro* studies indicate that in the presence of ATP, RecA promotes the strand exchange of single-strand DNA fragments with homologous duplex DNA. The reaction has three distinct steps: (i) RecA polymerizes on the single-strand DNA, (ii) the nucleo-protein filament binds the duplex DNA and searches for a homologous region, (iii) the strands are exchanged.

Reaction Conditions: Rec A Reaction Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Molecular Weight: 37,973 kDa.

Concentration: 2 mg/ml

T4 Gene 32 Protein

#M0300S	100 µg
#M0300L	500 µg

- *Increase yield and processivity of reverse transcriptase during RT-PCR*
- *Increase yield and specificity of PCR products from soil samples*
- *Stabilization and marking of ssDNA structures*
- *Available in a glycerol-free format*
- *Used in Recombinase Polymerase Amplification (RPA)*

Description: T4 Gene 32 Protein is a single-stranded DNA (ssDNA) binding protein required for bacteriophage T4 replication and repair. It cooperatively binds to and stabilizes transiently formed regions of ssDNA and plays an important structural role during T4 phage replication. It also has been used extensively to stabilize and mark regions of ssDNA for electron microscopic examination of intracellular DNA structures. Recently, it has been shown to improve restriction enzyme digestion, improve the yield and efficiency of reverse transcription (RT) reactions during RT-PCR, enhance T4 DNA polymerase activity, as well as increase the yield of PCR products.

Reaction Conditions: NEBuffer 4, 37°C. Heat inactivation: 65°C for 20 minutes.

Molecular Weight: 33,506 daltons.

Concentration: 10 mg/ml

ET SSB

#M2401S	50 µg

- *Improve the processivity of DNA polymerase*
- *Stabilization and marking of ssDNA structure*
- *Increase the yield and specificity of PCR*
- *Increase the yield and processivity of RT during RT-PCR*
- *Improve DNA sequencing through regions with strong secondary structures*

Description: ET SSB (Extreme Thermostable Single-Stranded DNA Binding Protein) is a single-stranded DNA binding protein isolated from a hyperthermophilic microorganism, which remains fully active after incubation at 95°C for 60 minutes. Due to the extreme thermostability, ET SSB can be used in applications that require extremely high temperature conditions, such as nucleic acid amplification and sequencing.

Unit Definition: Sold by mass of pure protein as determined by OD_{280}.

Molecular Weight: 16 kDa.

Concentration: 500 µg/ml

Note: ET SSB is active in any polymerase buffer. Add 200 ng of ET SSB per 50 µl reaction.

rCutSmart NEB r1.1 Recommended Buffer — Cloned at NEB — Recombinant Enzyme — rAlbumin Recombinant Albumin — SAM Requires SAM — 37° Incubation Temperature — 65° Heat Inactivation

Cloning Plasmids and DNAs

Cloning Plasmid/DNA	NEB #	Features	Concentration	MW/Size	Size
pBR322 Vector	N3033S N3033L	• Commonly used cloning vectors • Amp resistance	1,000 µg/ml	2.83 x 10^6 Da 4,361 bp	50 µg 250 µg
pUC19 Vector	N3041S N3041L	• Commonly used cloning vectors • Amp resistance	1,000 µg/ml	1.75 x 10^6 Da 2,686 bp	50 µg 250 µg
M13mp18 RF I DNA	N4018S	• Phage vectors derived from bacteriophage M13 • DNA, covalently closed circular • 13 Unique RE sites with β-gal gene • Blue/white selection	100 µg/ml	7,249 bp	10 µg
M13mp18 Single-stranded DNA	N4040S		250 µg/ml	7,249 bp	10 µg
Lambda DNA	N3011S N3011L	• Commonly used DNA substrate	500 µg/ml	31.5 x 10^6 Da 48,502 bp	250 µg 1,250 µg
Lambda DNA (dam$^-$)	N3013S N3013L	• Commonly used DNA substrate	500 µg/ml	31.5 x 10^6 Da 48,502 bp	250 µg 1,250 µg
ΦX174 RF I DNA	N3021S N3021L	• Commonly used DNA substrate • Covalently closed circular form of φX174	1,000 µg/ml	3.5 x 10^6 Da 5,386 bp	30 µg 150 µg
ΦX174 RF II DNA	N3022L	• Commonly used DNA substrate • Double-stranded nicked circular form of φX174	1,000 µg/ml	3.5 x 10^6 Da 5,386 bp	150 µg
ΦX174 Virion DNA	N3023S N3023L	• Single-stranded viral DNA	1,000 µg/ml	1.7 x 10^6 Da 5,386 bp	50 µg 250 µg

NEB offers a selection of common cloning plasmids and DNAs for use as substrates. Additional information for many of these DNAs can be found in the Technical Reference section or at www.neb.com.

M13KO7 Helper Phage

#N0315S 1.8 ml

- *Production of single-stranded phagemid DNA for sequencing and mutagenesis*

Description: M13KO7 is a derivative of M13 phage with the origin of replication from P15A and the kanamycin resistance gene from Tn903 both inserted within the M13 origin of replication, which is able to replicate in the absence of phagemid DNA. In the presence of phagemid bearing a wild-type M13 or f1 origin, single-stranded phagemid is packaged preferentially and secreted into the culture medium. This allows easy production of single-stranded phagemid DNA for mutagenesis or sequencing. M13KO7 carries the kanamycin resistance marker.

Source: M13KO7 Phage supernatant is isolated from infected E. coli ER2738 by a standard procedure.

Concentration: 1 x 10^{11} pfu/ml

Note: NEB does not recommend the use of M13KO7 as a cloning vector. For cloning peptides displayed on M13 phage, we recommend the Ph.D.™ Peptide Display Cloning System.

Meagan joined NEB in 2024 as a Senior Web Development Services Manager.

Programmable Nucleases

Site-specific gene modification and highly-specific *in vitro* cutting is enabled by nucleases that can be easily programmed with nucleic acids. In addition to programmed with nucleic acids. In addition to RNA-guided Cas enzymes, *Tth* Argonaute can be programmed with DNA, further expanded the range of available tools.

Tth Argonaute (TtAgo)

#M0665S 50 pmol

- *Short 16-18 nucleotide 5´-phosphorylated ssDNA guides are cost effective and can be phosphorylated with T4 Polynucleotide Kinase*
- *Guide/target sequence selection is not limited by the requirement of an adjacent sequence motif*
- *Highly active on ssDNA and most dsDNA substrates (generates a nick in dsDNA substrates), with mild activity on ssRNA substrates*

Description: *Thermus thermophilus* argonaute (TtAgo) is a programmable DNA-endonuclease which requires a short 5´-phosphorylated single-stranded DNA guide to target its activity to a specific corresponding sequence on a substrate. TtAgo introduces one break in the phosphodiester backbone of the complementary substrate between positions 10 and 11 of the DNA guide.

Source: *Thermus thermophilus* argonaute (TtAgo) is purified from an *E. coli* strain that carries a cloned gene from the Gram-negative thermophilic bacterium *Thermus thermophilus* which is expressed as a recombinant N-terminal 6X His-tagged fusion.

Note: Visit www.neb.com/M0665 for usage guidelines.

NEW

EnGen® SpRY Cas9

#M0669T 500 pmol
#M0669M 2,500 pmol

Companion Products:

EnGen Spy Cas9 HF1
#M0667T 500 pmol
#M0667M 2,500 pmol

EnGen sgRNA Synthesis Kit, *S. pyogenes*
#E3322V 10 reactions
#E3322S 20 reactions

EnGen Mutation Detection Kit
#E3321S 25 reactions

Description: EnGen SpRY Cas9 from *Streptococcus pyogenes* is an engineered, RNA-guided, DNA endonuclease that catalyzes site-specific cleavage of double-stranded DNA (dsDNA). Targeting requires a ~100 nucleotide single guide RNA (sgRNA) with complementarity to the 20-nucleotide region immediately upstream of a protospacer adjacent motif (PAM) on the dsDNA substrate. EnGen SpRY Cas9 encodes 11 point mutations (A61R, L1111R, D1135L, S1136W, G1218K, E1219Q, N1317R, A1322R, R1333P, R1335Q, T1337R) designed to diminish the requirement for a PAM. Unlike the canonical 5´-NGG-3´ PAM requirement of wild-type Spy Cas9, SpRY Cas9 has been demonstrated to have almost no PAM requirement *in vitro*, cleaving at many sites with a 5´-NNN-3´ PAM (although it exhibits a preference for 5´-NRN-3´ over 5´-NYN-3´ PAMs *in vivo*). DNA cleavage by EnGen SpRY Cas9 produces a double-stranded break occurring 3 nucleotides upstream of the PAM. EnGen SpRY Cas9 contains Simian virus 40 (SV40) T antigen nuclear localization sequence (NLS) on the C-terminus of the protein.

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 5 minutes.

Concentration: 20 µM

Note: 20 µM is equal to 3.25 mg/ml

NEW

Cryogenic Cold Storage Box

#T1041S 4 Box

- *Supplied as a pack of 4 boxes*
- *Designed for storing and protecting 1mL to 2 mL vials and tubes*
- *Featuring a hinged lid design and labeled grid numbers*

Description: The Cryogenic Cold Storage Box is designed to protect and store microcentrifuge vials and tubes at -80°C to room temperature (not recommended for liquid nitrogen storage). These cryo boxes are composed of fiberboard/cardboard material and can hold up to 81 items with the included adjustable slats that can be moved or removed to adapt to different tube sizes.

rCutSmart r3.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | rAlbumin Recombinant Albumin | SAM Requires SAM | 37° Incubation Temperature | 65° Heat Inactivation

Competent Cell Selection Chart for Cloning

	NEB® 5-alpha Competent *E. coli* NEB #C2987	NEB Turbo Competent *E. coli* NEB #C2984	NEB 5-alpha F′*I*^{q} Competent *E. coli* NEB #C2992	NEB 10-beta Competent *E. coli* NEB #C3019	*dam-/dcm-* Competent *E. coli* NEB #C2925	NEB® Stable Competent *E. coli* NEB #C3040
Features						
Versatile	•					•
Fast growth (< 8 hours)		•				
Toxic gene cloning			•			•
Large plasmid/BAC cloning				•		•
Dam/Dcm-free plasmid growth					•	
Retroviral/lentiviral vector cloning						•
recA^{-}	•		•	•		•
endA^{-}	•	•	•	•	•	•
Formats						
Chemically competent	•	•	•	•	•	•
Electrocompetent				•		
Subcloning	•					
96-well format*	•			•		
384-well format*	•					
12 x 8-tube strips*	•					

* Other strains are available upon request. For more information, contact custom@neb.com.

Monarch Nucleic Acid Purification Kits

Monarch kits provide fast and reliable purification of high quality DNA and RNA from a variety of sources using best-in-class silica-column, magnetic bead and innovative glass bead technology. DNA and RNA purified with Monarch kits is highly pure and suitable for use in a wide range of applications, including IVT RNA synthesis, sequencing, cloning, PCR and other enzymatic manipulations. Monarch kits are developed for performance and with sustainability in mind; they use significantly less plastic and are packaged in responsibly-sourced, recyclable material. For flexibility, select Monarch kit components are available separately. Learn more at NEBmonarch.com.

Product	NEB #	Size
Monarch Spin Plasmid Miniprep Kit	T1110S T1110L	50 preps 250 preps
Monarch Spin DNA Gel Extraction Kit	T1120S T1120L	50 preps 250 preps
Monarch Spin PCR & DNA Cleanup Kit (5 µg)	T1130S T1130L	50 preps 250 preps
Monarch Spin gDNA Extraction Kit	T3010S T3010L	50 preps 150 preps
Monarch HMW DNA Extraction Kit for Tissue	T3060S T3060L	5 preps 50 preps
Monarch HMW DNA Extraction Kit for Cells & Blood	T3050S T3050L	5 preps 50 preps
Monarch Spin RNA Isolation Kit (Mini)	T2110S	50 preps
Monarch Spin RNA Cleanup Kit (10 µg)	T2030S T2030L	10 preps 100 preps
Monarch Spin RNA Cleanup Kit (50 µg)	T2040S T2040L	10 preps 100 preps
Monarch Spin RNA Cleanup Kit (500 µg)	T2050S T2050L	10 preps 100 preps
Monarch Mag Viral DNA/RNA Extraction Kit	T4010S T4010L	600 preps 1,800 preps
Columns Available Separately		
Monarch Spin Columns S2D and Tubes	T1117L	100 preps
Monarch Spin Columns S2A and Tubes	T2047L	100 columns
Monarch Spin Columns S2C and Tubes	T3017L	100 preps
Monarch Spin Columns S1A and Tubes	T2037L	100 columns
Monarch Spin Columns S2B and Tubes	T2057L	100 columns

Monarch kit components are available separately.
See Nucleic Acid Purification Chapter for details.

Learn more about
Martin's work in a recent
Labconscious interview.

VIEW

Laboratory chemical and medical waste
Credit: eplisterra, Adobe Stock

Shaping a Legacy with a Blueprint for Sustainability

In 2013, Martin Farley embarked on a journey that would position him as a leading figure in sustainable science. Noticing a glaring absence of sustainability initiatives within European laboratories, Farley became the region's first dedicated employee working on sustainable science. His passion for making a tangible difference in the face of climate change propelled him to pioneer efforts that have since transformed laboratory practices and policies on an international scale.

Farley's initial foray into this field began at the University of Edinburgh, where he interned in sustainable labs as part of his Master's program in the Netherlands. Recognizing the fragmented nature of sustainability efforts, he co-founded the Laboratory Efficiency Action Network (LEAN) in the UK, which would bring together individuals passionate about sustainable science. LEAN served as a platform for professionals to share resources and case studies and collaborate on initiatives, effectively building a community where none had existed before.

However, Farley realized that networking alone wasn't enough. Laboratories needed a structured framework to implement sustainable practices effectively. This led him to create the Laboratory Efficiency Assessment Framework (LEAF) while working at University College London (UCL). LEAF is a comprehensive program amalgamating best practices into an accessible laboratory certification system. It covers many sustainability actions, from practical steps like turning off equipment and proper waste management to more complex considerations like integrating sustainability into purchasing decisions. It uniquely connects research reproducibility to sustainability, emphasizing that irreproducible experiments waste resources and increase environmental impact.

By November 2023, the LEAF program had been formally adopted by 105 institutions — the largest green lab certification globally. Its success also caught the attention of major UK research funders like the Wellcome Trust and Cancer Research UK, which began incorporating green lab certifications into their grant requirements — a testament to LEAF's credibility and impact.

Over the past decade, Farley has delivered over 700 talks, ranging from lab group discussions to larger institutional presentations. His dedication to education and outreach has been instrumental in shifting mindsets and encouraging scientists to adopt sustainable practices in their daily work. Farley has co-authored several publications related to sustainable research, including groundbreaking work on life cycle assessments (LCAs) of lab consumables.

Farley acknowledges the inherent resource intensity of Western lifestyles and feels a personal responsibility to be part of the solution. *"We have to do the good we can because we only have one life,"* he said, highlighting his deeply rooted commitment to environmental stewardship.

In his current role as the Associate Director of Environmental Sustainability at UK Research and Innovation (UKRI), Farley is leading efforts to create open-access frameworks for sustainability in diagnostic laboratories — a global first.

Farley's journey illustrates how one individual's passion and dedication can catalyze significant change. By addressing sustainability at both the grassroots and institutional levels, he has fostered a culture of environmental consciousness in laboratories, influenced policy and funding structures, and provided a blueprint for integrating sustainability into research.

Martin Farley
Sustainable Science Leader and LEAF Founder,
London, UK
2024 Passion in Science
Environmental Stewardship Award

Nucleic Acid Purification

Monarch® has evolved

Nucleic acid purification is an important step in molecular biology workflows and there are many commercially-available solutions from which to choose. Our Research and Development team meets regularly with customers to better understand what could be done to continually improve upon current nucleic acid purification kits. Your feedback helped us improve our line of Monarch Nucleic Acid Purification kits, which have been optimized for exceptional value with uncompromising quality.

Monarch kits are available for DNA and RNA extraction and cleanup, plasmid purification, and gel extraction. Our Monarch kits utilize unique column designs and scalable magnetic bead platforms, which enable the isolation of highly-pure nucleic acids, free from contaminants and often in low volumes. Our novel glass bead-based solution for extraction of high molecular weight DNA supports long read sequencing. Monarch kits are supported by a variety of validated, user-friendly protocols to support multiple workflows and applications.

We know that it can be difficult to be environmentally friendly in the lab, where sterility and convenience are of utmost importance. At times, it may seem that sustainability and benchwork are at odds with each other. Although we can't completely solve this problem, we can make changes to our product design to help move toward the goal of a greener lab, and that's exactly what we did with the design of our Monarch kits. Wherever possible, these kits use less plastic, as well as responsibly-sourced and recyclable packaging. The columns have thinner walls, reducing total plastic usage without affecting performance. All bottles were carefully chosen to minimize plastic usage, and the kit boxes are made from 100% post consumer content and are designed to be reused and/or recycled.

Let's work together to clean up the world of nucleic acid purification, one prep at a time.

Featured Products

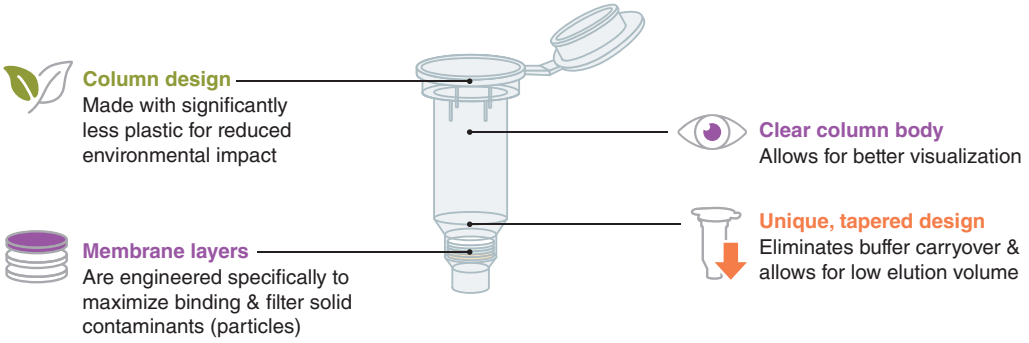
Featured Tools & Resources

Monarch Kit Components
Available separately. See individual product pages for details.

Monarch Nucleic Acid Purification Kits Have Evolved

Improved performance by design

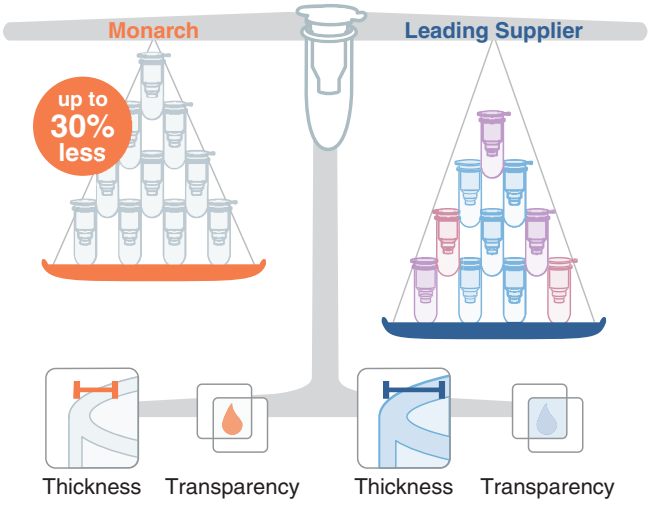
Not all columns are created equal (some are better)! Our updated Monarch kits include a redesigned column, which delivers higher purity and yield, while still using less plastic. These new columns are manufactured using state-of-the-art equipment and processes, which together ensures that our Monarch kits provide unmatched performance.



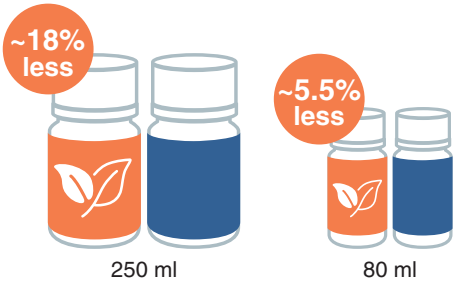
Better sustainability by design

Monarch kits have always been designed with sustainability in mind. By considering all aspects of development, our kits have been made even better for the environment while ensuring they are better for your budget. We've further reduced the amount of materials and waste used in the manufacture and packaging of these kits, which means we're able to pass those cost savings on to you. Packaging has been streamlined, concise protocol cards replace printed materials, and sustainable and recyclable materials are used throughout.

COLUMNS & COLLECTION TUBES
Weight in plastic



REAGENT BOTTLES
Weight in plastic



To learn more, visit **NEBMonarch.com**

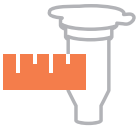
Make the better choice and migrate to Monarch

Workflows for detecting, analyzing, amplifying or manipulating DNA and RNA often require extraction and purification from a biological sample and/or enzymatic reactions. Monarch nucleic acid purification kits provide fast and reliable purification of high-quality DNA and RNA from a variety of sources using best-in-class silica column, magnetic bead and novel glass-bead based technologies. DNA and RNA purified with Monarch Kits is highly-pure and suitable for use in a wide variety of downstream applications including sequencing, cloning, PCR and other enzymatic manipulations. Monarch kits are all designed with sustainability in mind; they use less plastic whenever possible and are packaged in responsibly-sourced, recyclable material. For convenience, all Monarch kit components are also available separately.

Reduced lab waste

Significantly less plastic as compared to leading supplier

Monarch kits still deliver high yields, purity and performance



Thinner-walled columns

Reduction in total plastic without affecting performance

Buffer bottles

Carefully designed to minimize plastic usage

Flexible purchasing options

Buffers and columns sold separately

Purchase only what you need and avoid wasted materials

Same performance, design and formulations

Standalone products are the same components that are included in complete kits

MONARCH® Sustainability

No excessive packaging

Sturdy, reusable boxes at just the right size

Carefully designed to eliminate empty space, versatile Monarch boxes can be reused anywhere

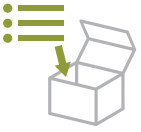
Concise protocol cards replace printed manuals

Manuals are available online

Sustainable & recyclable packaging

Sourced for recyclability

All components are purposefully sourced for recyclability



Instructions for recycling kit components

Can be found online

Recycled paper

Used to make the kit boxes, inserts and paper materials

Eco-friendly printing

Printing of boxes and packaging powered by green sustainable sources such as wind

To learn more, visit **neb.com/monarchsustainability**

NEW

Monarch® Spin Plasmid Miniprep Kit

#T1110S	50 preps
#T1110L	250 preps

Companion Products:

Monarch Spin Columns S2D and Tubes
#T1117L 100 preps

Monarch Spin Collection Tubes
#T2118L 100 tubes

Monarch Spin gDNA Extraction Kit
#T3010S 50 preps
#T3010L 150 preps

Monarch Spin DNA Gel Extraction Kit
#T1120S 50 preps
#T1120L 250 preps

Exonuclease V (RecBCD)
#M0345S 1,000 units
#M0345L 5,000 units

Monarch Buffer BZ
#T1114L 168 ml

Monarch Buffer WZ
#T1115L 26 ml

Monarch RNase A
#T3018L 1 ml

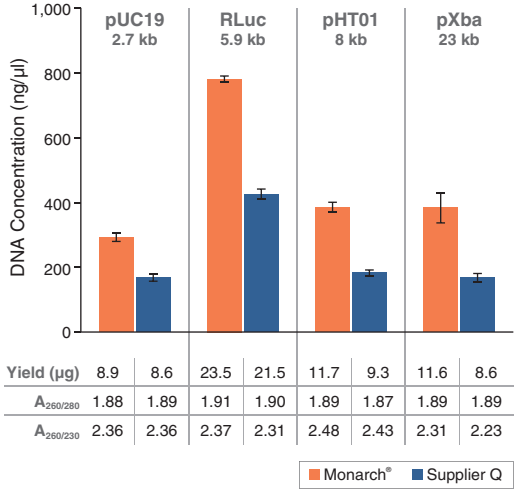
- *Prevent buffer retention and salt carry-over with optimized column design*
- *Monitor completeness of certain steps using colored buffer system*
- *No need to add RNase before starting*
- *Elute in low volumes*

Description: The Monarch Spin Plasmid Miniprep Kit offers a unique method for plasmid preparation and purification, enabling extraction of highly concentrated plasmid DNA from various bacterial strains, with yields up to 20 µg of high-quality DNA. This new version features upgraded spin columns precision engineered to uniquely allow for low elution, in as little as 30 µl, for highly concentrated DNA in downstream application such as sequencing, restriction digestion, transformation, transfection, and more.

Kit Includes:

- Spin Columns S2D
- Spin Collection Tubes
- Buffer B1
- Buffer B2
- Buffer B3
- Buffer BZ
- Buffer WZ
- Buffer EY
- RNase A

Specifications	
Culture Volume	1-5 ml, not to exceed 15 OD units
Binding Capacity	up to 20 µg
Plasmid Size	up to 25 kb
Typical Recovery	up to 20 µg. Yield depends on plasmid copy number, host strain, culture volume, and growth conditions.
Elution Volume	≥ 30 µl
Purity	$A_{260/280}$ and $A_{260/230}$ ≥ 1.8
Protocol Time	9.5–12.5 minutes of spin and incubation time
Compatible Downstream Applications	restriction digestion and other enzymatic manipulations, transformation, transfection, DNA sequencing, PCR, labeling, cell-free protein synthesis, etc.



	pUC19		RLuc		pHT01		pXba	
Yield (µg)	8.9	8.6	23.5	21.5	11.7	9.3	11.6	8.6
$A_{260/280}$	1.88	1.89	1.91	1.90	1.89	1.87	1.89	1.89
$A_{260/230}$	2.36	2.36	2.37	2.31	2.48	2.43	2.31	2.23

Monarch Spin Plasmid Miniprep Kit consistently produces more concentrated plasmid DNA with equivalent or better yield and purity compared to the leading supplier. *DNA concentration, yield and purity are higher with Monarch Spin Plasmid Miniprep Kit than leading supplier's kit, across different plasmids. Preps were performed according to recommended protocols using ~1 ml (OD600 = 3) aliquot for pUC19 and pHT01 and ~2 ml (OD600 = 6) aliquot for Rluc and pXba of the same overnight cultures. Concentrations of plasmid were measured using a Trinean DropSense 16.*

NEW

Monarch® Spin PCR & DNA Cleanup Kit (5 µg)

#T1130S	50 preps
#T1130L	250 preps

Companion Products:

Monarch Spin DNA Gel Extraction Kit
#T1120S 50 preps
#T1120L 250 preps

Monarch Spin Columns S1A and Tubes
#T2037L 100 columns

Monarch Spin Plasmid Miniprep Kit
#T1110S 50 preps
#T1110L 250 preps

Monarch Spin Collection Tubes
#T2118L 100 tubes

Monarch Buffer BZ
#T1114L 168 ml

Monarch Buffer WZ
#T1115L 26 ml

- *New version of PCR and DNA cleanup kit, featuring upgraded spin columns precision-engineered for high performance*
- *Elute in as little as 5 µl for highly concentrated DNA, with yields up to 5 µg*
- *Prevent buffer retention and salt carry over with unique, optimized column design*
- *No need to monitor pH*
- *Protocol modification for oligonucleotide cleanup is provided, allowing purification of ssDNA, oligonucleotide and other small DNA fragments*
- *Reduce hands-on time with faster protocols and less spin time*
- *Significantly less plastic by design in columns and kit compared to leading suppliers*

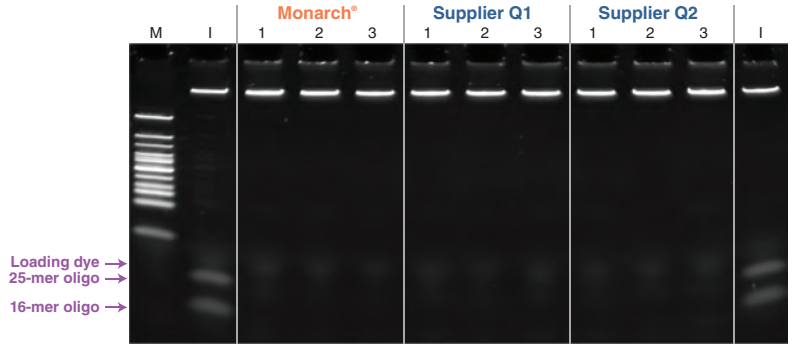
Description: The Monarch Spin PCR & DNA Cleanup Kit (5 µg) offers an efficient method for purification and concentration up to 5 µg of high-quality, double-stranded and single-stranded DNA from enzymatic reactions such as PCR, restriction digestion, ligation, and reverse transcription. This new version features upgraded spin columns precision-engineered to uniquely allow for low elution, in as little as 5 µl, for highly concentrated DNA in downstream applications such as sequencing, restriction digestion, library construction, labeling and other enzymatic manipulations. The kit includes spin columns, collection tubes, buffers, and other reagents in compact and streamlined packaging for silica membrane-based PCR purification and DNA cleanup. Our optimized buffer system allows efficient binding and purification of the DNA, without a need to monitor pH. These kits contain uniquely designed columns and bottles to significantly reduce plastic usage compared to leading suppliers, while maintaining structural integrity for centrifugation, vacuum manifolds and other steps. The kit features Monarch spin columns that are manufactured using state of the art automation machinery for high quality and consistency.

Kit Includes:

- Spin Columns S1A
- Spin Collection Tubes
- Buffer BZ
- Buffer WZ
- Buffer EY

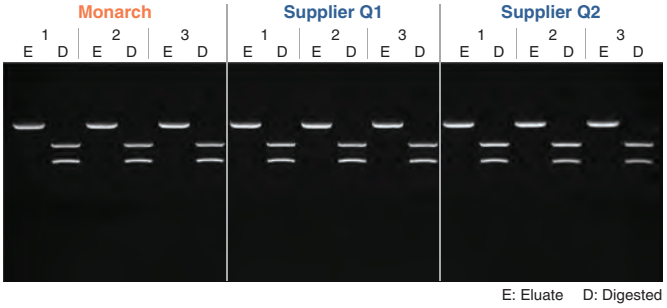
Specifications	
Binding Capacity	5 µg
DNA Size Range	~50 bp–25 kb DNA ≥ 15 bp to 25 kb (dsDNA) and DNA ≥ 18 nt to 10 kb (ssDNA) can also be purified using the Oligonucleotide Cleanup Protocol
Elution Volume	≥ 5 µl
Typical Recovery	DNA 50 bp–10 kb 70–90% DNA 16–23 kb 50–70% ssDNA ≥ 16 nt and dsDNA ≥ 12 bp 70–85%
Protocol Time:	5 minutes

A. Primer removal



M: Marker I: Input sample before cleanup

B. Salt-sensitive restriction enzyme digestion



E: Eluate D: Digested

DNA purified using Monarch Spin PCR & DNA Cleanup Kit (5 µg) are high-quality and suitable for downstream applications. *Use of the Monarch Spin PCR & DNA Cleanup Kit effectively cleans up DNA suitable for downstream applications, performing as well as a leading supplier's kits.*

A. *TBE PAGE data demonstrating primer removal. A 2 kb fragment was spiked with two oligonucleotides (16-mer, 25-mer) to a final concentration of 1 µM. The mixture was purified as per recommended protocols. Equivalent fractions of the original mixture and the eluted DNA were resolved in a 20% TBE polyacrylamide gel at 100V for 1 hour and stained with SYBR Gold. M = Low Molecular Weight DNA Ladder (NEB #N3233).*

B. *Agarose gel data demonstrating downstream application by restriction digestion with a salt-sensitive restriction enzyme. Three replicates of purified DNA and subsequent restriction enzyme digest were performed. 4 µg of a 3 kb DNA fragment was purified and 1 µg was digested with DraIII-HF (NEB #R3510). Equivalent fractions of the input DNA and digestion reaction were resolved in a 1.2% TBE agarose gel.*

NEW

Monarch® Spin DNA Gel Extraction Kit

#T1120S	50 preps
#T1120L	250 preps

Companion Products:

Monarch Spin PCR & DNA Cleanup Kit (5 µg)

#T1130S	50 preps
#T1130L	250 preps

Monarch Spin Columns S1A and Tubes

#T2037L	100 columns

Monarch Spin Plasmid Miniprep Kit

#T1110S	50 preps
#T1110L	250 preps

Monarch Spin Collection Tubes

#T2118L	100 tubes

Monarch Buffer WZ

#T1115L	26 ml

Monarch Buffer BY

#T1121L	230 ml

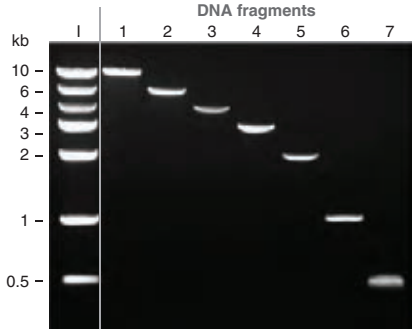
- *Elute in as little as 5 µl*
- *Prevent buffer retention and salt carry-over with optimized column design*
- *Save time with fast user-friendly protocol*
- *Purchase optimized kit formats or buffers & columns separately for your convenience*

Description: The Monarch Spin DNA Gel Extraction Kit offers an efficient method for DNA gel extraction and purification, enabling up to 5 µg of high-quality DNA. This new version features upgraded spin columns precision-engineered to uniquely allow for low elution, in as little as 5 µl, for highly concentrated DNA in downstream applications such as sequencing, restriction digestion, library construction, labeling and other enzymatic manipulations. Our optimized gel dissolving buffer allows efficient gel dissolving and DNA binding onto the silica matrix, without a need to monitor pH or add isopropanol. These kits contain uniquely designed columns and bottles to significantly reduce plastic usage compared to leading suppliers, while maintaining structural integrity for centrifugation, vacuum manifolds and other steps. The kit features Monarch spin columns that are manufactured using state of the art automation machinery for high quality and consistency.

Kit Includes:

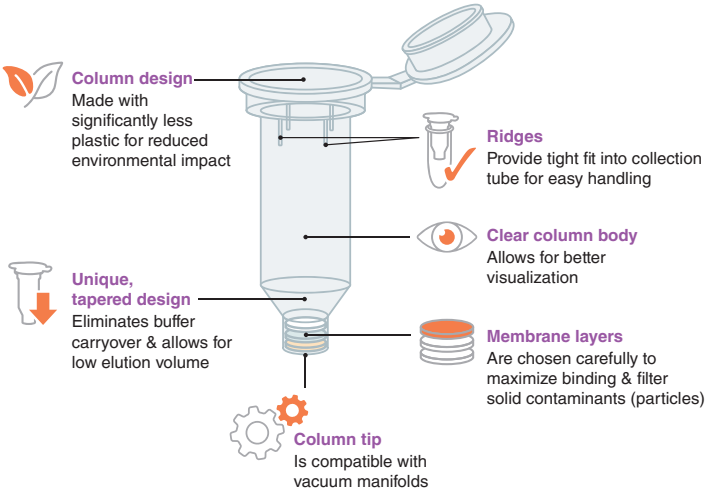
- Spin Columns S1A
- Spin Collection Tubes
- Buffer BY
- Buffer WZ
- Buffer EY

Specifications	
Binding Capacity	5 µg
DNA Size Range	50 bp–25 kb
Elution Volume	≥ 5 µl
Typical Recovery	DNA 50 bp–10 kb 70–90% DNA 11–25 kb 50–70%
Protocol Time:	15 minutes



I: Input DNA mixture

Monarch Spin DNA Gel Extraction Kit is effective for a wide range of DNA sizes. *A mixture of 7 DNA fragments ranging from 0.5 kb to 10 kb was prepared and resolved on a 1.2% w/v TBE agarose gel. Each fragment was manually excised from the agarose gel and processed using the Monarch Spin DNA Gel Extraction Kit. The elution of each fragment was resolved on a new gel with the original mixture for comparison.*



Features of the Monarch Spin Column S1A. *NEB Monarch's unique column design allows high-quality DNA purification with low-elution volume. The column is designed and made with significantly less plastic for reduced environmental impact.*

Monarch® Spin gDNA Extraction Kit

#T3010S	50 preps
#T3010L	150 preps

Companion Products:

Monarch HMW DNA Extraction Kit for Tissue
#T3060S 5 preps
#T3060L 50 preps

Monarch HMW DNA Extraction Kit for Cells & Blood
#T3050S 5 preps
#T3050L 50 preps

Monarch Spin Columns S2C and Tubes
#T3017L 100 preps

Monarch gDNA Tissue Lysis Buffer
#T3011L 34 ml

Monarch gDNA Cell Lysis Buffer
#T3012L 20 ml

Monarch gDNA Blood Lysis Buffer
#T3013L 20 ml

Monarch gDNA Binding Buffer
#T3014L 65 ml

Monarch gDNA Wash Buffer
#T3015L 60 ml

Monarch gDNA Elution Buffer
#T3016L 34 ml

Monarch RNase A
#T3018L 1 ml

Monarch Spin Collection Tubes
#T2118L 100 tubes

Proteinase K, Molecular Biology Grade
#P8107S 2 ml

- *Purify high quality gDNA from a wide variety of sample types (cells, blood, tissues and more)*
- *Experience extremely low residual RNA contamination (typically <1%)*
- *Isolate high molecular weight gDNA (peak size typically ≥ 50 kb)*
- *Take advantage of user-friendly protocols with fast and efficient lysis steps*
- *Additionally, use the kit to clean up genomic DNA*
- *Enjoy the flexibility to purchase kit components separately*

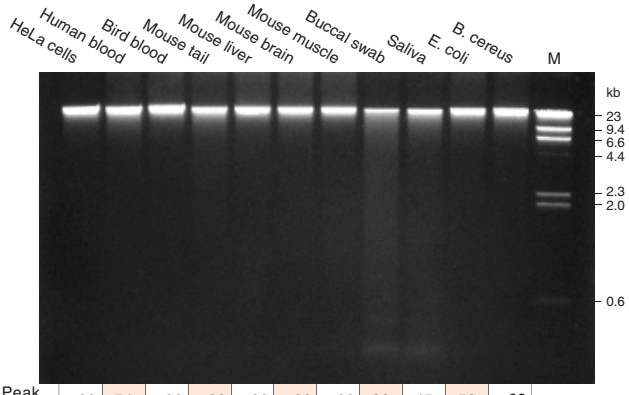
Description: The Monarch Spin gDNA Extraction Kit is a comprehensive solution for cell lysis, RNA removal, and purification of intact genomic DNA (gDNA) from a wide variety of biological samples, including cultured cells, blood, and mammalian tissues. Additionally, bacteria and yeast can be processed with extra steps to enhance lysis in these tough-to-lyse samples. Protocols are also included to enable purification from clinically-relevant samples, such as saliva and cheek swabs, as well as rapid cleanup of previously extracted gDNA. Purified gDNA has high quality metrics, including $A_{260/280} > 1.8$ and $A_{260/230} > 2.0$, high DIN scores and minimal residual RNA. The purified gDNA is suitable for downstream applications, such as endpoint PCR, qPCR and library prep for next generation sequencing (NGS). Typical peak size is 50–70 kb, making this kit an excellent choice upstream of next generation sequencing (NGS) platforms.

Kit Includes:

- gDNA Tissue Lysis Buffer
- gDNA Cell Lysis Buffer
- gDNA Blood Lysis Buffer
- gDNA Binding Buffer
- gDNA Wash Buffer
- gDNA Elution Buffer
- Spin Columns S2C
- Spin Collection Tubes
- Proteinase K, Molecular Biology Grade
- RNase A

Specifications	
Input	• Cultured mammalian cells: up to 5 x 10^6 cells • Mammalian whole blood: 100 µl • Tissue: up to 25 mg, depending on tissue type • Bacteria: up to 2 x 10^9 • Yeast: up to 5 x 10^7 • Saliva: up to 500 µl • Buccal swabs • Genomic DNA requiring cleanup
Binding Capacity	30 µg genomic DNA
Yield	Varies depending on sample type*
Genomic DNA Size	Peak size > 50 kb for most sample types; may be lower for saliva and buccal swabs
RNA Content	< 1% (with included RNase A treatment)
Purity	$A_{260/280} \geq 1.8$, $A_{260/230} \geq 2.0$

*See "Guidelines for Choosing Sample Inputs" in the technical reference section or at **www.neb.com/MonarchgDNAInputs**.



	HeLa cells	Human blood	Bird blood	Mouse tail	Mouse liver	Mouse brain	Mouse muscle	Buccal swab	Saliva	E. coli	B. cereus
Peak size (kb)	> 60	54	> 60	> 60	> 60	> 60	> 60	39	45	53	> 60
DIN	9.3	9.0	9.2	8.7	9.4	9.3	9.2	6.6	7.4	8.7	8.9
A_{260}/A_{280}	1.86	1.85	1.87	1.85	1.85	1.87	1.87	1.85	1.86	1.86	1.88
A_{260}/A_{230}	2.30	2.20	2.40	2.36	2.34	2.47	2.40	2.00	2.17	2.33	2.04

The Monarch Spin gDNA Extraction Kit efficiently purifies high-quality, high molecular weight gDNA from a variety of sample types. *100 ng of genomic DNA from each sample was loaded on a 0.75% agarose gel. gDNA was isolated following the standard protocols for blood, cultured cells and tissue, and the supplemental protocols for buccal swabs, saliva, Gram- and Gram+ bacteria. Starting material used: 1 x 10^6 HeLa cells, 100 µl human blood, 10 µl bird blood, 10 mg frozen tissue powder, 1 buccal swab, 500 µl saliva and ~1 x 10^9 bacterial cells. Lambda DNA-Hind III digest (NEB #N3012) was used as a marker in the last lane (M). Purified gDNA samples were analyzed using a Genomic DNA ScreenTape® on an Agilent Technologies® 4200 TapeStation®. Samples typically yield peak sizes 50–70 kb and DINs of ~9. The cell fractions processed in the buccal swab and saliva preps contain dead cells, as expected, causing a smear like pattern with typical low molecular weight apoptotic bands.*

Monarch® HMW DNA Extraction Kits

Monarch HMW DNA Extraction Kit for Tissue
#T3060S 5 preps
#T3060L 50 preps

Monarch HMW DNA Extraction Kit for Cells & Blood
#T3050S 5 preps
#T3050L 50 preps

Companion Products:

Monarch Pestle Set
#T3000L 100 sets

Monarch 2 ml Tubes
#T3003L 100 tubes

Monarch DNA Capture Beads
#T3005L 200 beads

Monarch Bead Retainers
#T3004L 100 sets

Monarch RBC Lysis Buffer
#T3051L 160 ml

Monarch gDNA Elution Buffer II
#T3056L 24 ml

Monarch HMW gDNA Tissue Lysis Buffer
#T3061L 62 ml

Monarch Protein Separation Solution
#T3062L 36 ml

Monarch Precipitation Enhancer
#T3055L 10 ml

Monarch Spin Collection Tubes
#T2118L 100 tubes

Proteinase K, Molecular Biology Grade
#P8107S 2 ml

Monarch RNase A
#E3018S 1 ml

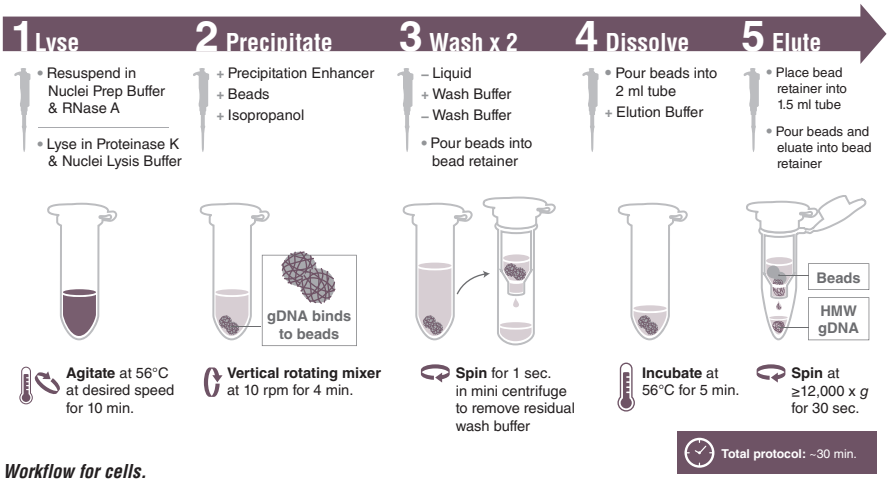
- *Fast workflow (cells: 30 min, blood: 60 min, tissue/bacteria: 90 min)*
- *Extract DNA into the megabase (Mb size range with cells, blood, soft organ tissues and bacteria*
- *Tune DNA size based on agitation speed during lysis*
- *Achieve best-in-class yields and purity*
- *Consistently achieve reproducible results*
- *Effectively remove RNA*
- *Elute DNA easily and completely*

Try the NEBNext® Companion Module for Oxford Nanopore Technologies Ligation Sequencing (NEB #E7180) for library prep after extraction.

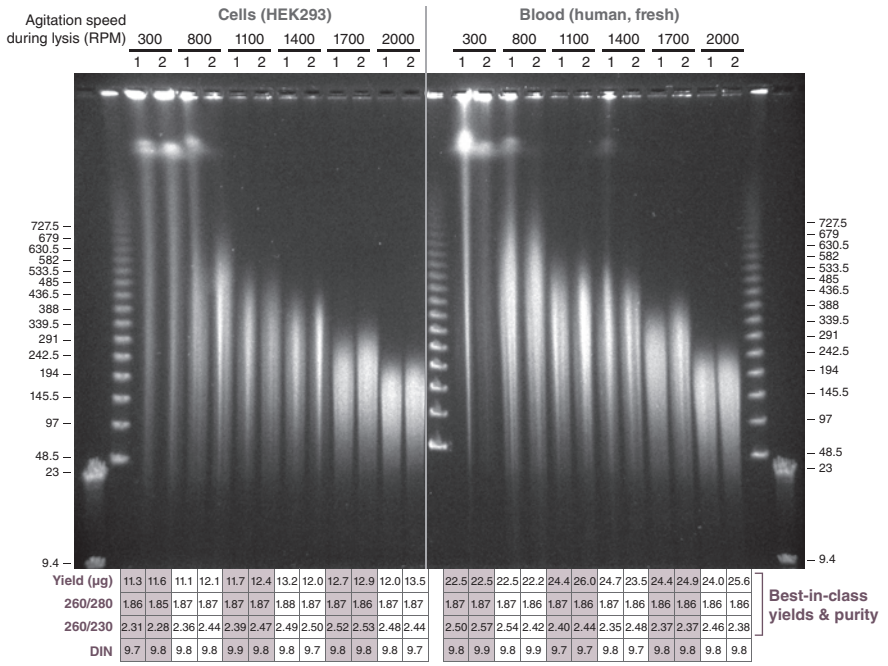
Description: Monarch HMW DNA Extraction Kits provide a rapid and reliable process for extracting high molecular weight DNA (HMW DNA) from biological samples including cells, blood, tissue, bacteria and other sample types. Utilizing an optimized process that combines gentle cell lysis with a tunable fragment length generation, followed by precipitation of the extracted DNA onto the surface of large glass beads, the prep proceeds rapidly. DNA size ranges from 50-250 kb for the standard protocol and into the Mb range on several sample types when the lowest agitation speeds are used. Purified DNA is recovered in high yield with excellent purity, including nearly complete removal of RNA. Purified HMW gDNA is easy to dissolve and is suitable for a variety of downstream applications including long-read sequencing (e.g., PacBio® and Oxford Nanopore Technologies®).

Kits Include:

- DNA Capture Beads & Bead Retainers
- 2 ml Tubes & Spin Collection Tubes
- RNase A
- Proteinase K, Molecular Biology Grade
- RBC Lysis Buffer (NEB #T3050 only)
- gDNA Nuclei Prep & Nuclei Lysis Buffers (NEB #T3050 only)
- Precipitation Enhancer (NEB #T3050 only)
- Protein Separation Solution (NEB #T3060 only)
- Pestles & Pestle Tubes (NEB #T3060 only)
- HMW gDNA Tissue Lysis Buffer (NEB #T3060 only)
- gDNA Wash Buffer & Elution Buffer II



Workflow for cells.



DNA fragment size is tunable based on agitation speed during lysis. *Preps were performed on duplicate aliquots of 1 x 106 HEK 293 cells and 500 µl fresh human blood. Samples were agitated at the indicated speed during the lysis step to control the fragmentation of the DNA. Equal amounts of DNA from the replicates (cells: 500 ng; blood: 650 ng) were resolved by PFGE (1% agarose gel, 6 V/cm, 13°C for 20 hours, switch times ramped from 0.5–94 seconds on a BioRad® CHEF-DR III System). Yield and purity ratios of the individual preps are shown in the accompanying tables. Lambda PFG Ladder and Lambda DNA-Hind III Digest (NEB #N0341 and #N3012) were used as molecular weight standards. Yield, purity ratios and DINs of the individual preps are shown in the accompanying tables.*

NEW

Monarch® Mag Viral DNA/RNA Extraction Kit

#T4010S	100 preps
#T4010L	600 preps
#T4010X	1,800 preps

Companion Products:

Monarch Spin RNA Isolation Kit (Mini)
#T2110S 50 preps

Monarch Spin gDNA Extraction Kit
#T3010S 50 preps
#T3010L 150 preps

Monarch StabiLyse DNA/RNA Buffer
#T2111L 145 ml

Monarch Buffer BX
#T2041L 80 ml

Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit
#E3019S 96 reactions

- *Designed for hands-free extraction of viral DNA and/or RNA.*
- *Compatible with manual and automated high-throughput workflows*
- *Tested for saliva, milk and respiratory swab sample types*
- *Compatible with wastewater samples, after enrichment steps (not supplied)*
- *Suitable for qPCR/RT-qPCR, ddPCR, library prep for sequencing/NGS and other downstream applications*
- *Includes carrier RNA for sensitive detection*

Description: The Monarch Mag Viral DNA/RNA Extraction Kit provides a rapid and reliable magnetic bead-based process for extracting viral nucleic acids. The kit combines the efficiency of silica-based nucleic acid purification with the ease of use of magnetic beads. Manual and automated workflows allow samples to be processed in microfuge tubes or 96-well plates. Kit sizes align to 96-well formats (100 preps, 600 preps, and 1800 preps), and the protocol is compatible with high throughput automation on a variety of platforms, including the KingFisher Flex magnetic particle processor and Agilent Bravo and MGISP liquid handler platforms.

Kit Includes:

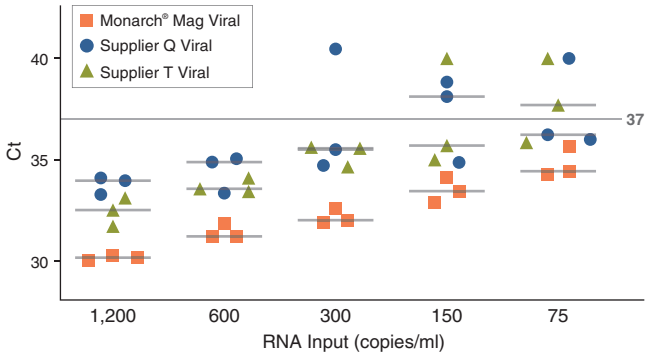
- Mag Beads M1
- Carrier RNA
- Monarch StabiLyse DNA/RNA Buffer
- Buffer BX
- Nuclease-free Water
- Proteinase K, Molecular Biology Grade

Specifications	
Purification format	Magnetic bead
Processing format	Manual or automated
Sample purification (representative examples)	Viral DNA and RNA* from respiratory viruses (enveloped and non-enveloped, dsDNA and ssRNA)
Sample sources	Saliva, respiratory swab in viral transport media (VTM)**
Sample input volume	Up to 200 µl**
Carrier supplier	Poly A carrier RNA***
Binding capacity	Up to 3 µg
Elution volume	33–100 µl
Tested automation platforms	KingFisher Flex; Agilent Bravo and MGISP liquid handlers
Compatible downstream applications	qPCR, RT-qPCR, ddPCR, library prep for NGS

* Viral DNA and RNA are purified in parallel. Preparation of DNA-free RNA or RNA-free DNA requires further treatment with the appropriate nuclease (not supplied).

** The sample input volume may be scalable to accommodate larger sample volumes. Further workflow optimization may be required.

*** Use of carrier RNA is recommended for recovery of low amounts of viral nucleic acid. Carrier RNA should be omitted if the downstream application utilizes poly(A) RNA enrichment; however, viral nucleic acid recovery may be reduced.



Performance comparison of Monarch Mag Viral DNA/RNA Extraction Kit with other suppliers demonstrates high reproducibility and sensitivity of the Monarch kit. *Mock samples representing decreasing viral loads were prepared using Heat-inactivated SARS-CoV-2 (ATCC) in VTM (Hardy Diagnostics®). Extraction was performed using Monarch Mag Viral DNA/RNA Extraction Kit and similar kits from two other suppliers. RT-qPCR was performed using NEB #E3019 and BioRad CFX96 Touch Real-Time PCR Detection System. Monarch Mag Viral DNA/RNA Extraction Kit showed consistently low Cts and reproducible data, even at low viral loads, compared to the competitor kits tested.*

Monarch® Spin RNA Cleanup Kits

Monarch Spin RNA Cleanup Kit (10 µg)
#T2030S 10 preps
#T2030L 100 preps

Monarch Spin RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

Monarch Spin RNA Cleanup Kit (500 µg)
#T2050S 10 preps
#T2050L 100 preps

Companion Products:

Monarch Spin Columns S1A and Tubes
#T2037L 100 columns

Monarch Spin Columns S2A and Tubes
#T2047L 100 columns

Monarch Spin Columns S2B and Tubes
#T2057L 100 columns

Nuclease-free Water
#B1500S 25 ml
#B1500L 100 ml

- *Choose from 3 different binding capacities and flexible elution volumes*
- *Quickly and easily purify large quantities of high quality RNA from in vitro transcription (IVT) reactions*
- *Efficiently remove unincorporated nucleotides from your RNA sample*

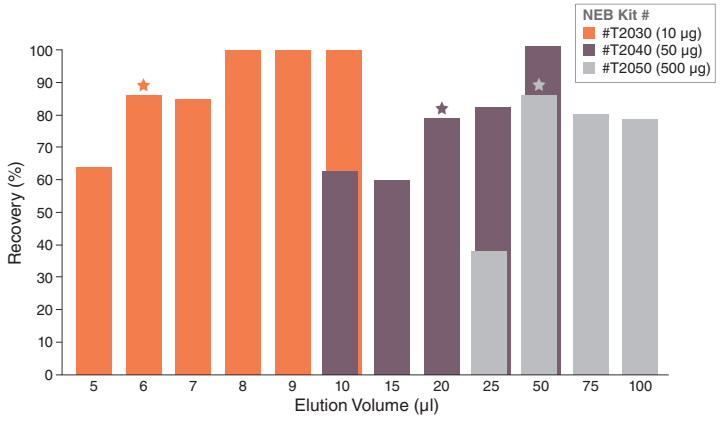
Great for RNA cleanup following *in vitro* transcription with HiScribe® Kits.

Description: The Monarch Spin RNA Cleanup Kits provide a fast and simple silica spin column-based solution for RNA cleanup and concentration after any enzymatic reaction (including *in vitro* transcription, DNase I treatment, capping and labeling) and after other purification methods such as phenol/chloroform extraction. The Monarch Spin RNA Cleanup Kits are available in 3 different binding capacities: 10 µg, 50 µg and 500 µg. Each kit contains unique columns, all designed to prevent buffer retention and ensure no carryover of contaminants, enabling low-volume elution of highly-pure RNA. Following the standard protocol, RNA ≥ 25 nt is purified with this kit; however, a modified protocol is available to enable the binding of RNA as small as 15 nt (including miRNAs).

Kits Include:

- Spin Columns (S1A, S2A, S2B)
- Buffer BX
- Buffer WX
- Collection Tubes
- Nuclease-free Water

Monarch Spin RNA Cleanup Kit	NEB #T2030 (10 µg)	NEB #T2040 (50 µg)	NEB #T2050 (500 µg)
Binding Capacity	10 µg	50 µg	500 µg
RNA Size Range	≥ 25 nt (≥ 15 nt with modified protocol)		
Typical Recovery	70–100%		
Eluion Volume	≥ 6 µl	20–50 µl	50–100 µl
Purity	$A_{260/280}$ > 1.8 and $A_{260/230}$ > 1.8		
Protocol Time	5 minutes of spin and incubation time		10–15 minutes of spin and incubation time



Recovery of RNA from Monarch Spin RNA Cleanup Kits with Varying Elution Volumes. *rRNA (10, 50 or 500 µg, respectively of 16S and 23S Ribosomal Standard from E. coli, Sigma) was purified using a Monarch RNA Cleanup Kit (10 µg, NEB #T2030) (50 µg, NEB #T2040) (500 µg, NEB #T2050). Nuclease-free water was used to elute the RNA. The percent recovery of the RNA was calculated from the resulting A260 as measured using a Trinean® DropSense® 16. ~80% of RNA can be efficiently recovered in 6 µl from the Monarch Spin RNA Cleanup Kit (10 µg, NEB #T2030), 20 µl from the Monarch Spin RNA Cleanup Kit (50 µg, NEB #T2040), and 50 µl from the Monarch Spin RNA Cleanup Kit (500 µg, NEB #T2050).*

NEW

Monarch® Spin RNA Isolation Kit (Mini)

#T2110S 50 preps

Companion Products:

Monarch Spin Columns S2A and Tubes
#T2047L 100 columns

Monarch Spin Columns S2C and Tubes
#T3017L 100 preps

Monarch Spin Collection Tubes
#T2118L 100 tubes

Monarch StabiLyse DNA/RNA Buffer
#T2111L 145 ml

Monarch Buffer WZ
#T1115L 26 ml

- *Use with a wide variety of sample types*
- *Purify RNA of all sized, including miRNA & small RNA >20 nucleotides*
- *Includes DNase I, specialized columns, Proteinase K, and a stabilization reagent*
- *Efficiently remove contaminating genomic DNA*
- *Save money with value pricing for an all-in-one kit*

Description: The Monarch Spin RNA Isolation Kit (Mini) is a comprehensive solution for sample preservation, cell lysis, gDNA removal, and purification of total RNA from various biological samples, including cultured cells, mammalian tissues, microbes, plants, insects and blood. With just this single kit, users can purify up to 100 µg of high-quality total RNA from multiple sample types, from standard as well as tough-to-lyse samples, with included enzymes, reagents, and specialized columns. The kit uniquely enables binding capacities like RNA purification mini kits, combined with the low elution volumes of micro kits. Cleanup of enzymatic reactions and purification of RNA from TRIzol® -extracted samples is also possible using this kit. Purified RNA has metrics with $A_{260/280}$ and $A_{260/230}$ ratios typically > 2.0, high RNA integrity scores, and minimal residual gDNA. This kit captures RNA ranging in size from full-length rRNAs down to intact miRNAs. Purified RNA is suitable for downstream applications such as RT-qPCR, cDNA synthesis, RNA-seq, and RNA hybridization-based technologies.

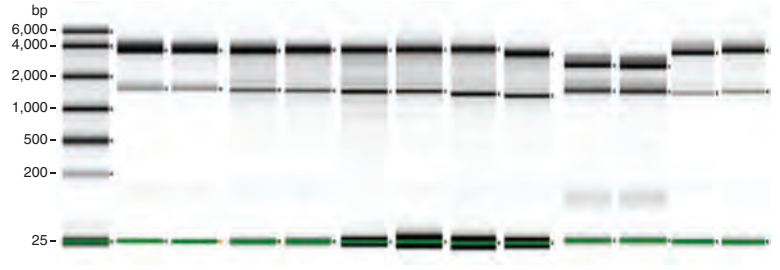
Kit Includes:

- Spin Columns S2C
- Spin Columns S2A
- Spin Collection Tubes
- Monarch StabiLyse DNA/RNA Buffer
- Buffer BX
- Buffer WZ
- DNase I Reaction Buffer
- DNase I, Lyophilized
- Proteinase K, Molecular Biology Grade
- Nuclease-free Water

Specifications	
Binding Capacity	100 µg RNA
RNA Size	≥ 20 nt
Purity	$A_{260/280}$ and $A_{260/230}$ usually ≥ 1.8
Input Amount	up to 10^8 cells or 50 mg tissue*
Elution Volume	≥ 10 µl
Yield	varies depending on sample type
Compatible downstream applications	RNA Library prep for NGS, RT-PCR, RT-qPCR, Northern blots

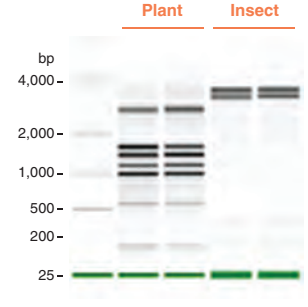
*See "Guidelines for Choosing Sample Input Amounts" in the technical reference section or at **www.neb.com/MonarchRNAInputs.**

A



RIN_e	10.0	10.0	8.9	9.2	8.8	9.6	9.4	9.6	10.0	10.0	10.0	10.0
$A_{260/280}$	2.08	2.08	2.09	2.10	2.10	2.10	2.15	2.09	2.15	2.13	2.07	2.08
$A_{260/230}$	2.36	2.31	2.21	2.27	2.27	2.08	2.13	2.15	2.39	2.35	2.08	2.09

B



$A_{260/280}$	2.11	2.11	2.15	2.16
$A_{260/230}$	2.38	2.34	2.04	1.95

Monarch Spin RNA Isolation Kit (Mini) is a versatile solution for high-quality RNA extraction from a wide variety of sample types. *RNA was extracted in duplicate using the Monarch Spin RNA Isolation Kit (Mini) from various samples representing a range of biological properties in the starting material, including fibrous, fatty and nuclease-rich mammalian tissues, tough-to-lyse samples such as Gram-positive bacteria, plants and insects, as well as enzymatic cleanup reactions. RNA quality was assessed using A_{260}/A_{280} and A_{260}/A_{230} ratios measured using a microvolume spectrophotometer (Lunatic®, Unchained Labs®). RNA integrity was measured using Agilent® automated electrophoresis systems according to sample types. For samples with known typical RNA profiles, Agilent RNA ScreenTape® was used on the TapeStation® 4200 (A). For samples with known atypical RNA profiles such as plant green tissue with plastidal content, and insects with ribosomal gene breaks, Agilent Bioanalyzer® 2100 used with NanoChip (B).*

View video to learn more.

WATCH

Students engaging in a reseach project
Credit: Rogelio Hernández López

Bringing Cutting-Edge Science Education to Local Communities

While pursuing his Ph.D. at Harvard University, Rogelio Hernández-López was struck by the stark contrast between the educational opportunities at elite institutions and those in his native Mexico. Reflecting on his own path from Mexico to one of the world's leading research universities, he felt a profound responsibility to bridge this gap. Together with several of his friends, he envisioned a program that would bring the excitement and rigor of cutting-edge science directly to students who might otherwise never have such opportunities.

In 2014, this vision materialized as Clubes de Ciencia Mexico (Science Clubs Mexico), a non-profit organization dedicated to expanding access to high-quality STEM education across Latin America. The initiative began modestly, with Hernández-López and a group of fellow graduate students organizing workshops in Mexico during their summer breaks. Their goal was to inspire curiosity and passion for science among high school and college students through hands-on, intensive courses.

The inaugural program was a resounding success, igniting enthusiasm among students and instructors alike. Recognizing the potential for broader impact, Hernández-López and his team expanded this model to other countries, including Bolivia, Colombia, Peru, Brazil, Paraguay and Spain. Over the past decade, the program has offered free access to advanced scientific education to over 20,000 students.

A hallmark of Clubes de Ciencia is its immersive, collaborative approach to learning. Workshops cover a diverse array of topics, from astrophysics and biotechnology to robotics and mathematics, and are co-taught by local instructors and international scientists from prestigious institutions. Students engage in real-world research projects, fostering critical thinking, problem-solving skills and teamwork. *"It's about changing the mindset,"* Hernández-López explained. *"We want students to experience the process of scientific inquiry, to ask questions, and to realize that they can contribute to science."*

Hernández-López's commitment to the program is unwavering, even as he balances a demanding career as an Assistant Professor at Stanford University, where he leads a lab focused on engineering cellular therapies for cancer treatment.

Clubes de Ciencia operates with a lean structure, relying heavily on volunteers, local committees and partnerships with educational institutions. Full-time staff coordinates logistics like booking flights, arranging accommodations and securing facilities, while a vast network of volunteers contributes to curriculum development, fundraising and operations. This decentralized model makes the program scalable and deeply rooted in the communities it serves.

One of the most inspiring aspects of Hernández-López's journey is how a simple idea grew into a transformative movement. *"If you had asked me at that time, I would have never imagined that it was going to grow the way it has,"* he admitted. The program's impact is evident in the stories of students who have pursued careers in STEM, inspired by their experiences in the workshops.

By leveraging his knowledge and resources, Rogelio Hernández-López and the Clubes de Ciencia teams have opened doors for thousands of young people, inspiring them to pursue science and promoting diversity, inclusivity and equal opportunity in STEM. His dedication bridges educational gaps, emphasizing the importance of mentorship, access to education and belief in every student's potential.

Rogelio Hernández López
Stanford University, Stanford, CA, USA
2024 Passion in Science
Science Mentorship and Advocacy Award

NEBNext® Reagents for Next Generation Sequencing

Leading the way in library preparation for next generation sequencing.

Library preparation is a critical part of the next generation sequencing workflow; successful sequencing requires the production of high quality libraries of sufficient yield.

As sequencing technologies continue to improve and capacities expand, the need for high performance sample prep is greater than ever, from decreasing input quantities and samples of lower quality, to supporting an expanding range of applications.

To meet these growing challenges, the NEBNext suite of products continues to evolve to support next generation sequencing with sample preparation tools that expand applications, streamline workflows, minimize inputs, and improve library quality and yields.

NEBNext reagents are available for library preparation for DNA, RNA, FFPE, small RNA, single cell and microbiome samples, for use with Illumina®, Oxford Nanopore Technologies®, Ultima Genomics®, Ion Torrent™ and other sequencing platforms. Kits for specialized applications including methylome analysis, virus sequencing and immune repertoire sequencing are also included in the NEBNext line.

Products are supplied in user-friendly formats including kits and modules. A fast-growing range of adaptors and primers is available separately, for maximized flexibility. Use of NEBNext products has been cited in tens of thousands of peer-reviewed publications.

For additional convenience and cost effectiveness in high-throughput workflows, NEBNext reagents are also available in bulk and customized formats. For more information, contact Custom@neb.com.

Find an overview of NGS library preparation.

Featured Products

Featured Tools & Resources

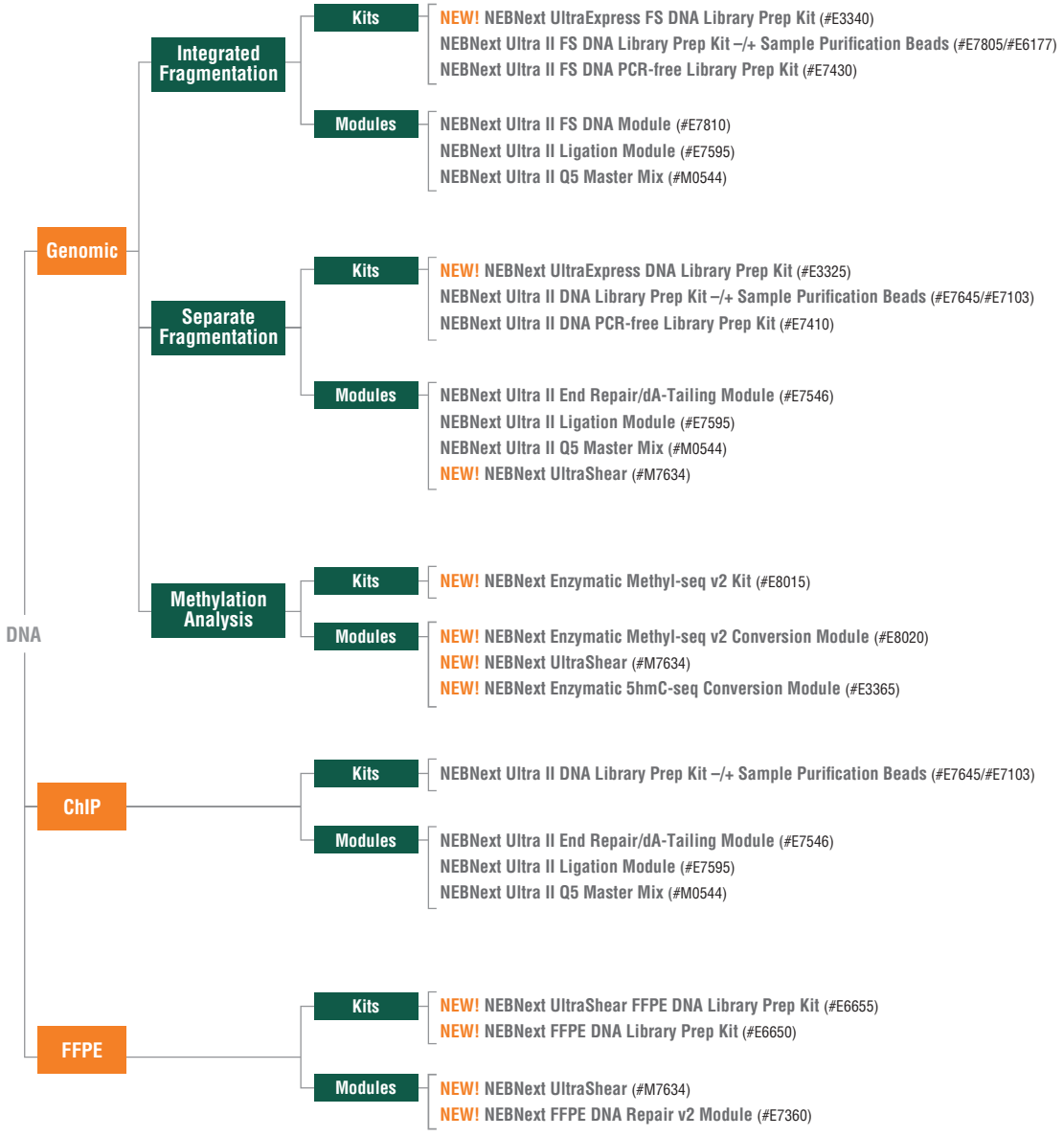
Visit **NEBNextSelector.neb.com** for help with selecting products.

Visit the **NEBNext Index Oligo Selector Tool** for guidance on optimal barcode combinations when multiplexing.

Visit **NEBNext.com** to keep up to date on everything NEBNext.

NEBNext DNA Product Selection Chart

Use the following chart to determine the best NEBNext products for your DNA library prep needs.
For the most up-to-date product and pricing information, visit **NEBNext.com**.



Multiplex Oligos

Single Index	Dual Index	Unique Dual Index	LV Unique Dual Index	Unique Dual Index UMIs
Set 1 (#E7335), Set 2 (#E7500), Set 3 (#E7710), Set 4 (#E7730), 96 Index Primers (#E6609)	Set 1(#E7600), Set 2 (#E7780)	Set 1 (#E6440), Set 2 (#E6442), Set 3 (#E6444), Set 4 (#E6446), Set 5 (#E6448), Primer Pairs for EM-seq (#E7140)	Set 1(#E3400), Set 2 (#E3402), Set 3 (#E3404), Set 4 (#E3406) Set 5 (#E3408), Set 2A (#E3390), Set 2B (#E3392)	DNA Set 1 (#E7395), DNA Set 2 (#E7874), DNA Set 3 (#E7876), DNA Set 4 (#E7878)

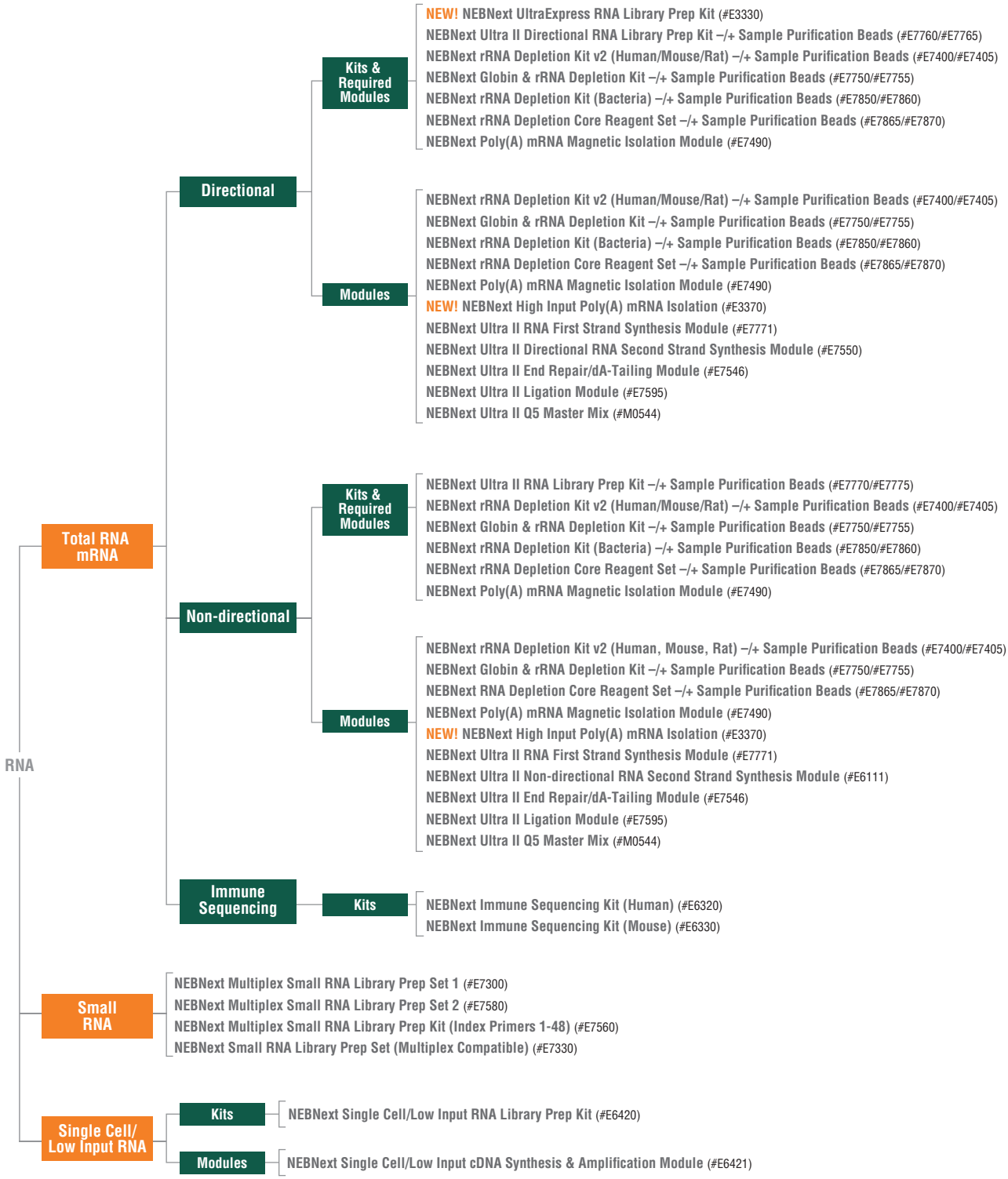
Reagents for the original Ultra workflow are also available.

NEBNext Selector

For help selecting products, try our online product selection tool at **NEBNextSelector.neb.com**

NEBNext RNA Product Selection Chart

Use the following chart to determine the best NEBNext products for your RNA sequencing needs.
For the most up-to-date product and pricing information, visit **NEBNext.com**.



Multiplex Oligos

Single Index	Dual Index	Unique Dual Index	Unique Dual Index UMIs
Set 1 (#E7335), Set 2 (#E7500), Set 3 (#E7710), Set 4 (#E7730), 96 Index Primers (#E6609)	Set 1(#E7600), Set 2 (#E7780)	Set 1 (#E6440), Set 2 (#E6442), Set 3 (#E6444), Set 4 (#E6446), Set 5 (#E6448), Primer Pairs for EM-seq (#E7140)	RNA Set 1 (#E7416)

Reagents for the original Ultra workflow are also available.

NEW

NEBNext UltraExpress® FS DNA Library Prep Kit

#E3340S	24 reactions
#E3340L	96 reactions

Companion Products:

Monarch Spin gDNA Extraction Kit

#T3010S	50 preps
#T3010L	150 preps

NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 5)

#E6448S	96 reactions
#E6448L	384 reactions

NEBNext Library Quant Kit for Illumina

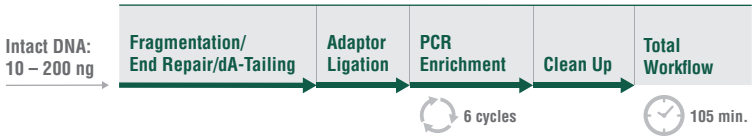
#E7630S	100 reactions
#E7630L	500 reactions

NEBNext Magnetic Separation Rack

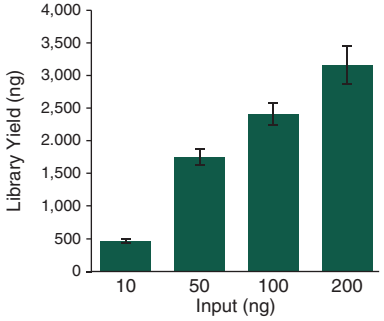
#S1515S	24 tubes

- *Fast workflow (<2 hours)*
- *FS (Fragmentation System) reagents include enzymatic fragmentation, end prep, and dA-tailing with a single enzyme mix*
- *Fewer steps and consumables*
- *Fewer cleanups*
- *Wide input range (10-200 ng intact DNA)*
- *Single protocol for all inputs*
- *Automation-friendly workflows*

Description: The NEBNext UltraExpress FS DNA Library Prep Kit is the latest generation of NEBNext DNA library prep for intact DNA inputs, with a fast, streamlined workflow to generate high yields of high-quality libraries. The workflow with incorporated enzymatic fragmentation step allows processing of samples with a wide range of input amounts of intact DNA using a single protocol, without adjustment of reaction conditions.



NEBNext UltraExpress FS DNA Library Prep workflow.



The NEBNext UltraExpress FS DNA Library Prep Kit provides robust library yields over a wide input range. *Libraries were prepared in triplicate from 10, 50, 100 and 200 ng of a 9:1 Human NA19240 genomic DNA (Coriell Institute for Medical Research) and Escherichia coli gDNA (Lofstrand Labs Limited) mixed sample, using the NEBNext UltraExpress FS DNA single-protocol workflow (e.g., same adaptor amount and 6 PCR cycles for all input amounts). Yields exceeded the minimum requirement (40 ng) for a single Illumina® NovaSeq® 6000 run to achieve whole genome sequencing with at least 30X coverage.*

NEW

NEBNext UltraExpress® DNA Library Prep Kit

#E3325S	24 reactions
#E3325L	96 reactions

Companion Products:

NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 5)

#E6448S	96 reactions
#E6448L	384 reactions

NEBNext Library Quant Kit for Illumina

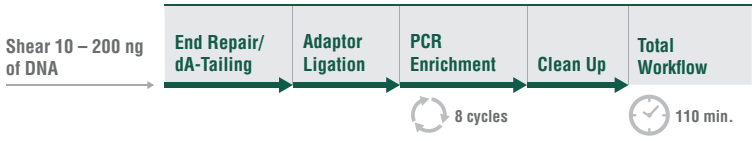
#E7630S	100 reactions
#E7630L	500 reactions

NEBNext Magnetic Separation Rack

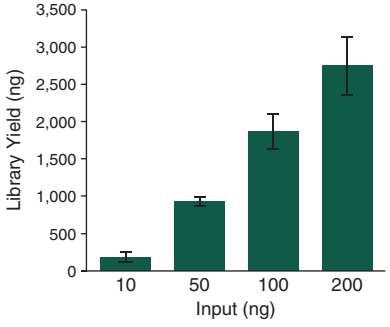
#S1515S	24 tubes

- *Fast workflow (<2 hours)*
- *Fewer steps and consumables*
- *Fewer cleanups*
- *Wide input range (10-200 ng pre-sheared DNA)*
- *Single protocol for all inputs*
- *Automation-friendly workflows*

Description: The NEBNext UltraExpress DNA Library Prep Kit is the latest generation of NEBNext DNA library prep, with a fast, streamlined workflow to generate high yields of high-quality libraries. The workflow allows processing of samples with a wide range of input amounts of pre-sheared DNA using a single protocol, without adjustment of reaction conditions.



NEBNext UltraExpress DNA Library Prep workflow



The NEBNext UltraExpress DNA Library Prep Kit provides robust library yields over a wide input range. *Libraries were prepared from 10, 50, 100 or 200 ng of Human NA19240 genomic DNA (Coriell Institute for Medical Research) using the same adaptor amount and 8 PCR cycles. Yields exceeded the minimum requirement (40 ng) for a single Illumina® NovaSeq® 6000 run to achieve whole genome sequencing with at least 30X coverage.*

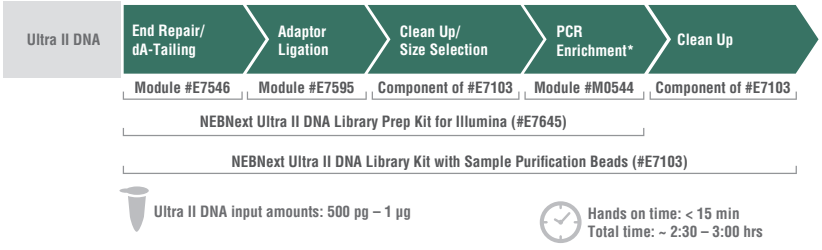
The heart of the matter – NEBNext® Ultra™ II Workflow

As sequencing technologies continue to improve and applications expand, the need for compatibility with ever-decreasing input amounts and sub-optimal sample quality grows. Reliability and high performance are critical, along with faster turnaround, higher throughput and automation compatibility.

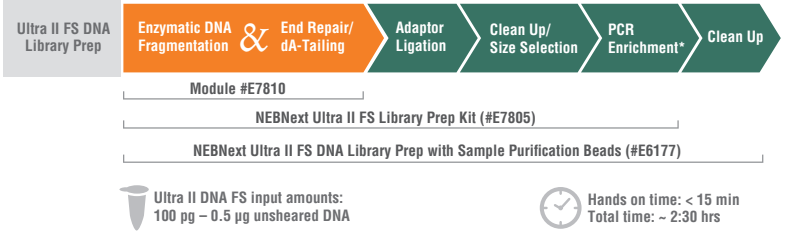
The NEBNext Ultra II workflow lies at the heart of NEB's portfolio for next generation sequencing library preparation. NEBNext Ultra II kits and modules for Illumina are the perfect combination of reagents, optimized formulations and simplified workflows, enabling you to generate DNA or RNA libraries of the highest quality and yield, even when starting from extremely low input amounts.

- *Learn one central workflow and apply it to a suite of different applications*
- *Save time with streamlined modular workflows, reduced hands-on time and automation compatibility*
- *Benefit from low input amount requirements, fewer PCR cycles and uniform GC coverage in all applications*

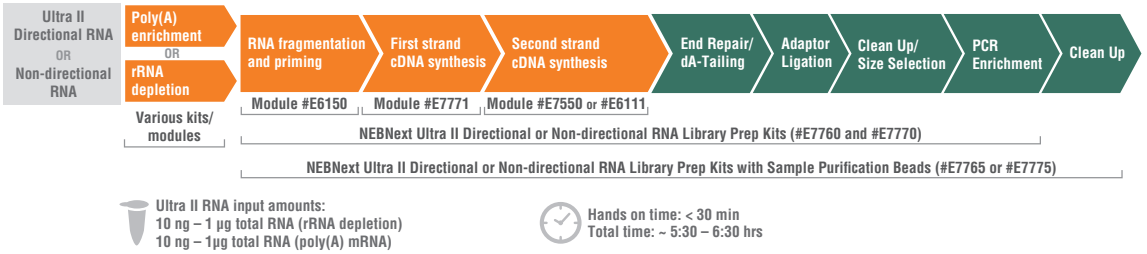
The Ultra II workflow is central to many NEBNext solutions, including:

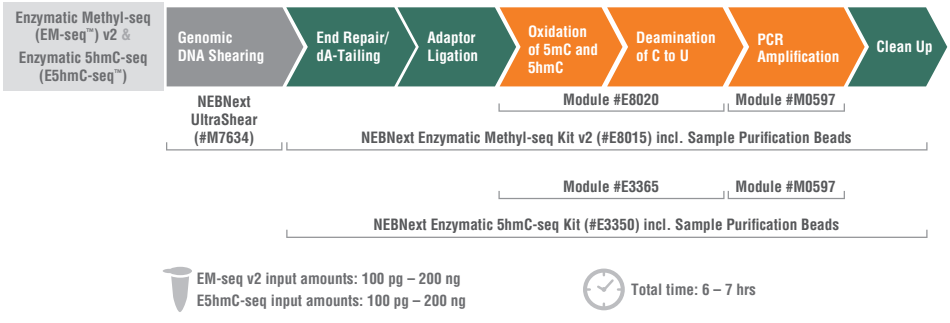


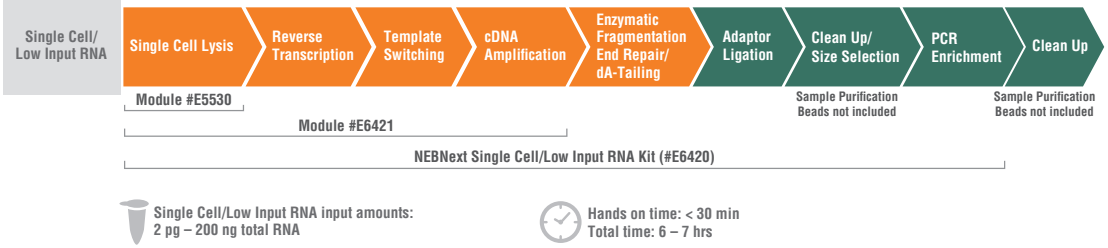
**PCR-free workflows are also available*



**PCR-free workflows are also available*







NEBNext® Ultra™ II DNA, FS and PCR-free DNA Library Prep Kits for Illumina®

NEBNext Ultra II DNA Library Prep Kit for Illumina
#E7645S 24 reactions
#E7645L 96 reactions

NEBNext Ultra II DNA Library Prep with Sample Purification Beads
#E7103S 24 reactions
#E7103L 96 reactions

NEBNext Ultra II DNA PCR-free Library Prep Kit for Illumina
#E7410S 24 reactions
#E7410L 96 reactions

NEBNext Ultra II FS DNA Library Prep Kit for Illumina
#E7805S 24 reactions
#E7805L 96 reactions

NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads
#E6177S 24 reactions
#E6177L 96 reactions

NEBNext Ultra II FS DNA PCR-free Library Prep Kit for Illumina
#E7430S 24 reactions
#E7430L 96 reactions

NEBNext Ultra II FS DNA PCR-free Library Prep with Sample Purification Beads
#E7435S 24 reactions
#E7435L 96 reactions

- *Get more of what you need, with the highest library yields*
- *Generate high quality libraries even with limited amounts of DNA, as low as 500 pg*
- *Prepare libraries from ALL of your samples, including GC-rich targets and FFPE DNA samples*
- *Save time with streamlined workflows, reduced hands-on time, and automation compatibility*
- *Access reliable and easy-to-use, scalable enzymatic DNA fragmentation, integrated into the workflow with the FS kit*
- *Enjoy the flexibility and reliability of the gold standard SPRIselect size selection and clean-up beads, supplied in just the amounts you need*

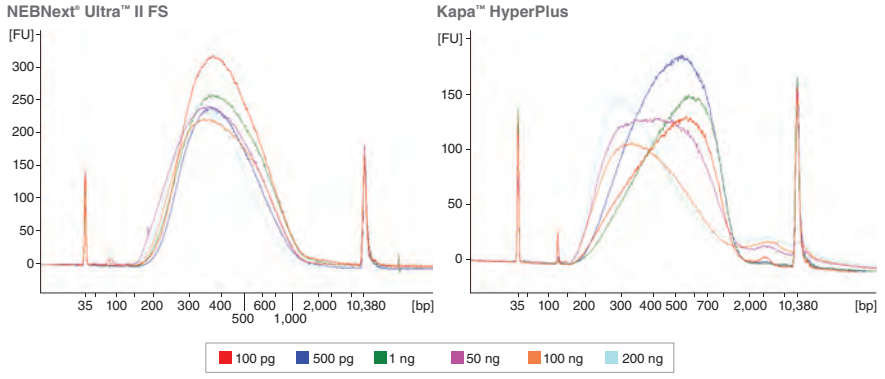
Visit **NEBNextUltraII.com** for more information, including our technical notes and protocol videos

Description: NEBNext Ultra II DNA Library Prep Kits for Illumina meet the challenge of constructing high quality libraries from ever-decreasing input quantities, enabling high yield preparation of high quality libraries from 100 picograms to 1 microgram of input DNA. Ultra II kits use a fast, streamlined, automatable workflow and enable use of fewer PCR cycles while also improving GC coverage. The kit is also effective with challenging samples such as FFPE DNA.

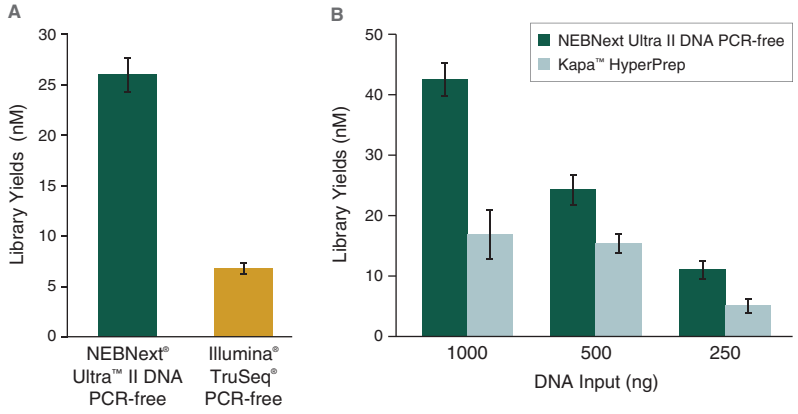
The Ultra II FS DNA Library Prep Kit combines robust enzymatic DNA fragmentation with end repair and dA-tailing, integrated into a streamlined library prep workflow.

PCR-free kits are now available for both the Ultra II DNA and Ultra II FS DNA workflows.

The Ultra II FS kits are available with or without SPRIselect® beads.



NEBNext Ultra II FS DNA provides consistent fragmentation regardless of input amount. *Libraries were prepared from Human NA19240 genomic DNA using the input amounts shown. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. For Kapa HyperPlus®, input DNA was cleaned up with 3X beads prior to library construction, as recommended, and a 20-minute fragmentation time. Library size was assessed using the Agilent® Bioanalyzer®. Low input (1 ng and below) libraries were loaded on the Bioanalyzer without a dilution. High input libraries were loaded with a 1:5 dilution in 0.1X TE.*

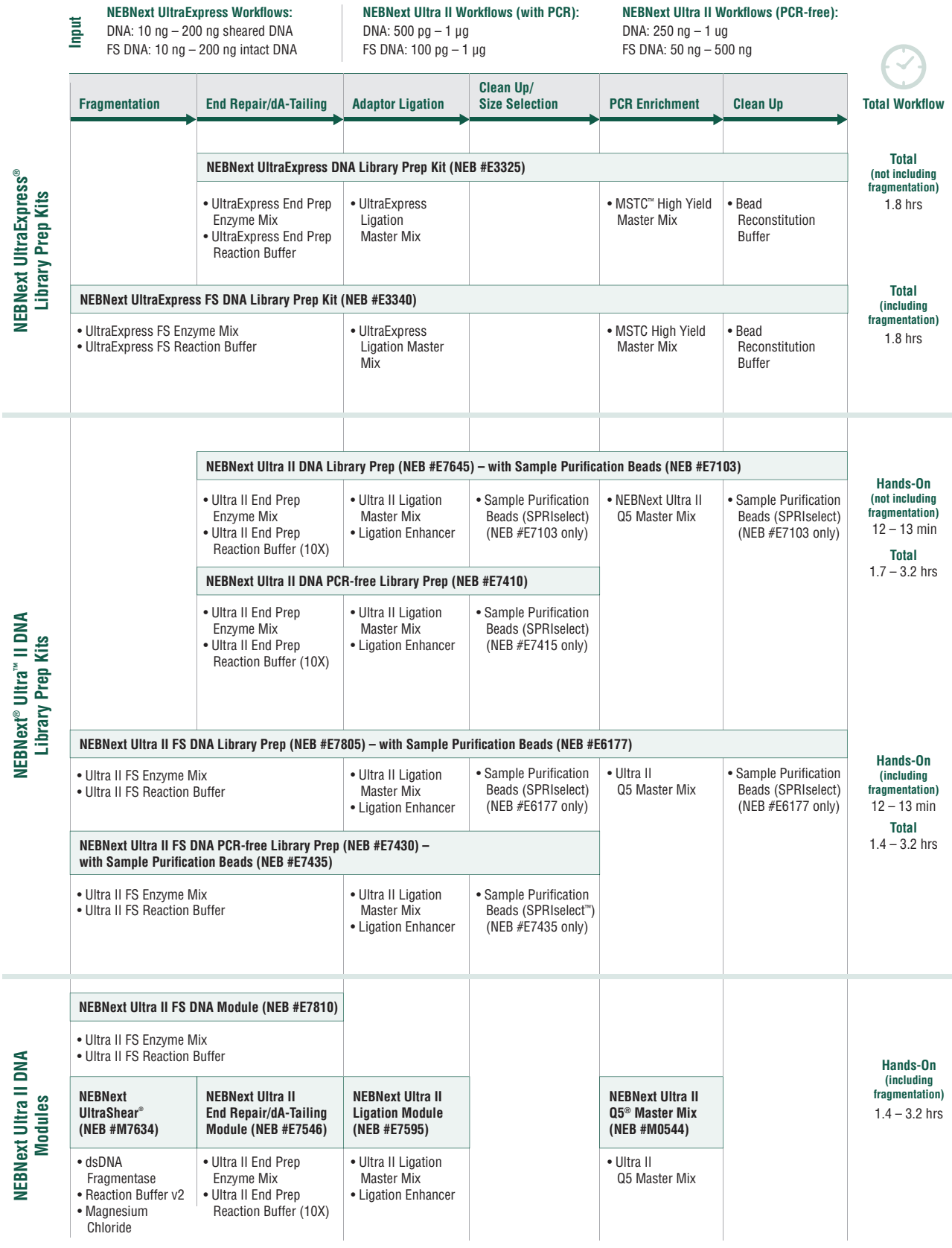


NEBNext Ultra II DNA PCR-free Library Prep Kit generates libraries with higher yields.
A. *PCR-free libraries were prepared with NA19240 genomic DNA (Coriell Institute) using NEBNext Ultra II DNA PCR-free and Illumina TruSeq® PCR-free library prep kits and size selected for 350 bp inserts. DNA inputs were 1 µg.*

B. *Libraries of 150-200 bp inserts were prepared using NEBNext Ultra II DNA PCR-free and Roche Sequencing Kapa HyperPrep library prep kits coupled with Covaris shearing without size selection. NEBNext Unique Dual Index UMI Adaptors DNA Set 1, IDT for Illumina (TruSeq DNA UD Indexes) and Kapa Dual-Indexed Adaptors were used for the NEBNext, Illumina, and Kapa kits, respectively, following manufacturers' recommendations.*

NEBNext® DNA Library Prep Reagents

NEBNext UltraExpress® and NEBNext Ultra™ II Kits for DNA are available with or without integrated enzymatic DNA fragmentation. NEBNext Multiplex Oligos (Adaptors and Primers) are available in a range of options; learn more at **www.neb.com/oligos**. In addition to stringent QCs on individual components, the NEBNext DNA kits are also functionally validated by preparation of a library, followed by Illumina sequencing.



NEBNext Enzymatic Methyl-seq v2 (EM-seq™)

NEW
NEBNext Enzymatic Methyl-seq v2 Kit
#E8015S 24 reactions
#E8015L 96 reactions

NEW
NEBNext Enzymatic Methyl-seq v2 Conversion Module
#E8020S 24 reactions
#E8020L 96 reactions

Companion Products:

NEBNext Enzymatic Methyl-seq Kit
#E7120S 24 reactions
#E7120L 96 reactions

NEBNext UltraShear
#M7634S 24 reactions
#M7634L 96 reactions

NEBNext LV Unique Dual Index Primers Set 2A
#E3390S 24 reactions

NEBNext LV Unique Dual Index Primers Set 2B
#E3392S 24 reactions

NEBNext LV Unique Dual Index Primers Set 1
#E3400S 96 reactions

NEBNext LV Unique Dual Index Primers Set 2
#E3402S 96 reactions

NEBNext LV Unique Dual Index Primers Set 3
#E3404S 96 reactions

NEBNext LV Unique Dual Index Primers Set 4
#E3406S 96 reactions

NEBNext LV Unique Dual Index Primers Set 5
#E3408S 96 reactions

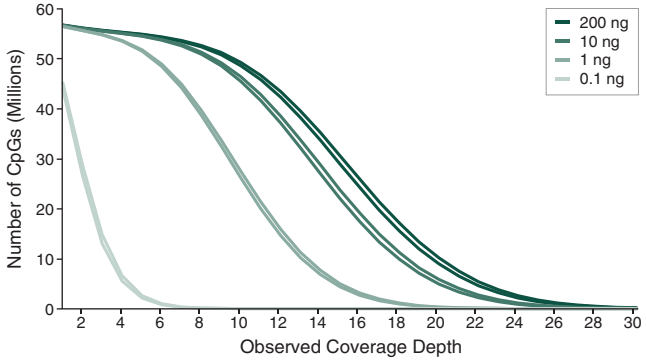
NEBNext Magnetic Separation Rack
#S1515S 24 tubes

- *Superior sensitivity of detection of 5mC and 5hmC*
- *0.1 ng - 200 ng input range*
- *Detection of more CpGs with fewer sequencing reads*
- *Even GC coverage*
- *High performance library preparation and larger library insert sizes*
- *Index primers supplied separately*
- *Enzymatic fragmentation of DNA compatible with EM-seq workflows can be achieved using NEBNext UltraShear® (NEB #M7634)*

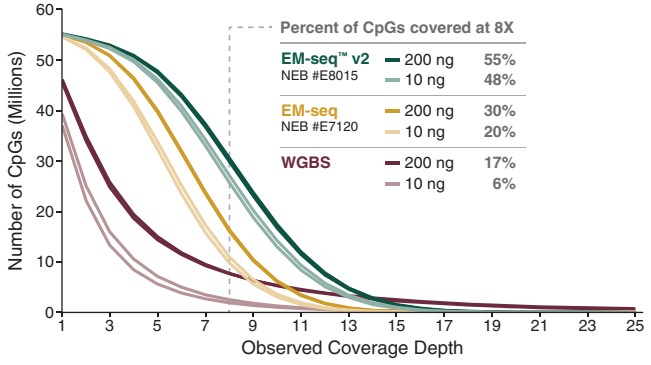
Description: NEBNext Enzymatic Methyl-seq (EM-seq™) is a high-performance enzyme-based alternative to bisulfite conversion for the identification of 5mC and 5hmC. Unlike bisulfite conversion, this highly efficient method minimizes DNA damage, resulting in superior detection of methylated cytosines, with fewer sequencing reads.

The new NEBNext Enzymatic Methyl-seq v2 Kit has a wider input range (as low as 100 pg) and a faster, more streamlined workflow than the original EM-seq kit (NEB #E7120).

The NEBNext Enzymatic Methyl-seq v2 Kit includes conversion reagents, library prep reagents and the EM-seq Adaptor. Multiple sets of the required index primers (NEBNext LV Unique Dual Index Primers) are available separately, enabling greater flexibility in multiplexing.



EM-seq™ v2 exhibits high CpG coverage across a range of inputs. *EM-seq™ v2 libraries were prepared from 200–0.1 ng of NA12878 DNA (sheared to 350 bp using Covaris® ME220), spiked with unmethylated lambda and CpG-methylated pUC19. Libraries were sequenced on an Illumina® NovaSeq® 6000 (2 x 150 bases). Approximately 910 million reads for each library were aligned to a composite human T2T, lambda and pUC19 reference genome using bwa-meth. The T2T genome covers a maximum of 67.8 million CpGs when the top and bottom strands are counted independently. EM-seq covered over 56 million CpG sites for 200–1 ng inputs and roughly 45 million CpG sites for 0.1 ng input libraries.*



NEBNext EM-seq™ v2 identifies more CpGs than WGBS and the original EM-seq, at lower sequencing coverage depth. *EM-seq™ v2 (NEB #E8015), EM-seq (NEB #E7120) and WGBS libraries were prepared from 200 ng and 10 ng of NA12878 DNA (sheared to ~350 bp), spiked with unmethylated lambda and CpG-methylated pUC19. Libraries were sequenced on an Illumina NovaSeq 6000. For accurate comparison of the original EM-seq and WGBS data with EM-seq v2 data, we evaluated data from approximately 625 million 100 base reads for each library aligned to a composite human T2T, lambda and pUC19 reference genome using bwa-meth. The T2T genome covers a maximum of 67.8 million CpGs when the top and bottom strands are counted independently. EM-seq v2 and EM-seq covered over 54 million CpG sites for both 200 ng and 10 ng inputs; however, WGBS libraries covered only 46 million and 39 million for 200 ng and 10 ng inputs respectively at 1X coverage. The dashed lines represent coverage of 8X. The table lists the percentage of CpG sites covered by different libraries at 8X coverage level.*

View the EM-seq workflow.

NEBNext® Enzymatic 5hmC-seq (E5hmC-seq™)

NEW
NEBNext Enzymatic 5hmC-seq Kit
#E3350S 24 reactions
#E3350L 96 reactions

NEW
NEBNext Enzymatic 5hmC-seq Conversion Module
#E3365S 24 reactions
#E3365L 96 reactions

Companion Products:

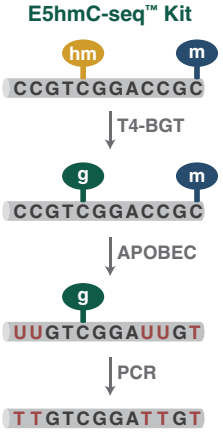
NEBNext LV Unique Dual Index Primers Set 2B
#E3392S 24 reactions

NEBNext LV Unique Dual Index Primers Set 3
#E3404S 96 reactions

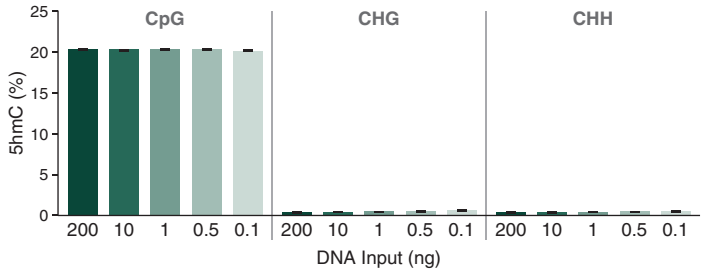
NEBNext UltraShear
#M7634S 24 reactions
#M7634L 96 reactions

- *Specific detection of 5hmC at the single base level*
- *Enzyme-based workflow enables high yields and high-quality data*
- *0.1 ng – 200 ng inputs*
- *Minimal GC bias*
- *E5hmC-seq and EM-seq data can be combined*
- *Conversion module available separately*

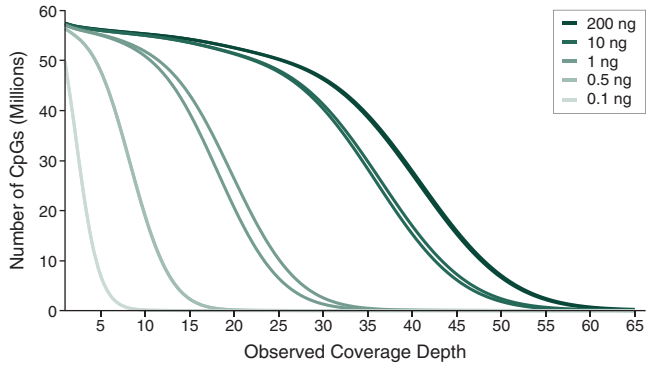
Description: While NEBNext Enzymatic Methyl-seq (EM-seq) detects both 5mC and 5hmC, it does not distinguish between them. Specific detection of 5hmC sites is now enabled by the NEBNext Enzymatic 5hmC-seq Kit (E5hmC-seq™). The kit includes NEBNext Ultra II library prep reagents, and 5hmC is detected using a two-step enzymatic conversion workflow (Figure 1), that minimizes damage to DNA and allows discrimination of 5hmC from both cytosine and 5mC after Illumina sequencing. E5hmC-seq data can also be subtracted from EM-seq data, allowing determination of the precise location of individual 5mC and 5hmC sites.



E5hmC-seq™ conversion method. *To enable specific 5hmC detection, 5hmC is first glucosylated using T4-BGT. 5mC and unmodified cytosine are then deaminated by APOBEC to thymine and uracil, respectively, while the protected 5hmC is not converted. During Illumina® sequencing, 5hmCs are represented as cytosine, while cytosine and 5mCs are represented as thymine.*



5hmC detected by E5hmC-seq™ in human brain gDNA is consistent across inputs. *200–0.1 ng of human brain genomic DNA was sheared to 350 bp (Covaris® ME220) and E5hmC-seq libraries were prepared and sequenced on an Illumina NovaSeq® 6000 (2 x 150 bases). Approximately 1.9 billion (200 ng, 10 ng and 1 ng) or 715 million (0.5 ng and 0.1 ng) reads for each library were aligned to a composite human T2T, lambda and T4 reference genome using bwa-meth, and methylation information was extracted from the alignments using MethylDackel. Values shown are the average of two technical replicates and error bars show standard deviation. Detected 5hmC levels are similar between all inputs in the CpG, CHH and CHG contexts.*



E5hmC-seq™ exhibits high CpG coverage across a range of inputs. *200–0.1 ng of human brain genomic DNA was sheared to 350 bp (Covaris ME220) and E5hmC-seq libraries were prepared and sequenced on an Illumina NovaSeq 6000 (2 x 150 bases). Approximately 1.9 billion (200 ng, 10 ng and 1 ng) or 715 million (0.5 ng and 0.1 ng) reads for each library were aligned to a composite human T2T, lambda and T4 reference genome using bwa-meth. Methylation information was extracted from the alignments using MethylDackel and reported in methylkit format across all three contexts. Using the CpG specific file a cumulative coverage plot was generated for CpG sites covered using E5hmC-seq libraries across all inputs. The T2T genome covers a maximum of 67.8 million CpGs when the top and bottom strands are counted independently. E5hmC-seq covered over 56 million CpG sites for 0.5 ng to 200 ng inputs and roughly 48 million CpG sites for 0.1 ng input libraries.*

NEBNext® FFPE DNA Library Prep & UltraShear® FFPE DNA Library Prep Kits

NEW
NEBNext FFPE DNA Library Prep Kit
#E6650S 24 reactions
#E6650L 96 reactions

NEW
NEBNext UltraShear FFPE DNA Library Prep Kit
#E6655S 24 reactions
#E6655L 96 reactions

Companion Products:

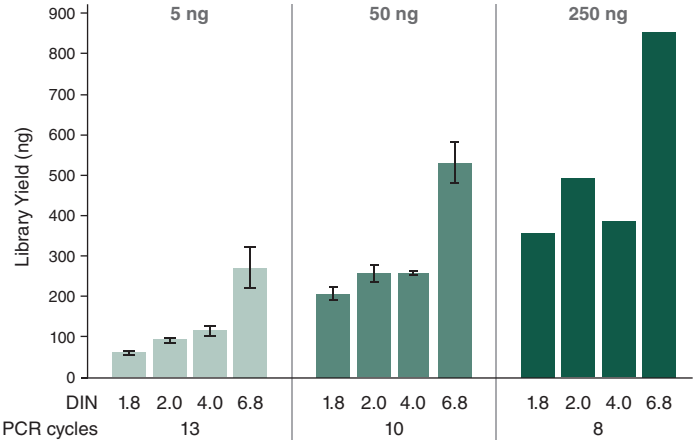
NEBNext Multiplex Oligos for Illumina
(96 Unique Dual Index Primer Pairs)
#E6440S 96 reactions
#E6440L 384 reactions

NEBNext Multiplex Oligos for Illumina
(96 Unique Dual Index Primer Pairs Set 3)
#E6444S 96 reactions
#E6444L 384 reactions

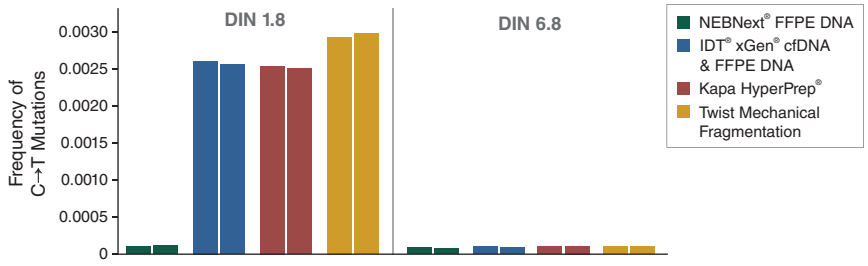
- *Includes FFPE DNA repair reagents plus optimized library prep reagents and protocol*
- *Optional NEBNext UltraShear enzymatic fragmentation*
- *Increased library yields*
- *Improved sequencing metrics*
- *Greater sensitivity of somatic variant calling*
- *Automation-friendly workflows*

Description: FFPE DNA poses many challenges for library preparation, including characteristically low input amounts and highly variable damage from fixation, storage, and extraction methods. Regions of interest are often enriched using hybrid capture-based approaches – these workflows require high inputs of diverse, uniform DNA libraries.

The NEBNext FFPE DNA Library Prep Kit includes the NEBNext FFPE DNA Repair v2 Module, an optimized cocktail of enzymes designed to repair FFPE DNA, library prep reagents featuring a new polymerase master mix, and a protocol optimized for FFPE DNA. The NEBNext UltraShear FFPE DNA Library Prep Kit also includes NEBNext UltraShear, a new solution designed for enzymatic fragmentation of challenging samples (e.g., FFPE DNA). This enzymatic shearing solution further increases library yields and quality, while eliminating artifacts typical of other enzymatic fragmentation approaches.



The NEBNext FFPE DNA Library Prep Kit enables robust library preparation from a range of sample inputs and quality. *Libraries were prepared from 5, 50 or 250 ng of Covaris®-sheared normal tissue FFPE DNA ranging in quality from DNA Integrity Number (DIN) 1.8 to 6.8 with the indicated PCR cycles. Libraries were made in triplicate for 5 and 50 ng input and 1 replicate for 250 ng. Each bar represents the average of triplicates with error bars indicating standard deviation for the 5 and 50 ng inputs. Robust library yields were obtained across sample qualities and input amounts. Most target enrichment workflows require 200 ng of library for hybrid capture input, and sufficient library yield can be obtained using a minimum of 50 ng FFPE DNA with the NEBNext FFPE DNA Library Prep Kit.*



NEBNext FFPE DNA Library Prep Kit reduces damage-derived sequencing artifacts. *Libraries were prepared from 50 ng of Covaris-sheared normal tissue FFPE DNA of either low (DIN 1.8) or high (DIN 6.8) quality using the NEBNext FFPE DNA Library Prep Kit and other library prep kits as shown. Libraries were sequenced on the Illumina® NextSeq® 500 (2 x 76 bases). Libraries were downsampled to 600,000 paired-end reads, mapped using bowtie2 (version 2.3.2.2) to the GrCh38 reference, and duplicates marked using Picard MarkDuplicate (version 1.56.0). The average frequency of C→T mutations at each C position in Read 2 was calculated for two technical replicates using Tasmanian (version 1.0.7). C→T mutations arising from cytosine deamination damage in low quality FFPE DNA are effectively repaired by the NEBNext FFPE DNA Repair v2 mix included in the NEBNext FFPE DNA Library Prep Kit. Other kits show a high level of C→T artifacts in low quality FFPE DNA (DIN 1.8) due to a lack of DNA damage repair.*

NEBNext® FFPE DNA Repair v2 Module

#E7360S	24 reactions
#E7360L	96 reactions

Companion Product:

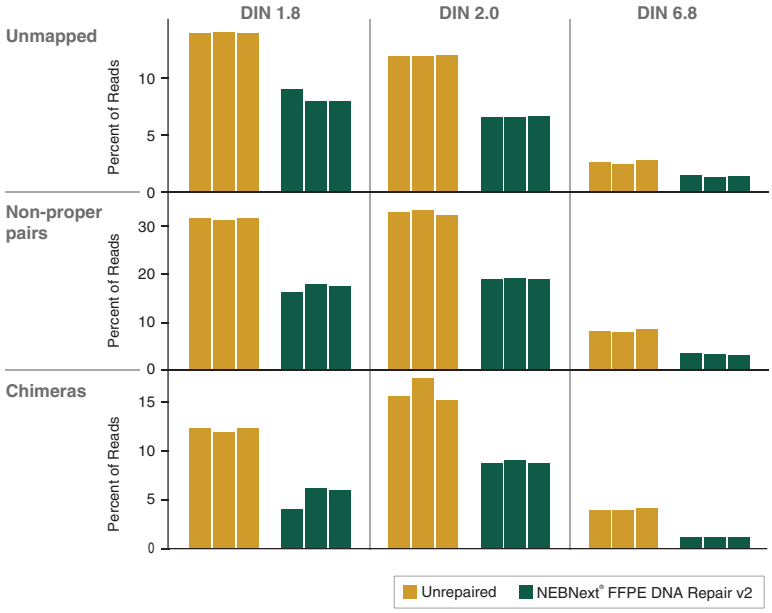
NEBNext FFPE DNA Repair Mix

#M6630S	24 reactions
#M6630L	96 reactions

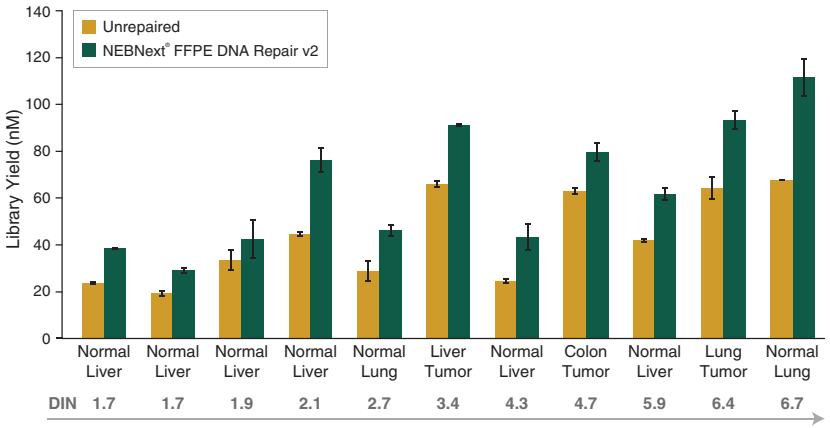
- *Higher repair efficiency with FFPE DNA*
- *A more convenient reaction buffer containing all the required buffer components for both efficient FFPE DNA repair and downstream end repair and dA-tailing*
- *No cleanup is required between repair and library prep, through the use of Thermolabile Proteinase K*

Description: The methods used for fixation and storage of Formalin-Fixed, Paraffin-Embedded (FFPE) DNA samples cause significant damage, making it challenging to obtain high quality sequence data. The NEBNext FFPE DNA Repair v2 Module is an optimized cocktail of enzymes designed to repair FFPE DNA, and supplied with optimized reagents to enable a streamlined workflow for NGS library preparation.

The NEBNext FFPE DNA Repair v2 Module improves upon the performance of the original NEBNext FFPE DNA Repair Mix, and offers higher efficiency, a more streamlined workflow, a more convenient reaction buffer and no cleanup is required between repair and library prep.



The NEBNext FFPE DNA Repair v2 Module improves library quality metrics including mapping rate, properly paired reads, and chimeric reads. *Libraries were prepared with 50 ng of three different quality normal liver FFPE DNA samples in triplicate, either untreated or treated with the NEBNext FFPE DNA Repair v2 Module before library preparation using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Libraries were sequenced on the Illumina NextSeq® 500. Paired-end reads were downsampled to 1 million reads and mapped to the GRCh38 human reference (RefSeq 884148) using Bowtie2 (v2.3.2). Mapped reads were analyzed with MarkDuplicates (v1 56.0) and Picard SAM/BAM alignment summary metrics (v1.56.0). Treatment with the NEBNext FFPE DNA Repair v2 Module increases the mapping rate and decreases the level of non-properly paired and chimeric reads.*



The NEBNext FFPE DNA Repair v2 Module enables robust library preparation from a broad range of FFPE DNA sample qualities. *Libraries were prepared with 25 ng of Covaris® acoustic-sheared FFPE DNA samples of different qualities and tissue sources. The NEBNext FFPE DNA Repair v2 Module was used, followed by NEBNext Ultra II DNA library preparation (NEB #E7645) with 9 PCR cycles. Libraries were quantified using the Agilent® HS D1000 TapeStation®. The NEBNext FFPE DNA Repair v2 Module improves the yield of FFPE libraries by varying degrees depending on the quality and damage types present in the input DNA. Error bars indicate the standard deviation of two replicates for each library sample.*

NEBNext UltraShear®

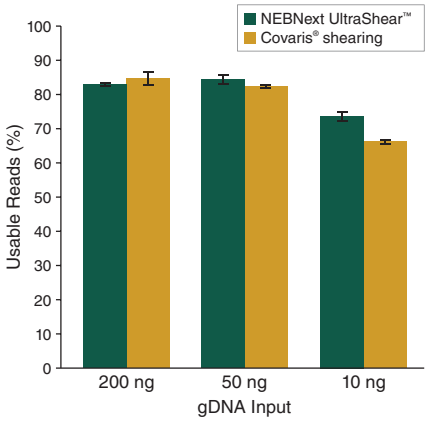
#M7634S 24 reactions
#M7634L 96 reactions

- *Compatible with methylation analysis workflows, including NEBNext® Enzymatic Methyl-seq (EM-seq™)*
- *Compatible with FFPE DNA*
- *Fast workflow with minimal hands-on time*
- *For methylation analysis, improves CpG coverage and sequencing metrics*
- *For FFPE DNA, increases usable reads and coverage uniformity*

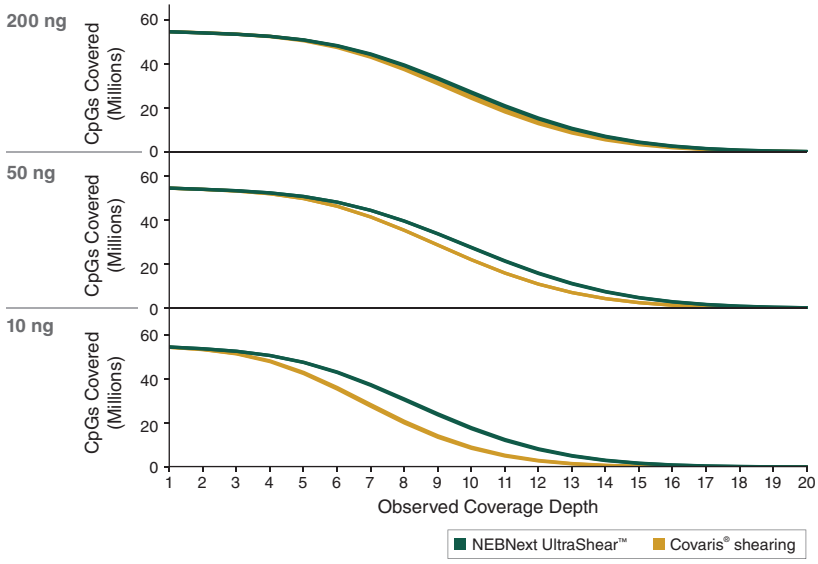
Note that the NEBNext Ultra II FS DNA Library Kit for Illumina (NEB #E7805, #E6177) is recommended for Illumina library prep with high quality genomic DNA, and provides a streamlined workflow.

Description: Enzymatic fragmentation of DNA as part of the library prep workflow provides many advantages compared to mechanical shearing. However, specialized fragmentation reagents are required for enzymatic shearing of challenging samples such as FFPE DNA, and in order to maintain methylation marks on samples for methylome analysis.

NEBNext UltraShear is a mix of enzymes that has been designed and optimized to fragment these sample types upstream of library preparation. This improves library yields and diversity, and allows retention of methylation marks, while eliminating sequencing artifacts typical of other enzymatic fragmentation approaches.



NEBNext UltraShear increases EM-seq library yields. *200 ng, 50 ng and 10 ng of NA12878 DNA spiked with control DNA used in the EM-seq workflow (CpG-methylated pUC19 DNA and unmethylated lambda DNA) were fragmented by either NEBNext UltraShear (20 minutes at 37°C) or Covaris ME220 (350 bp protocol) followed by EM-seq library preparation. Library yields were quantified using Agilent TapeStation with the High Sensitivity D1000 ScreenTape. EM-seq libraries fragmented by NEBNext UltraShear have higher yields than Covaris for the same number of PCR cycles for each input (200 ng = 4 cycles; 50 ng = 6 cycles; 10 ng = 8 cycles).*



Improved CpG coverage in EM-seq libraries produced using NEBNext UltraShear. *200 ng, 50 ng and 10 ng of NA12878 DNA spiked with control DNA (CpG-methylated pUC19 DNA and unmethylated lambda DNA) used in the NEBNext EM-seq workflow was fragmented by either NEBNext UltraShear (20 minutes at 37°C) or Covaris ME220 (350 bp protocol) followed by EM-seq library preparation. Technical replicates were generated for each input amount. All libraries were sequenced on the same flowcell of an Illumina NovaSeq 6000 (2x100 bases). 725 million reads were sampled (seqtk) from each library for methylation analysis. Reads were adaptor trimmed (fastp), aligned to the GRCh38 reference (bwa-meth), and duplicate marked (Picard MarkDuplicates) before calling methylation using MethylDackel. NEBNext UltraShear and Covaris fragmentation used ahead of the NEBNext EM-seq workflow yielded a similar number of CpGs (~54 million) at minimum 1X coverage. At minimum 10X coverage, more CpGs are identified when NEBNext UltraShear is used, due to improved library diversity and coverage evenness.*

NEBNext® dsDNA Fragmentase®

#M0348S 50 reactions
#M0348L 250 reactions

Companion Product:

NEBNext dsDNA Fragmentase Reaction Buffer v2
#B0349S 6 ml

- *Generation of dsDNA fragments for sequencing on next generation sequencing platforms*

Description: NEBNext dsDNA Fragmentase generates dsDNA breaks in a time-dependent manner to yield 50–1,000 bp DNA fragments depending on reaction time. NEBNext dsDNA Fragmentase contains two enzymes, one randomly generates nicks on dsDNA and the other recognizes the nicked site and cuts the opposite DNA strand across from the nick, producing dsDNA breaks. The resulting DNA fragments contain short overhangs, 5´-phosphates, and 3´-hydroxyl groups. The random nicking activity of NEBNext dsDNA Fragmentase has been confirmed by preparing libraries for next-generation sequencing. A comparison of the sequencing results between gDNA prepared with NEBNext dsDNA fragmentase and with mechanical shearing demonstrates that the NEBNext dsDNA Fragmentase does not introduce any detectable bias during the sequencing library preparation and no difference in sequence coverage is observed using the two methods.

NEW

NEBNext UltraExpress® RNA Library Prep Kit

#E3330S	24 reactions
#E3330L	96 reactions

Companion Products:

NEBNext Poly(A) mRNA Magnetic Isolation Module

#E7490S	24 reactions
#E7490L	96 reactions

NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)

#E7400S	6 reactions
#E7400L	24 reactions
#E7400X	96 reactions

NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 5)

#E6448S	96 reactions
#E6448L	384 reactions

NEBNext Library Quant Kit for Illumina

#E7630S	100 reactions
#E7630L	500 reactions

NEBNext Magnetic Separation Rack

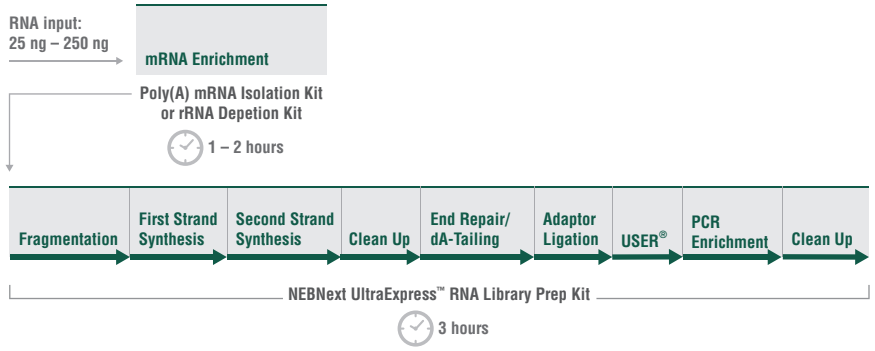
#S1515S	24 tubes

Monarch Spin RNA Isolation Kit (Mini)

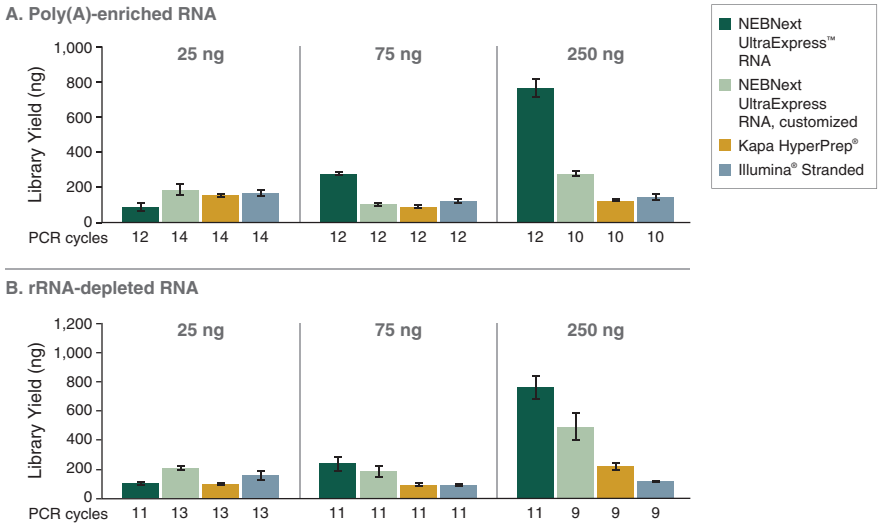
#T2011S	50 preps

- *Fast workflow (3 hours)*
- *Fewer steps and consumables*
- *Fewer cleanups*
- *Single protocol for all inputs*
- *Compatible with a range of sample types including bacterial RNA, human whole blood and FFPE RNA*
- *Automation-friendly workflows*

Description: The NEBNext UltraExpress RNA Library Prep Kit is the latest generation of NEBNext RNA library prep, with a fast, streamlined workflow. The kit is compatible with mRNA isolation and rRNA depletion workflows and a wide range of sample types. With a 3-hour library prep protocol, the kit enables creation of high-quality RNA libraries in a single day, in conjunction with mRNA or rRNA depletion kits.



High quality RNA-Seq libraries in a day.



The NEBNext UltraExpress® RNA Library Prep Kit produces high library yields across a range of inputs.

A. Poly(A)-containing mRNA was isolated from Universal Human Reference RNA (UHRR) (Agilent®), using the NEBNext® Poly(A) mRNA Magnetic Isolation Module (NEB #E7490). Libraries were prepared using the NEBNext UltraExpress® RNA Library Prep Kit, Kapa mRNA HyperPrep® Kit or Illumina® Stranded mRNA Kit. The NEBNext UltraExpress RNA Library Prep Kit was used with a single adaptor dilution (50X) and 12 PCR cycles for all inputs, or with customized adaptor dilutions (20X for 76–250 ng, 100X for 25–75 ng) and PCR cycle numbers (listed in figure).

B. Ribosomal RNA (rRNA) was depleted from UHRR, and libraries were prepared using the UltraExpress RNA Library Prep Kit (preceded by the NEBNext rRNA Depletion Kit (Human/Mouse/Rat – NEB #E7400), Kapa HyperPrep Kit with RiboErase, or Illumina Stranded Total RNA Library Prep Kit with Ribo-Zero® Plus. The NEBNext UltraExpress RNA Library Prep Kit was used with a single adaptor dilution (50X) and 11 PCR cycles for all inputs, or with customized adaptor dilutions (20X for 76–250 ng, 100X for 25–75 ng) and PCR cycle numbers (listed in figure).

The total RNA input amount and number of PCR cycles are indicated. Library yields calculated from an average of three replicates are shown with error bars indicating the standard deviation between replicates.

NEBNext® Ultra™ II Library Prep Kits for RNA

NEBNext Ultra II Directional RNA Library Prep Kit for Illumina

#E7760S	24 reactions
#E7760L	96 reactions

NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads

#E7765S	24 reactions
#E7765L	96 reactions

NEBNext Ultra II RNA Library Prep Kit for Illumina

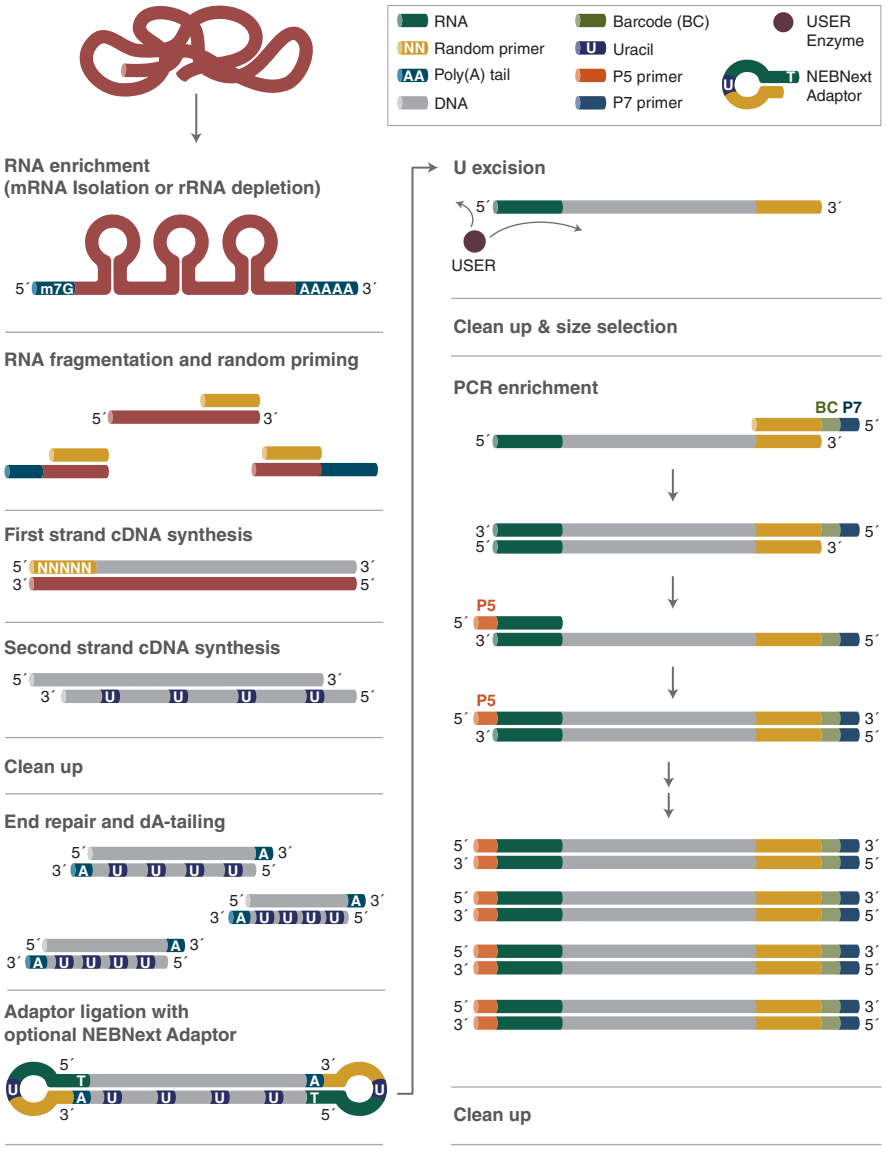
#E7770S	24 reactions
#E7770L	96 reactions

NEBNext Ultra II RNA Library Prep with Sample Purification Beads

#E7775S	24 reactions
#E7775L	96 reactions

- *Get more of what you need, with the highest library yields*
- *Generate high quality libraries with limited amounts of RNA: 10 ng–1 µg Total RNA (polyA mRNA workflow); 10 ng–1 µg (rRNA depletion workflow)*
- *Minimize bias, with fewer PCR cycles required*
- *Maximize the flexibility to order reagents for your specific workflow needs*
- *Directional (strand-specific, using the "dUTP method") and non-directional workflow options available*
- *rRNA Depletion and poly(A) mRNA isolation reagents are available separately*
- *Save time with streamlined workflows, reduced hands-on time, and automation compatibility*
- *Enjoy the reliability of the gold standard SPRIselect size selection and clean-up beads, supplied in just the amounts you need*
- *Rely on robust performance, even with low quality RNA*

Description: Do you need increased sensitivity and specificity from your RNA-seq experiments? Do you have ever-decreasing amounts of input RNA? To address these challenges, our latest generation of RNA library prep kits generate several fold higher yields of high quality libraries and enable use of lower input amounts and fewer PCR cycles. The kits have streamlined, automatable workflows and are available for directional (strand-specific, using the "dUTP method") and non-directional library prep, with the option of SPRISelect beads for size-selection and clean-up steps.



NEBNext Ultra II Directional RNA Library Prep Kit workflow.

View the animation describing NEBNext Ultra II Directional RNA Workflow.

NEBNext® Library Prep RNA Reagents

NEBNext RNA Kits are available for directional (strand-specific) and non-directional library preparation, and for bulk RNA and single cell samples. These kits utilize streamlined workflows and have been designed for performance with input amounts as low as 10 ng. Note that reagents for rRNA depletion and poly(A) mRNA enrichment are supplied separately, as are adaptors and primers. In addition to stringent QCs on individual components, the NEBNext RNA kits are functionally validated by preparation of a library, followed by Illumina sequencing.

mRNA Enrichment/RNA Depletion (optional)

- rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB #E7400, #E7405)
- rRNA Depletion Kit (Bacteria) (NEB #E7850, #E7860)
- Globin & rRNA Depletion Kit (Human/Mouse/Rat) (NEB #E7750, #E7755)
- NEBNext RNA Depletion Core Reagent Set (NEB #E7865, #E7870)
- Poly(A) mRNA Magnetic Isolation Module (NEB #E7490)
- High Input Poly(A) mRNA Isolation (NEB #E3370)

Input — **Poly(A) mRNA Workflow:** 10 ng – 1 µg | **rRNA Depletion Workflow:** 10 ng – 1 µg

	Fragmentation	First Strand cDNA Synthesis	Second Strand cDNA Synthesis	End Repair/ dA Tailing	Adaptor Ligation	Size Selection	PCR Enrichment	Clean Up	Total Workflow
NEBNext UltraExpress® Kits	**NEBNext UltraExpress RNA Library Prep Kit (NEB #E3330)**								
	• NEBNext UltraExpress RNA Fragmentation Mix • NEBNext UltraExpress Strand Specificity Reagent • Nuclease-free Water		• NEBNext UltraExpress Second Strand Master Mix • Cleanup: 0.1X TE	• NEBNext UltraExpress End Prep Enzyme Mix • NEBNext UltraExpress End Prep Reaction Buffer	• NEBNext UltraExpress Ligation Master Mix • NEBNext Adaptor Dilution Buffer • NEBNext UltraExpress USER® Enzyme		• NEBNext MSTC™ High Yield Master Mix	• 0.1X TE • NEBNext Bead Reconstitution Buffer	**Total** 3.0 hrs 3.5 hrs* 5.0 hrs**
NEBNext® Ultra™ II Directional Kits	**NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB #E7760) – with Sample Purification Beads (NEB #7765)**								
	• First Strand Synthesis Reaction Buffer • First Strand Synthesis Enzyme Mix • Random Primers • Strand Specificity Reagent		• Second Strand Synthesis Reaction Buffer with dUTP Mix • Second Strand Synthesis Enzyme Mix • Nuclease-free Water	• End Prep Enzyme Mix • End Repair Reaction Buffer	• Ultra II Ligation Master Mix • Ligation Enhancer • Adaptor Dilution Buffer	• Sample Purification Beads (SPRIselect™) – NEB #E7765 only	• Ultra II Q5 Master Mix	• Sample Purification Beads (SPRIselect) – NEB #E7765 only	**Hands-On** 27 min **Total** 5.5 – 5.7 hrs * 6.6 – 6.8 hrs **
NEBNext Ultra II Non-directional Kits	**NEBNext Ultra II RNA Library Prep Kit for Illumina (NEB #E7770) – with Sample Purification Beads (NEB #E7775)**								
	• First Strand Synthesis Reaction Buffer • First Strand Synthesis Enzyme Mix • Random Primers • Strand Specificity Reagent		• Second Strand Synthesis Reaction Buffer • Second Strand Synthesis Enzyme Mix • Nuclease-free Water	• End Prep Enzyme Mix • End Repair Reaction Buffer	• Ultra II Ligation Master Mix • Ligation Enhancer • Adaptor Dilution Buffer	• Sample Purification Beads (SPRIselect) – NEB #E7775 only	• Ultra II Q5 Master Mix	• Sample Purification Beads (SPRIselect) – NEB #E7775 only	**Hands-On** 27 min **Total** 5.5 – 5.7 hrs * 6.6 – 6.8 hrs **
NEBNext Ultra II Modules	**Magnesium RNA Fragmentation Module (NEB #E6150)** • RNA Fragmentation Buffer • RNA Fragmentation Stop Solution	**Ultra II RNA First Strand Synthesis Module (NEB #E7771)** • First Strand Synthesis Reaction Buffer • First Strand Synthesis Enzyme Mix • Random Primers • Strand Specificity Reagent	**Ultra II Directional RNA Second Strand Synthesis Module (NEB #E7550)** • Second Strand Synthesis Enzyme Mix • Second Strand Synthesis Reaction Buffer with dUTP **Ultra II Non-directional RNA Second Strand Synthesis Module (NEB #E6111)** • Second Strand Synthesis Enzyme Mix • Second Strand Synthesis Reaction Buffer	**NEBNext Ultra II End Repair/ dA-Tailing Module (NEB #E7546)** • Ultra II End Prep Enzyme Mix • Ultra II End Prep Reaction Buffer	**NEBNext Ultra II Ligation Module (NEB #E7595)** • Ultra II Ligation Master Mix • Ligation Enhancer		**NEBNext Ultra II Q5 Master Mix (NEB #M0544)** • Ultra II Q5 Master Mix		

* Including poly(A) mRNA isolation
** Including rRNA depletion

NEBNext® rRNA Depletion Kits (Human/Mouse/Rat and Bacteria)

NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)

#E7400S	6 reactions
#E7400L	24 reactions
#E7400X	96 reactions

NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads

#E7405S	6 reactions
#E7405L	24 reactions
#E7405X	96 reactions

NEBNext rRNA Depletion Kit (Bacteria)

#E7850S	6 reactions
#E7850L	24 reactions
#E7850X	96 reactions

NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads

#E7860S	6 reactions
#E7860L	24 reactions
#E7860X	96 reactions

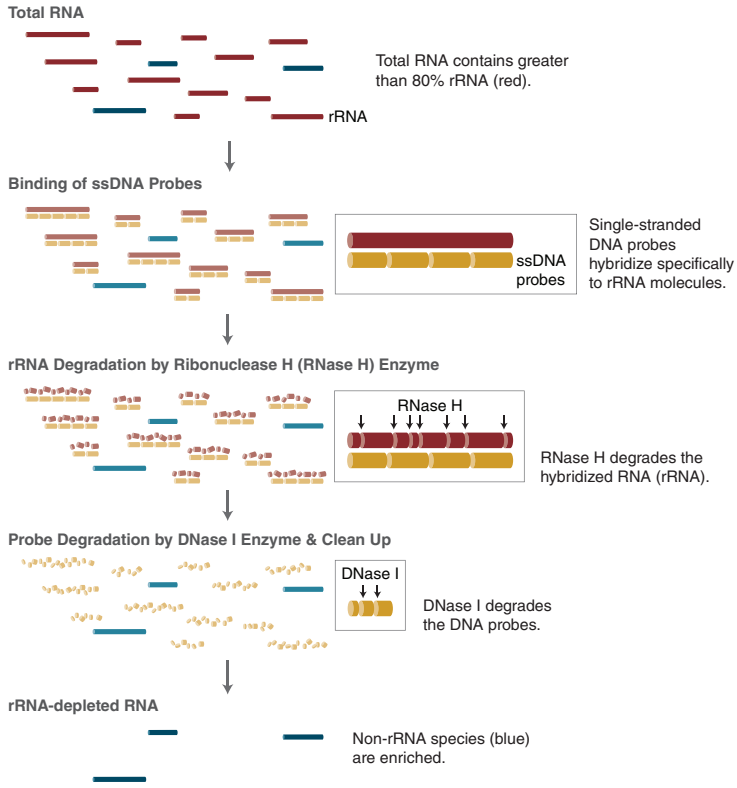
- *Suitable for low-quality (e.g., FFPE) and high-quality RNA*
- *Compatible with a broad range of input amounts: 10 ng–1 µg*
- *Superior depletion of abundant RNAs, with retention of RNAs of interest*
- *Fast workflow: 2 hours, with less than 10 minutes hands-on time*
- *Depleted RNA is suitable for RNA-seq, random-primed cDNA synthesis, or other downstream RNA analysis applications*
- *Available with optional Agencourt® RNAClean® XP Beads*

Description: The NEBNext rRNA Depletion Kit (Human/ Mouse/Rat) employs an RNase H-based method (1,2) to deplete cytoplasmic (5S, 5.8S, 18S, 28S, human ITS, ETS) and mitochondrial (12S and 16S) rRNA from human total RNA preparations.

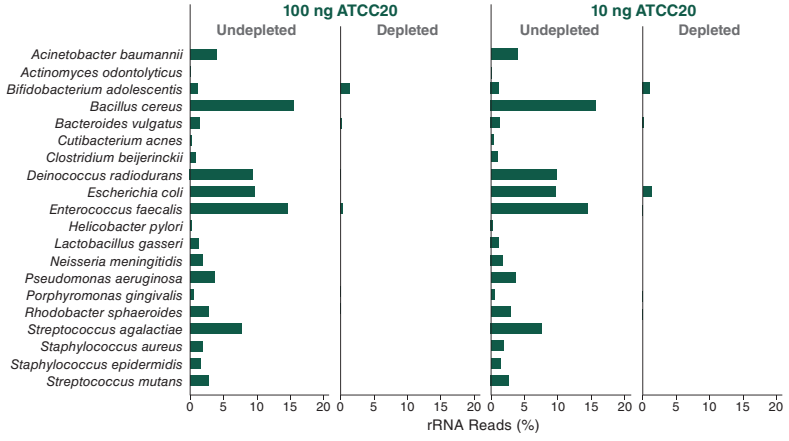
Specific enrichment of bacterial mRNAs is challenging due to their lack of poly(A) tails, precluding the use of oligo d(T)-based enrichment methods. For these samples, specific removal of bacterial rRNAs is an efficient way to enrich for RNAs of interest.

The NEBNext rRNA Depletion Kit (Bacteria) targets removal of rRNA (5S, 16S and 23S) from gram-positive and gram-negative organisms, from monocultures or samples with mixed bacterial species.

(1) Adiconis, X. et al (2013) Nature Methods, 10, 623–629.
(2) Morlon, J.D. et al (2012) PLoS One, 77 e42882.



NEBNext rRNA Depletion Kit workflow.



Depletion of ribosomal RNA enriches for RNAs of interest, and maintains expression correlation, across a mock community of bacterial species and a range of input amounts. *Total RNA was extracted from a lyophilized pool of 20 different bacterial organisms (ATCC® #MSA-2002). Ribosomal RNA was depleted using the NEBNext rRNA Depletion Kit (Bacteria). RNA-seq libraries were prepared from untreated and depleted RNA using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina followed by paired-end sequencing (2 x 75 bp). 4 Million read pairs were sampled (seqtk) from each library, mapped to a composite genome (Bowtie 2.3.2) before counting reads on genes (htseq-count) and correlating their levels. Effective depletion of sequences overlapping with annotated rRNA regions was observed at 100 ng and 10 ng of input RNA for most of the organisms. Correlation analysis of the transcripts indicates consistent transcript expression regardless of treatment or input amount.*

NEBNext® Globin & rRNA Depletion Kits

NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)

#E7750S	6 reactions
#E7750L	24 reactions
#E7750X	96 reactions

NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads

#E7755S	6 reactions
#E7755L	24 reactions
#E7755X	96 reactions

- *Efficient, specific depletion of globin mRNA and rRNA*
- *Suitable for low- and high-quality RNA*
- *Broad range of input amounts: 10 ng–100 µg*
- *Superior deletion of abundant RNAs*
- *Fast workflow: 2 hours, with less than 10 minutes hands-on time*

GLOBIN-ZERO® is a registered trademark of Illumina, Inc.

Description: The great majority of RNA in blood samples is comprised of globin mRNA as well as cytoplasmic and mitochondrial ribosomal RNAs (rRNA). These highly abundant RNA species can conceal the biological significance of less abundant transcripts, and so their efficient and specific removal is desirable.

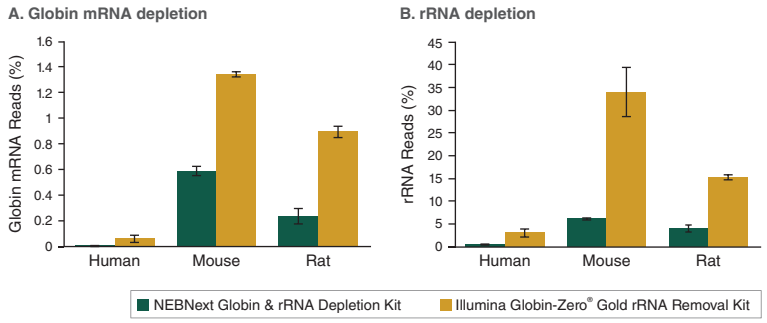
The NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) employs the NEBNext RNase H-based RNA depletion workflow to deplete the following:

- Globin mRNA (HBA1/2, HBB, HBD, HBM, HBG1/2, HBE1, HBQ1 and HBZ)
- Cytoplasmic rRNA (5S, 5.8S, 18S, 28S, ITS and ETS)
- Mitochondrial rRNA (12S and 16S)

The kit is effective with human, mouse and rat total RNA preparations, both intact and degraded. The resulting depleted RNA is suitable for RNA-seq, random-primed cDNA synthesis, or other downstream RNA analysis.

This kit can also be used following poly(A) mRNA enrichment (e.g., using the NEBNext poly(A) mRNA Magnetic Isolation Module, NEB #E7490), so that the final depleted RNA contains only mRNA of interest and no non-coding RNA.

This kit is available with or without RNAClean beads.



NEBNext Globin & rRNA Depletion Kit efficiently removes Globin mRNA and rRNA. *Ribosomal RNA (rRNA) and globin mRNA were depleted from Human, Mouse, and Rat Whole Blood Total RNA (100 ng) using the NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) (NEB #E7750) or the Globin-Zero® Gold rRNA Removal Kit (Illumina #GZG1224). Libraries were prepared from the depleted RNA using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina and sequenced on an Illumina NextSeq instrument (2 x 75 bp). Reads were down sampled to 20 million reads per sample for analysis, and were identified as globin mRNA (A) or rRNA (B) using Mirabait (6 or more, 25-mers). The data represents an average of 3-4 replicates. Error bars indicate standard error.*

Customized Depletion of Unwanted RNA

NEBNext RNA Depletion Core Reagent Set

#E7865S	6 reactions
#E7865L	24 reactions
#E7865X	96 reactions

NEBNext RNA Depletion Core Reagent Set with RNA Sample Purification Beads

#E7870S	6 reactions
#E7870L	24 reactions
#E7870X	96 reactions

- *Compatible with a broad range of input amounts: 10 ng–1 µg*
- *Suitable for low-quality or high-quality RNA*
- *Fast workflow: 2 hours, with less than 10 minutes hands-on time*

Description: In RNA-seq, highly expressed transcripts with minimal biological interest, such as ribosomal RNA (rRNA) can dominate readouts and mask detection of more informative low-abundance transcripts. This challenge is amplified when working with sample types for which pre-designed RNA depletion kits are not available. The NEBNext RNA Depletion Core Reagent set provides a customized approach to deplete unwanted RNA from any organism, using probe sequences designed with the user-friendly NEBNext Custom RNA Depletion Design Tool.

The efficient RNase-H-based workflow, and close tiling of probes designed using the online tool, enables effective depletion from both low- and high-quality RNA, with a broad range of input amounts.

STEP 1: Use the online NEBNext Custom RNA Depletion Design Tool to obtain custom probe sequences, by entering the sequence of your target RNA.

STEP 2: Order ssDNA probe oligonucleotides from your trusted oligo provider.

STEP 3: Use the probes with the NEBNext Custom RNA Depletion Core Reagent Set or in combination with other NEBNext RNA Depletion Kits

NEW **NEBNext Custom RNA Depletion Design Tool**

Design oligos for depletion of unwanted RNA from any organism, when used in the NEBNext RNA depletion workflow.

https://depletion-design.neb.com/

NEW

NEBNext® High Input Poly(A) mRNA Isolation Module

#E3370S 24 reactions

Companion Products:

NEBNext Poly(A) mRNA Magnetic Isolation Module
#E7490S 24 reactions
#E7490L 96 reactions

NEBNext Ultra II Directional RNA Library Prep Kit for Illumina
#E7760S 24 reactions
#E7760L 96 reactions

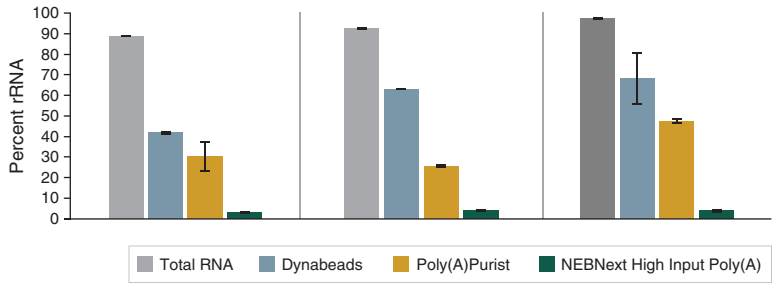
NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads
#E7765S 24 reactions
#E7765L 96 reactions

NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)
#E7400S 6 reactions
#E7400L 24 reactions
#E7400X 96 reactions

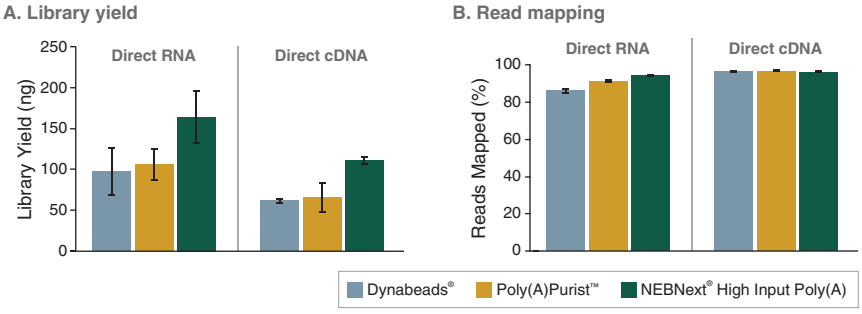
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads
#E7405S 6 reactions
#E7405L 24 reactions
#E7405X 96 reactions

- *High inputs: 5 –50 µg total RNA per reaction*
- *Low elution volume*
- *Fast workflow*
- *Automation compatible*

Description: The NEBNext High Input Poly(A) mRNA Isolation Module is designed to isolate intact poly(A)+ RNA from high inputs (5-50 µg per reaction) of previously isolated total RNA, using oligo $d(T)_{25}$-coupled paramagnetic beads. Intact mRNA can be obtained in approximately one hour, and eluted in small volumes. For inputs less than 5 µg, the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490) is recommended.



The NEBNext® High Input Poly(A) mRNA Isolation Module produces low ribosomal RNA retention, across sample types. *Poly(A) RNA was enriched using Dynabeads® mRNA Purification Kit (Invitrogen®), Poly(A) Purist™ MAG (Invitrogen) or the NEBNext High Input Poly(A) mRNA Magnetic Isolation Module from 50 µg Universal Human Reference RNA (UHR, Agilent) or RNA extracted from mouse kidney tissue or S. cerevisiae (yeast) using the Monarch Total RNA Miniprep Kit. Percent ribosomal RNA (rRNA) of total or poly(A)-enriched RNA samples was determined from sequencing of triplicate (UHR and mouse poly(A) samples) or duplicate (total RNA and yeast poly(A) RNA samples) experiments, with standard deviation. Libraries were prepared from 40 ng poly(A)-enriched RNA using the NEBNext Ultra II Directional RNA Library Prep Kit and sequenced on an Illumina NextSeq 550 instrument. Six million reads were sampled from each library.*



RNA from the NEBNext® High Input Poly(A) mRNA Isolation Module produces higher library yields for nanopore sequencing, with good read mapping. *400 ng of poly(A)-enriched Universal Human Reference (UHR) RNA, enriched using the stated methods, was prepared for Direct RNA Sequencing (ONT #SQK-RNA002) on a GridION® sequencer (Oxford Nanopore Technologies®). 100 ng of poly(A)-enriched UHR RNA, enriched using the stated methods, was prepared for Direct cDNA Sequencing (ONT SQK-DCS109) on a GridION sequencer.*

A. *Library yields were assessed using Qubit® dsDNA High Sensitivity Assay Kit (Invitrogen); shown are the average of replicates with standard deviation.*

B. *Average mapping percentages of reads from replicate Direct RNA and Direct cDNA sequencing runs with standard deviation.*

NEBNext® Poly(A) mRNA Magnetic Isolation Module

#E7490S 24 reactions
#E7490L 96 reactions

Description: The NEBNext Poly(A) mRNA Magnetic Isolation Module is designed to isolate intact poly(A)+ RNA from previously-isolated total RNA. The technology is based on the coupling of Oligo $d(T)_{25}$ to 1 µm paramagnetic beads which are then used as the solid support for the direct binding of poly(A)+ RNA. Thus, the procedure permits the manual processing of multiple samples and can be adapted for automated high-throughput applications. Additionally, magnetic separation technology permits elution of intact mRNA in small volumes eliminating the need for precipitating the poly(A)+ transcripts in the eluent. Intact poly(A)+ RNA which is fully representative of the mRNA population of the original sample can be obtained in less than one hour.

NEBNext® Single Cell/Low Input RNA Library Prep

NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina

#E6420S	24 reactions
#E6420L	96 reactions

NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module

#E6421S	24 reactions
#E6421L	96 reactions

NEBNext Single Cell Lysis Module

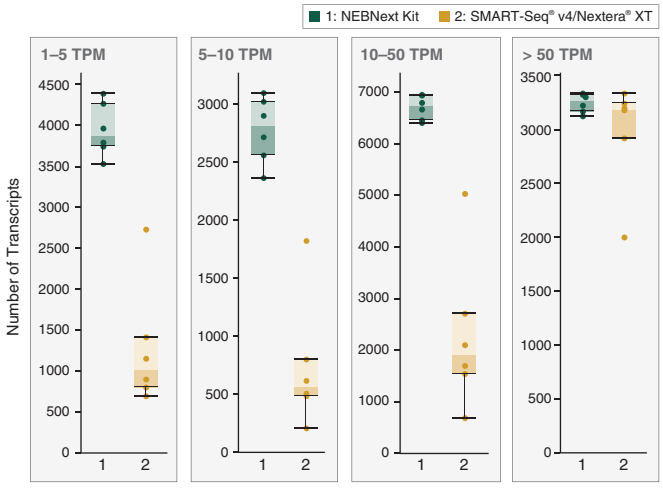
#E5530S	96 reactions

- *Generate the highest yields of high-quality full-length transcript sequencing libraries from single cells, or as little as 2 pg–200 ng total RNA*
- *Experience unmatched detection of low abundance transcripts*
- *Obtain full-length, uniform transcript coverage for a wide range of input amounts or sample types*
- *Use with cultured or primary cells, or total RNA*
- *Save time with a fast, streamlined workflow, minimal handling steps and hands-on time*
- *Utilize a single-tube protocol from cell lysis to cDNA*
- *Enzymatic DNA fragmentation, end repair and dA-tailing reagents are in a single enzyme mix, utilizing a single protocol, regardless of GC content*
- *Available with or without library construction reagents*

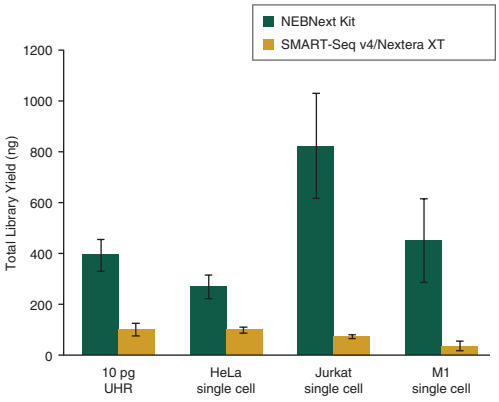
Description: The unique workflow of the NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina meets the demand for a highly sensitive, yet robust method that consistently generates high-quality, full-length transcript sequencing data from a single cell or ultra-low input RNA.

Optimized cDNA synthesis and amplification steps incorporate template switching, and utilize a unique protocol and suite of reagents.

cDNAs are generated directly from single cells or 2 pg–200 ng RNA, and even low-abundance transcripts are represented in the high yields of cDNA obtained. This is followed by library construction that incorporates the Ultra II FS enzymatic DNA fragmentation/end repair/dA-tailing mix in a simple and efficient workflow.



Increased transcript detection with the NEBNext Single Cell/Low Input RNA Library Prep Kit. *Sequencing libraries were generated from Jurkat single cells (6 replicates) using the NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Clontech # 634891) plus the Nextera® XT DNA Library Prep Kit (Illumina #FC-131-1096). Libraries were sequenced on an Illumina NextSeq 500 using paired-end mode (2 x 76 bp). TPM = Transcripts per Kilobase Million. Each dot represents the number of transcripts identified at the given TPM range, and each box represents the median, first and third quartiles per replicate and method. Salmon 0.6 was used for read mapping and quantification of all GENCODE v25 transcripts. Panels show the number of transcripts detected within the following TPM ranges: 1-5, 5-10, 10-50 and > 50 TPM. Increased identification of low abundance transcripts is observed with the NEBNext libraries.*



Higher library yields with the NEBNext Single Cell/Low Input RNA Library Prep Kit. *Sequencing libraries were generated from HeLa, Jurkat and M1 single cells or 10 pg of Universal Human Reference (UHR) RNA (Agilent #740000) with recommended amounts of ERCC RNA Spike-In Mix I (Thermo Fisher Scientific® #4456740). The NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Clontech #634891) plus the Nextera XT DNA Library Prep Kit (Illumina #FC-131-1096) were used. Error bars indicate standard deviation for 6-11 replicates. For the NEBNext workflow ~80% of the cDNA was used as input into sequencing library preparation, and libraries were amplified with 8 PCR cycles. For the SMART-Seq v4/Nextera XT workflow, as recommended, 125 pg of cDNA was used as input in sequencing library preparation and 12 PCR cycles were used for amplification. Error bars indicate standard deviation for 6-11 replicates.*

NEBNext® Small RNA Library Prep Kits

NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1)
#E7300S 24 reactions
#E7300L 96 reactions

NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2)
#E7580S 24 reactions
#E7580L 96 reactions

NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48)
#E7560S 96 reactions

NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible)
#E7330S 24 reactions
#E7330L 96 reactions

- *Minimal adaptor-dimer formation*
- *High yields*
- *Input RNA can be total RNA*
- *Suitable for methylated small RNAs (e.g, piRNAs) as well as unmethylated small RNAs*

Description: The novel NEBNext Small RNA workflow has been optimized to minimize adaptor-dimers while producing high-yield, high-diversity libraries. Adaptors and primers are included in the Small RNA kits, and multiplexing options are available. The Multiplex kit contains index primers, and the Multiplex-Compatible kit enables use with your own barcode primers.

Input 100 ng – 1 µg

3´ Adaptor Ligation	Primer Hybridization	5´ Adaptor Ligation	First Strand Synthesis	PCR Enrichment	Size Selection	Total Workflow
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1 NEB #E7300, Set 2 NEB #E7580)						**Hands-On Time** 30 min **Total Time** 6 hrs
• 3´ Ligation Enzyme Mix • 3´ Ligation Reaction Buffer (2X) • 3´ SR Adaptor	• SR RT Primer	• 5´ Ligation Enzyme Mix • 5´ Ligation Reaction Buffer (10X) • 5´ SR Adaptor • Nuclease-Free Water	• RNase Inhibitor, Murine • M-MuLV Reverse Transcriptase (RNase H⁻) • First Strand Synthesis Reaction Buffer	• LongAmp® *Taq* 2X Master Mix • SR Primer • Index Primers 1–12 (Set 1) • Index Primers 13–24 (Set 2)	• Gel Loading Dye, Blue (6X) • Quick-Load® pBR322 DNA-MspI Digest • DNA Gel Elution Buffer (1X) • Linear Acrylamide • TE Buffer	
NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48) (NEB #E7560)						**Hands-On Time** 30 min **Total Time** 6 hrs
• 3´ Ligation Enzyme Mix • 3´ Ligation Reaction Buffer (2X) • 3´ SR Adaptor	• SR RT Primer	• 5´ Ligation Enzyme Mix • 5´ Ligation Reaction Buffer (10X) • 5´ SR Adaptor • Nuclease-Free Water	• RNase Inhibitor, Murine • M-MuLV Reverse Transcriptase (RNase H⁻) • First Strand Synthesis Reaction Buffer	• LongAmp *Taq* 2X Master Mix • SR Primer • NEBNext Index 1-48 Primers for Illumina	• Gel Loading Dye, Blue (6X) • Quick-Load pBR322 DNA-MspI Digest • DNA Gel Elution Buffer (1X) • Linear Acrylamide • TE Buffer	
NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible) (NEB #E7330)						**Hands-On Time** 30 min **Total Time** 6 hrs
• 3´ Ligation Enzyme Mix • 3´ Ligation Reaction Buffer (2X) • 3´ SR Adaptor	• SR RT Primer	• 5´ Ligation Enzyme Mix • 5´ Ligation Reaction Buffer (10X) • 5´ SR Adaptor • Nuclease-Free Water	• RNase Inhibitor, Murine • M-MuLV Reverse Transcriptase (RNase H⁻) • First Strand Synthesis Reaction Buffer	• LongAmp *Taq* 2X Master Mix • SR Primer • Index Primer 1	• Gel Loading Dye, Blue (6X) • Quick-Load pBR322 DNA-MspI Digest • DNA Gel Elution Buffer (1X) • Linear Acrylamide • TE Buffer	

NEBNext® Magnetic Separation Rack

#S1515S 24 tubes

- *Fast separations in purification and size-selection steps in next generation sequencing workflows*
- *Small-scale separation of magnetic particles*
- *Anodized aluminum rack with Neodymium Iron Boron (NdFeB) rare earth magnets, the most powerful commercially available*
- *24 tube capacity: 8- and 12-strip 0.2 ml PCR tubes or individual 0.2 ml PCR tubes*

Description: Next generation sequencing library preparation workflows include magnetic bead-based purification and size-selection steps and it is important for library yield and quality that bead separation be highly efficient and fast.

The NEBNext Magnetic Separation Rack was designed for this application and contains rare earth Neodymium Iron Boron (NdFeB) magnets, the most powerful commercially available magnets, in an anodized aluminium rack. The rack holds 24 (0.2 ml) tubes, and is compatible with single tubes or strip tubes.

NEBNext Adaptors & Primers for Illumina

NEBNext® Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1)
#E7395S 96 reactions
#E7395L 384 reactions

NEW
NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 2)
#E7874S 96 reactions
#E7874L 384 reactions

NEW
NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 3)
#E7876S 96 reactions
#E7876L 384 reactions

NEW
NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 4)
#E7878S 96 reactions
#E7878L 384 reactions

NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors RNA Set 1)
#E7416S 96 reactions
#E7416L 384 reactions

NEW
NEBNext LV Unique Dual Index Primers Set 2A
#E3390S 24 reactions

NEW
NEBNext LV Unique Dual Index Primers Set 2B
#E3392S 24 reactions

NEW
NEBNext LV Unique Dual Index Primers Set 1
#E3400S 96 reactions

NEW
NEBNext LV Unique Dual Index Primers Set 2
#E3402S 96 reactions

NEW
NEBNext LV Unique Dual Index Primers Set 3
#E3404S 96 reactions

NEW
NEBNext LV Unique Dual Index Primers Set 4
#E3406S 96 reactions

NEW
NEBNext LV Unique Dual Index Primers Set 5
#E3408S 96 reactions

NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)
#E6440S 96 reactions
#E6440L 384 reactions

NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 2)
#E6442S 96 reactions
#E6442L 384 reactions

NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 3)
#E6444S 96 reactions
#E6444L 384 reactions

NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 4)
#E6446S 96 reactions
#E6446L 384 reactions

NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 5)
#E6448S 96 reactions
#E6448L 384 reactions

NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)
#E7140S 24 reactions
#E7140L 96 reactions

NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)
#E7600S 96 reactions

NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2)
#E7780S 96 reactions

NEBNext® Multiplex Oligos for Illumina® (Index Primers Set 1)
#E7335S 24 reactions
#E7335L 96 reactions

NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)
#E7500S 24 reactions
#E7500L 96 reactions

NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)
#E7710S 24 reactions
#E7710L 96 reactions

NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)
#E7730S 24 reactions
#E7730L 96 reactions

NEBNext Multiplex Oligos for Illumina (96 Index Primers)
#E6609S 96 reactions
#E6609L 384 reactions

NEBNext Adaptor Dilution Buffer
#B1430S 9.6 ml

Description: Designed for use in library prep for DNA, ChIP DNA and RNA (but not Small RNA), the NEBNext Multiplex Oligos for Illumina are an essential component of the NGS sample prep workflow. Offering a range of indexing strategies, from Unique Dual Index UMI Adaptors to the truncated, hairpin-loop NEBNext Adaptor, meant for use with UDI, dual, and single index primers, the NEBNext Multiplex Oligos can support NGS across a wide range of formats. Optimized for performance in recommended applications, there's an NEBNext indexing option tailored to you. NEBNext Oligos can be used with NEBNext products, and with other standard Illumina-compatible library preparation protocols.

Single or dual barcode primer options are available. Unique dual index primer pairs are available to address the "index hopping" seen with certain Illumina sequencing instruments.

Unique Dual Index UMI Adaptors (available for both DNA and RNA library prep) offer a ready-to-ligate adaptor for correction of PCR duplicates and errors, while improving the detection of single-nucleotide variants (SNVs). When read without the UMI sequence, the full-length adaptor enables PCR-free DNA library prep.

- *Index Primers are available for NGS library prep workflows that include an amplification step*
- *Index Adaptors enable PCR-free workflows and incorporation of UMIs for error correction/deduplication*
- *Extensively QC'd for purity and increased library yields*
- *Flexibility for use with NEBNext library preparation kits and other standard, Illumina-compatible library preparation methods*
- *Provided with index-pooling guidelines and sample sheets*

Multiplex Oligos Selection Chart

	SINGLE INDEX	DUAL INDEX	UNIQUE DUAL INDEX	UNIQUE DUAL INDEX UMIs
NEB PRODUCTS	NEB #E7335 NEB #E7730 NEB #E7500 NEB #E6609 NEB #E7710	NEB #E7600 NEB #E7780	NEB #E6440 NEB #E7140 NEB #E3404 NEB #E6442 NEB #E3390 NEB #E3406 NEB #E6444 NEB #E3392 NEB #E3408 NEB #E6446 NEB #E3400 NEB #E6448 NEB #E3402	NEB #E7395 NEB #E7878 NEB #E7874 NEB #E7416 NEB #E7876
Contains UMI	No	No	No	Yes
Addresses Index Hopping	No	No	Yes	Yes
Indexing Strategy	Index Primer	Index Primer	Index Primer	Index Adaptor
Adaptor Included	NEBNext Adaptor (Loop)	NEBNext Adaptor (Loop)	NEBNext Adaptor (Loop)	Unique Dual Index UMI Adaptor
Applications	DNA-seq, RNA-seq (except small RNA)	DNA-seq, RNA-seq (except small RNA)	DNA-seq, RNA-seq (except small RNA)	DNA-seq, RNA-seq (except small RNA)
Number of Indices for Multiplexing	up to 144	up to 384	up to 480	384 for DNA; 96 for RNA
Compatible with EM-seq™	Yes*	Yes*	Yes*	No
Compatible with EpiMark® Bisulfite Sequencing	Yes**	Yes**	Yes**	No
Number of Sets Available; Formats & Indices Available	Five; Sets 1-4 (12 indices/set): Individual vials 96 Index: premixed plate	Two; Individual vials containing 8 i5 primers and 12 i7 primers for combinatorial mixing	Thirteen; Sets are provided as either 24 or 96 indices in premixed, foil-sealed 96-well plates	Four sets for DNA, One set for RNA; Adaptors with 96 indices in premixed, foil-sealed 96-well plate (DNA-seq OR RNA-seq) and primers

* Requires use of the correct adaptor; choose based on the version of EM-seq being used. Original EM-seq (NEB #E7120) is not supplied with the NEBNext EM-seq Adaptor. The adaptor and indices must be purchased separately as part of NEBNext Unique Dual Index Pairs for EM-seq (NEB #E7140). Adaptors for use with NEBNext EM-seq v2 (NEB #E8015) and NEBNext E5hmC-seq (NEB #E3350) are provided as components in the library prep kits. Index primers for use with these kits are optimized for low-volume workflows and are available as NEBNext LV Unique Dual Index Primers (Sets 1–5).

** Requires use of NEBNext EM-seq adaptor from NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs, #E7140S/L.

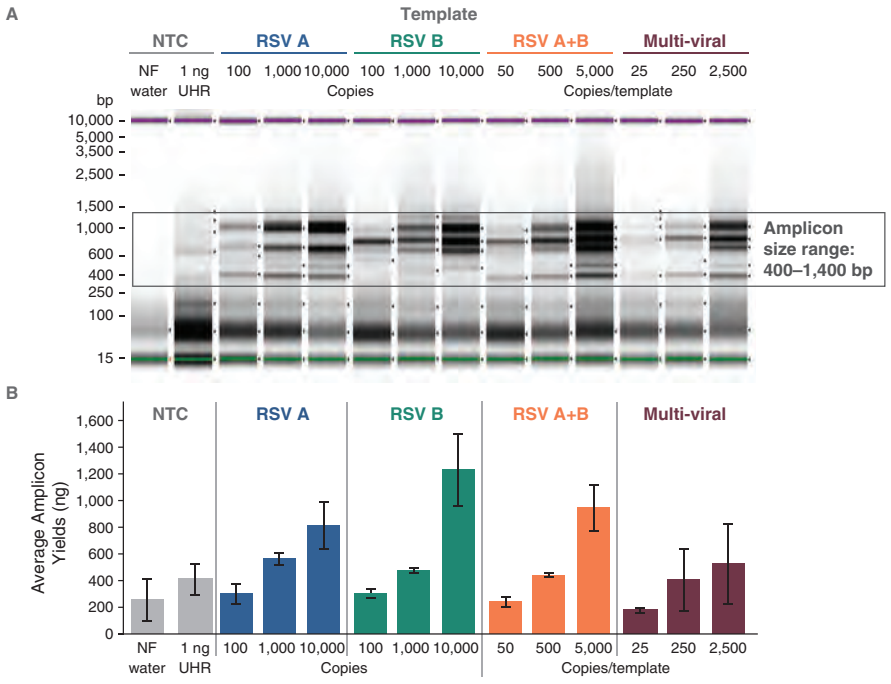
NEW

NEBNext® RSV Primer Module

#E9642S 24 reactions
#E9642L 96 reactions

- *Full-length coverage of RSV A and RSV B*
- *Intentionally designed, balanced, and functionally optimized primer sequences*
- *Flexibility to use with Illumina® or Oxford Nanopore Technologies® platforms*
- *Timely RSV variant surveillance with NEB's Primer Monitor tool*
- *Primer sequence information can be found in the NEBNext GitHub Repository*

Description: NEBNext RSV Primer Module is intended for use in cDNA synthesis, amplification, and library preparation upstream of Respiratory Syncytial Virus (RSV) sequencing. The Primer Module can be used upstream of both Illumina® and Oxford Nanopore Technologies® sequencing, when paired with LunaScript Multiplex One-Step RT-PCR Kit and a compatible library prep solution (e.g., NEBNext UltraExpress FS DNA Library Prep Kit or ONT library prep reagents). Note: This product is intended for research-use only.



NEBNext® RSV Primer Module coupled with LunaScript® Multiplex One-Step RT-PCR Kit provides robust RSV amplicon yields. *Overlapping amplicons were generated from 100–10,000 total copies of viral gRNA templates, with 1 ng Universal Human Reference RNA (UHR) background, using dual multiplexed RSV-targeting primer pools. The gRNA templates were RSV A gRNA (ATCC® VR1540), RSV B gRNA (ATCC VR-1580), a 1:1 [RSV A : RSV B] gRNA mix, or a 1:1:1:1 [RSV A : RSV B : Influenza A : SARS-CoV-2] mix.*

A. *1/10th diluted amplicons were run on TapeStation® using DS 5000 HS reagents without a cleanup. TapeStation traces show expected amplicon peaks within the 400 to 1,400 bp size range.*

B. *Average amplicon yields (n=3) within 400 to 1400 bp window determined via TapeStation Analysis of diluted post-RT-PCR amplicons.*

NEBNext® ARTIC Products for SARS-CoV-2 Sequencing

NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina)
#E7658L 96 reactions

NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies)
#E7660L 96 reactions

NEBNext ARTIC SARS-CoV-2 RT-PCR Module
#E7626L 96 reactions

- *Streamlined, high-efficiency protocol*
- *Ample amplicon yields from a wide range of viral genome inputs*
- *Improved SARS-CoV-2 genome coverage depth with a more balanced primer pool*
- *Available for Illumina and Oxford Nanopore Technologies sequencing platforms*
- *No requirement for amplicon normalization prior to Illumina library preparation*

Description: The NEBNext ARTIC kits were developed in response to the critical need for reliable and accurate methods for sequencing viral pathogens, specifically SARS-CoV-2. These kits, for long and short read sequencing, were based on the original work of the ARTIC Network (1). The ARTIC SARS-CoV-2 sequencing workflow is a multiplexed amplicon-based whole-viral-genome sequencing approach.

NEBNext ARTIC Companion kits include primers and reagents for RT-PCR from SARS-CoV-2 gRNA and downstream library preparation for Illumina and Oxford Nanopore Technologies sequencing.

The optimized primers and reagents for RT-PCR deliver uniform, ample amplicon yields from gRNA across a wide copy number range, and library prep and sequencing can be performed downstream of a single RT-PCR procedure.

For Illumina applications, a novel DNA polymerase formulation for the enrichment of next-generation sequencing libraries eliminates the need to normalize amplicon concentrations prior to library preparation. The NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina) incorporates enzymatic cDNA fragmentation, and generates libraries with inserts in the 150 bp range.

(1) Josh Quick 2020. nCoV-2019 sequencing protocol v2 (GunIt). protocols.io https://dx.doi.org/10.17504/protocols.io.bdp7i5rn

Reagents for Oxford Nanopore Technologies® Sequencing

NEW

NEBNext Companion Module v2 for Oxford Nanopore Technologies® Ligation Sequencing

#E7672S	24 reactions
#E7672L	96 reactions

NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies)

#E7660L	96 reactions

Companion Products:

Monarch HMW DNA Extraction Kit for Cells & Blood

#T3050S	5 preps
#T3050L	50 preps

Monarch HMW DNA Extraction Kit for Tissue

#T3060S	5 preps
#T3060L	50 preps

Monarch Spin gDNA Extraction Kit

#T3010S	50 preps
#T3010L	150 preps

NEBNext Magnetic Separation Rack

#S1515S	24 tubes

- *Includes Salt-T4® DNA Ligase & NEBNext FFPE DNA Repair Buffer v2*
- *Component volumes tailored for use with many SQK-LSK114 workflows*
- *Simplified ordering and inventory management*
- *Compatible with all devices: MinION®, GridION®, PromethION®, Flongle®*
- *No waste – no unnecessary buffers or excess reagents*

Description: Many NEBNext and NEB products are recommended for use in multiple sample prep workflows for Oxford Nanopore Technologies sequencing, for a range of sample types and applications.

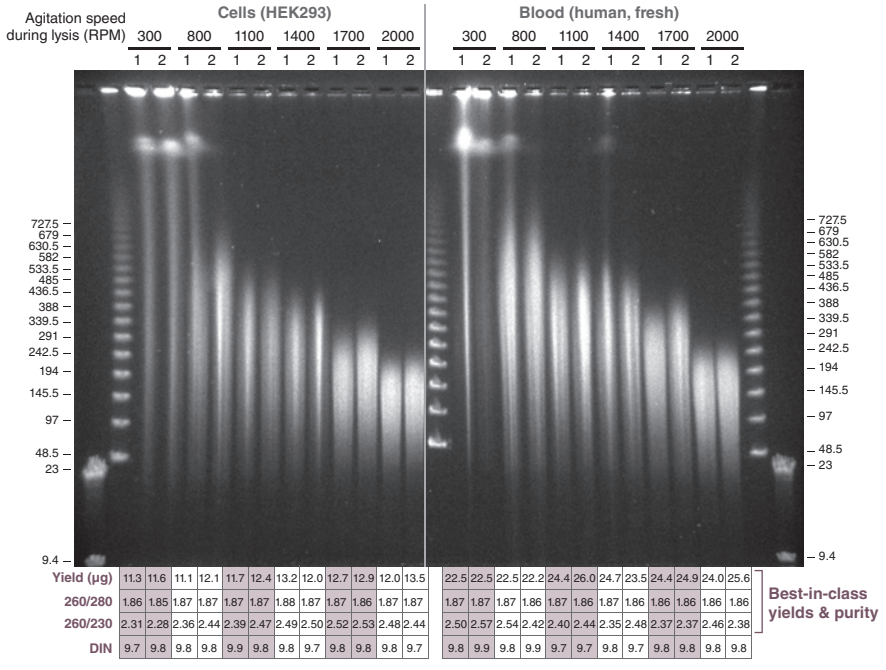
The NEBNext Companion Module v2 for Oxford Nanopore Technologies Ligation Sequencing includes the NEBNext DNA repair, end repair and ligations reagents recommended for Oxford Nanopore Technologies updated SQK-LSK114 singleplex ligation sequencing library prep protocols.

- Includes Salt-T4® DNA Ligase & NEBNext FFPE DNA Repair Buffer v2
- Component volumes tailored for use with many SQK-LSK114 workflows
- Simplified ordering and inventory management
- Compatible with all devices: MinION®, GridION®, PromethION®, Flongle®
- No waste – no unnecessary buffers or excess reagents

Also Available:

Monarch DNA Extraction for Oxford Nanopore Sequencing

Long read sequencing technologies, including Oxford Nanopore sequencing, require high quality extracted DNA. For the longest reads, the Monarch® HMW DNA Extraction kits enable isolation of DNA in the Mb range. The HMW DNA Extraction Kit for Tissue (NEB #T3060) is effective with a variety of tissues, bacteria and other samples (yeast, insect, amphibian), and the HMW DNA Extraction Kit for Cells & Blood (NEB #T3050) isolates HMW DNA from cultured cells and whole blood. When reads < 80 kb are required, the Monarch Genomic DNA Purification Kit (NEB #T3010) produces genomic DNA with a typical peak size of > 50 kb.



DNA fragment size is tunable based on agitation speed during lysis. *Preps were performed on duplicate aliquots of 1 x 10^6 HEK 293 cells and 500 µl fresh human blood. Samples were agitated at the indicated speed during the lysis step to control the fragmentation of the DNA. Equal amounts of DNA from the replicates (cells: 500 ng; blood: 650 ng) were resolved by PFGE (1% agarose gel, 6 V/cm, 13°C for 20 hours, switch times ramped from 0.5–94 seconds on a BioRad CHEF-DR III System). Yield and purity ratios of the individual preps are shown in the accompanying tables. Lambda PFG Ladder and Lambda DNA-Hind III Digest (NEB #N0341 and #N3012) were used as molecular weight standards. Yield, purity ratios and DINs of the individual preps are shown in the accompanying tables.*

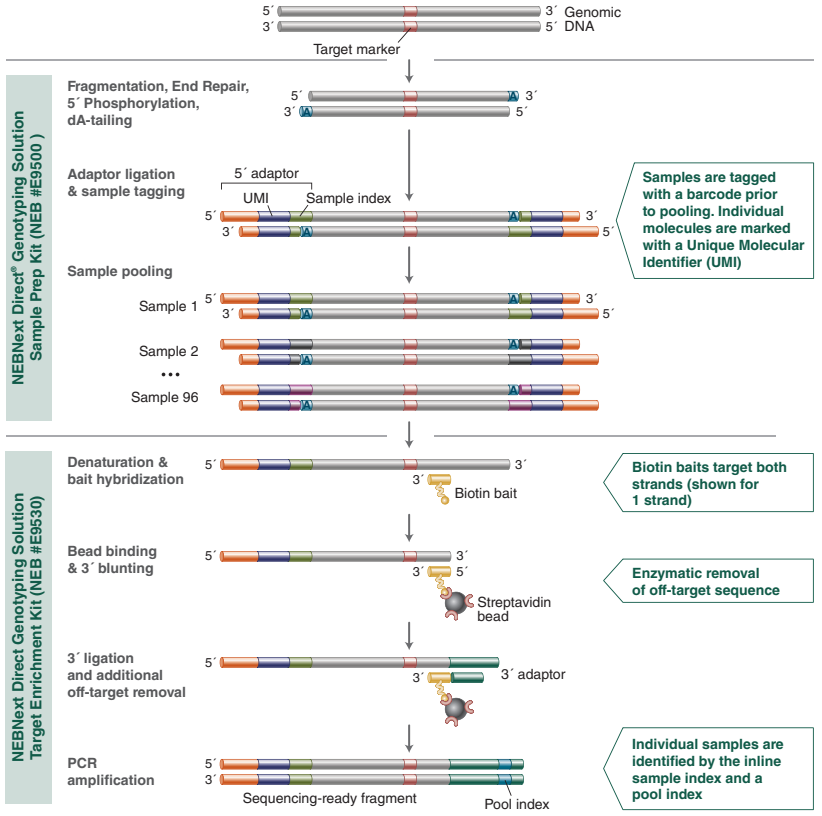
NEBNext Direct® Genotyping Solution

#E9530B-S	8 reactions
#E9500B-S	96 reactions
#N9530B	8 reactions

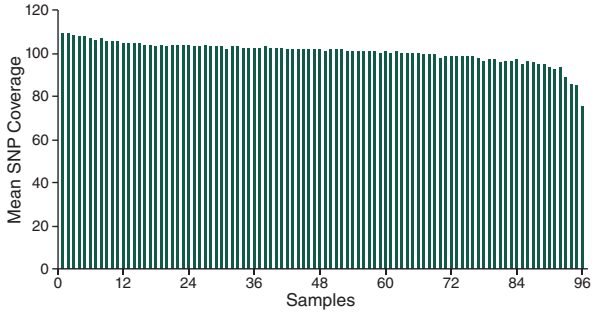
- *Ideal solution for genotyping hundreds to thousands of markers*
- *Reduce costs and streamline workflow through pre-capture pooling of up to 96 samples*
- *Maximize sequencer efficiency through dual barcode sample indexing plus Unique Molecular Identifier*
- *Unparalleled target coverage uniformity through unique capture-based enrichment*
- *Eliminate marker dropouts with finely tuned bait design*
- *Increase sample throughput using the 1-day, automatable workflow*

Visit **www.neb.com/E9500** to learn more.

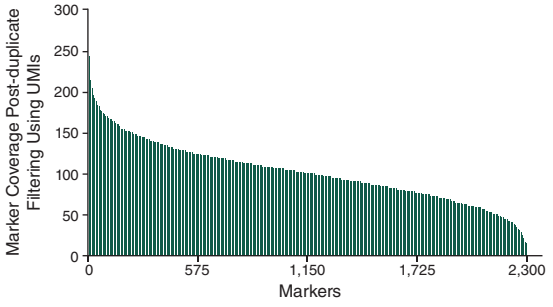
Description: The NEBNext Direct Genotyping Solution combines highly multiplexed, capture-based enrichment with maximum efficiency next-generation sequencing to deliver cost-effective, high-throughput, genotyping for a wide variety of applications. Applicable for marker ranges spanning 100-5,000 markers, pre-capture multiplexing of up to 96 samples combined with dual indexed sequencing allowing extremely high levels of sample multiplexing in a single Illumina sequencing run.



NEBNext Direct Genotyping Solution workflow.



NEBNext Direct Genotyping Solution demonstrates similar coverage across 96 pooled samples. *Mean SNP coverage of 2,309 SolCAP markers across 96 samples. 25 ng of purified tomato DNA was used for each sample. Samples were index-tagged and pooled prior to hybridization and Libraries were sequenced on an Illumina MiSeq with 20 cycles of Read 1 to sequence the 12 base UMI and 8 base sample index, and 75 cycles of Read 2 to sequence the targets.*



Mean Coverage across 2309 markers within a single sample. *Histogram of coverage across each of the 2,309 SolCAP markers demonstrates evenness of enrichment across targets and coverage levels sufficient for genotyping calls. These data represent enrichment of a single tomato sample pooled with 95 others prior to hybridization. 25 ng of purified tomato DNA was used for each sample. Samples were index-tagged and pooled prior to hybridization and libraries were sequenced on an Illumina MiSeq with 20 cycles of Read 1 to sequence the 12 base UMI and 8 base sample index, and 75 cycles of Read 2 to sequence the targets.*

NEBNext® Immune Sequencing Kits (Human & Mouse)

NEBNext Immune Sequencing Kit (Human)

#E6320S	24 reactions
#E6320L	96 reactions

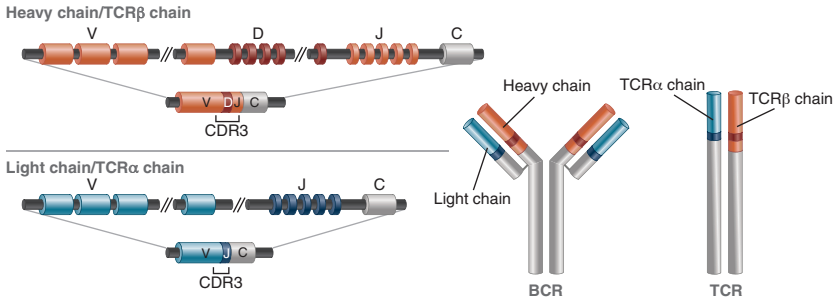
NEBNext Immune Sequencing Kit (Mouse)

#E6330S	24 reactions
#E6330L	96 reactions

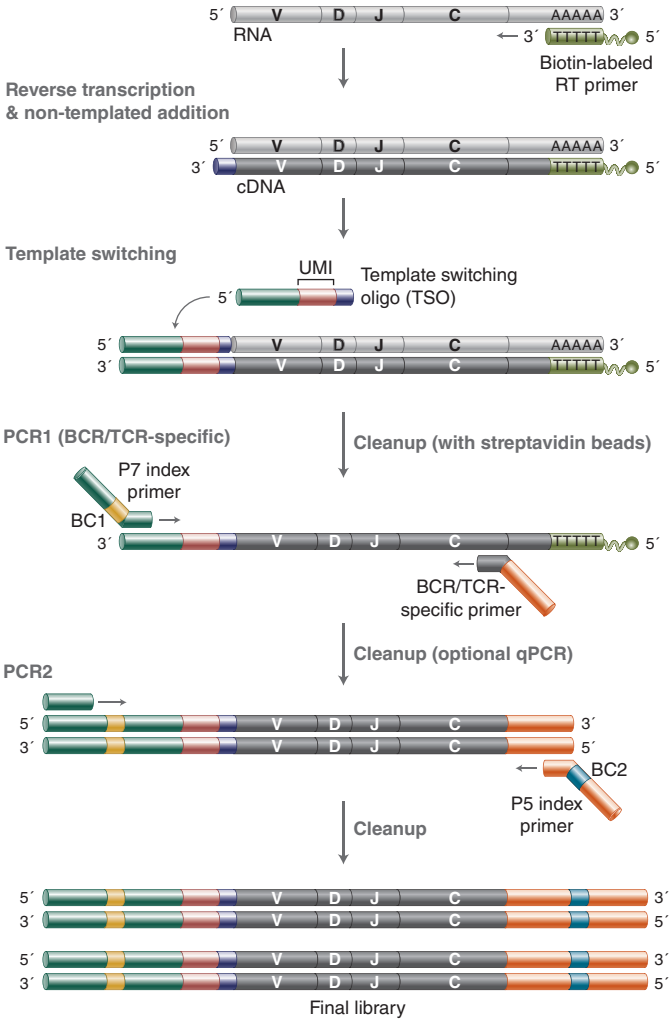
Description: The NEBNext Immune Sequencing Kits (Human & Mouse) enable exhaustive profiling of somatic mutations in the full-length immune gene repertoires of B cells and T cells, via the expression of complete antibody chains. A unique, UMI-based mRNA barcoding process allows PCR copies derived from an individual molecule to be converted to a consensus sequence. This improves sequence accuracy and eliminates PCR bias.

Immune repertoire sequencing is frequently used to analyze immune responses, both current and distant. Areas of particular interest include characterization of autoimmune diseases, oncology, discovery of neutralizing antibodies against infectious disease, tumor-infiltrating lymphocytes and use as a tool to study residual disease. Recent improvements in read lengths and throughputs of next-generation sequencing (NGS) platforms have resulted in a rise in the popularity of immune repertoire sequencing.

- *Generation of full-length variable sequences (including isotype information), allowing downstream antibody synthesis and functional characterization not possible with approaches sequencing only the CDR3 region*
- *Eliminated use of variable region primers, reducing primer pool complexity and realizing unbiased and simultaneous recovery of B-cell and T-cell receptor transcripts*
- *Minimized PCR bias and improved sequencing accuracy by allowing a consensus to be generated from duplicate sequencing reads originating from the same transcript; UMIs enable accurate quantitation of each clone present in the sample*
- *Optimized high target-capture efficiency for immune repertoire sequencing and analysis from sub-microgram quantities of total RNA*



Simplified representation of the structure of an antibody or TCR. *Simplified representation of the structure of an antibody or TCR showing the outcome of V(D)J recombination in mature lymphocytes.*



NEBNext Immune Sequencing Kit Workflow.

NEBNext® Microbiome DNA Enrichment Kit

#E2612S	6 reactions
#E2612L	24 reactions

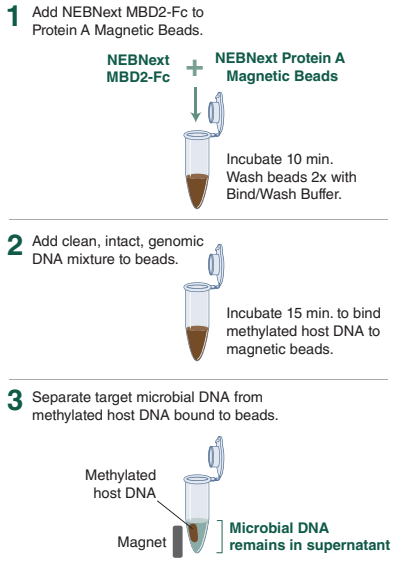
Description: The NEBNext Microbiome DNA Enrichment Kit facilitates separation of microbial DNA from methylated host DNA (including human) by selective binding and removal of the CpG methylated host DNA (1).

Functional Validation: Each set of reagents are functionally validated by enriching *E. coli* DNA from a mixture of *E. coli* and human DNA. Enrichment is evaluated through library construction and sequencing of the enriched sample on an Illumina sequencer.

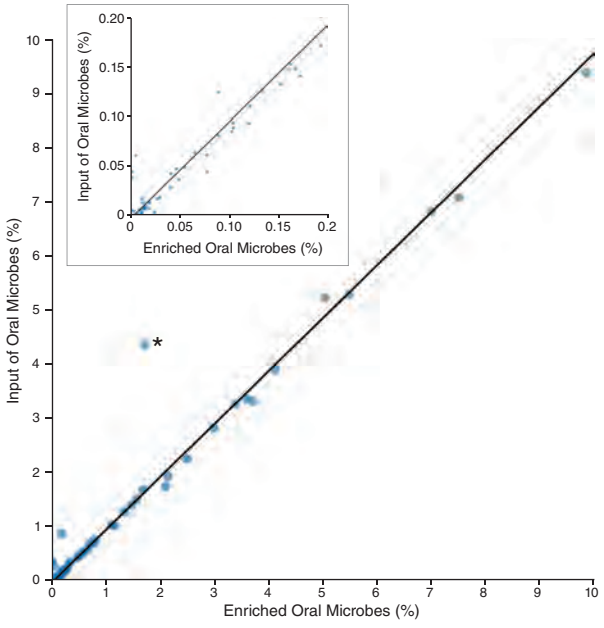
(1) Feehery, G.R., et al. (2013) *PLoS One*, 8: e76096
(2) Chen, T., et al. (2010) *Database*, Vol. 2010, Article ID baq013, doi: 10.1093/database/baq013
(3) Langmead, B., et al. (2009) *Genome Biol.* 10:R25 doi: 10.1186/gb-2009-10-3-r25

Kit Includes:

- NEBNext MBD2-Fc Protein
- NEBNext Bind/wash Buffer
- 16s rRNA Universal Gene Bacteria Control Primers
- RPL30 Human DNA Control Primers
- NEBNext Protein A Magnetic Beads

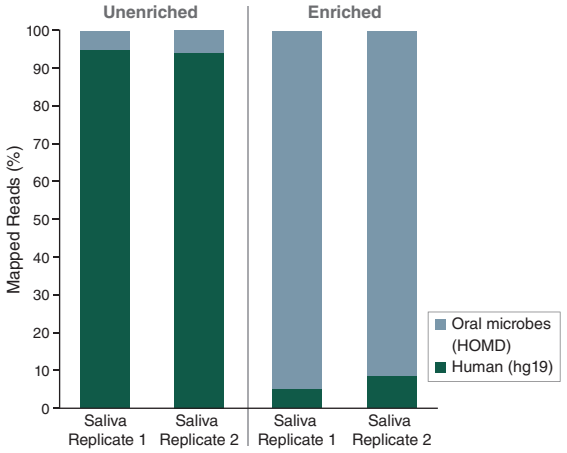


NEBNext Microbiome DNA Enrichment Kit workflow.



Microbiome Diversity is Retained after Enrichment with the NEBNext Microbiome DNA Enrichment Kit. *DNA was purified from pooled human saliva DNA (Innovative Research) and enriched using the NEBNext Microbiome DNA Enrichment Kit. Libraries were prepared from unenriched and enriched samples, followed by sequencing on the SOLiD 4 platform. The graph shows a comparison between relative abundance of each bacterial species listed in HOMD[2] before and after enrichment with the NEBNext Microbiome DNA Enrichment Kit. Abundance is inferred from the number of reads mapping to each species as a percentage of all reads mapping to HOMD. High concordance continues even to very low abundance species (inset). We compared 501M 50 bp SOliD 4 reads in the enriched dataset to 537M 50 bp SOLiD 4 reads in the unenriched dataset. Reads were mapped using Bowtie 0.12.7[3] with typical settings (2 mismatches in a 28 bp seed region, etc).*

** Niesseria flavescens – This organism may have unusual methylation density, allowing it to bind the enriching beads at a low level. Other Neisseria species (N. mucosa, N. sicca and N. elongata) are represented, but do not exhibit this anomalous enrichment.*



Salivary Microbiome DNA Enrichment. *DNA was purified from pooled human saliva DNA (Innovative Research) and enriched using the NEBNext Microbiome DNA Enrichment Kit. Libraries were prepared from unenriched and enriched samples and sequenced on the SOLiD 4 platform. The graph shows percentages of 500 M–537 M SOLiD™4 50 bp reads that mapped to either the Human reference sequence (hg19) or to a microbe listed in Human Oral Microbiome Database (HOMD)[2]. (Because the HOMD collection is not comprehensive, ~80% of reads in the enriched samples do not map to either database.) Reads were mapped using Bowtie 0.12.7[3] with typical settings (2 mismatches in a 28 bp seed region, etc.).*

NEBNext® Ultra™ II Q5® Master Mix

#M0544S	50 reactions
#M0544L	250 reactions
#M0544X	500 reactions

Companion Products:

NEBNext Q5 Hot Start HiFi PCR Master Mix

#M0543S	50 reactions
#M0543L	250 reactions

NEBNext High-Fidelity 2X PCR Master Mix

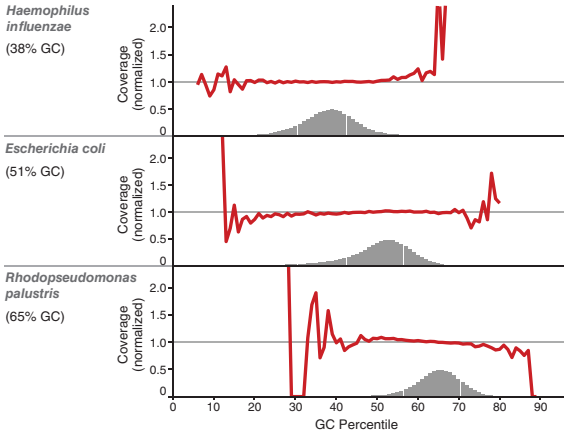
#M0541S	50 reactions
#M0541L	250 reactions

- *Next generation sequencing library preparation*
- *High-fidelity amplification*
- *Uniform GC coverage*
- *Improves sequencing library coverage of known difficult regions of the human genome*

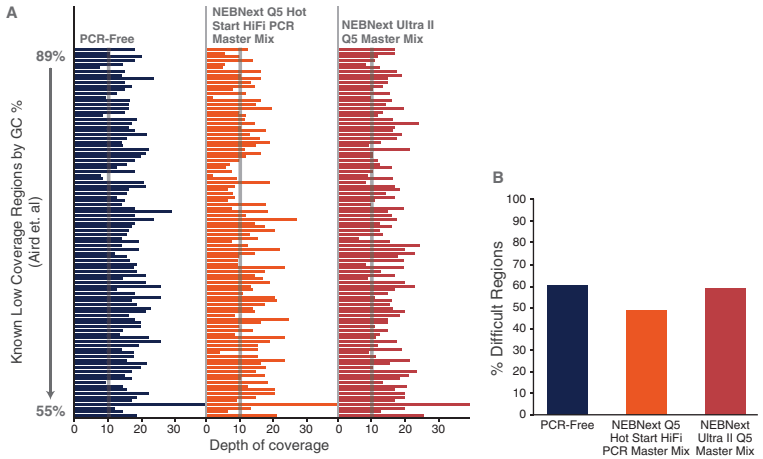
Description: The NEBNext Ultra II Q5 Master Mix is a new formulation of Q5 DNA Polymerase that has been optimized for robust, high-fidelity amplification of next-generation sequencing (NGS) libraries. This formulation further improves the uniformity of amplification of libraries, including superior performance with GC-rich regions.

The polymerase component of the master mix, Q5 High-Fidelity DNA Polymerase, is a novel thermostable DNA polymerase that possesses 3´→5´ exonuclease activity, and is fused to a processivity-enhancing Sso7d domain. Q5 also has the highest fidelity available (> 100-fold higher than that of *Taq* DNA Polymerase and ~12-fold higher than that of *Pyrococcus furiosus* (Pfu) DNA Polymerase), resulting in ultra-low error rates.

The NEBNext Ultra II Q5 Master Mix is an aptamer-based hot start formulation that allows convenient room temperature reaction set up. The convenient 2X master mix format contains dNTPs, Mg^{++} and a proprietary buffer, and requires only the addition of primers and DNA template for robust amplification. NEBNext Ultra II Q5 Master Mix is also included in the NEBNext Ultra II DNA Library Prep Kit for Illumina.



NEBNext Ultra II Q5 Master Mix provides uniform GC coverage for microbial genomic DNA with a broad range of GC composition. *Libraries were made using 100 ng of the genomic DNAs shown and the NEBNext Ultra II DNA Library Prep Kit. Libraries were amplified using the NEBNext Ultra II Q5 Master Mix, and sequenced on an Illumina MiSeq. GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each GC% is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library. NEBNext Ultra II Q5 Master Mix provides uniform GC coverage regardless of the GC content of the DNA.*



NEBNext Ultra II Q5 Master Mix provides improved coverage of known low-coverage regions of the human genome. *Libraries were prepared from Human NA19240 genomic DNA. One library was not amplified. The other two libraries were amplified using 5 cycles of PCR with NEBNext Q5 Hot Start HiFi PCR Master Mix (NEB #M0543) or with NEBNext Ultra II Q5 Master Mix (NEB #M0544). Libraries were sequenced on an Illumina NextSeq 500. 420 million 75 bp reads were randomly extracted from each dataset, representing an average coverage of 10X. Reads were mapped to the GRCh37 reference genome using Bowtie 2.2.4. Reads on each region were counted using bedtools v2.19.1. A: The number of reads overlapping distinct low coverage regions of the human genome (1) are shown for each library. B: From the 420 million 75 bp reads randomly extracted from each dataset, 10X coverage was expected. The % of difficult regions covered at > 10X are shown for each library. The NEBNext Ultra II Q5 Master Mix provides improved coverage of these known low coverage regions, without drop-outs, and shows similar coverage to the unamplified sample. (1) Popatov, V. and Ong, J.L. (2017). Examining Sources of Error in PCR by Single-Molecule Sequencing. PLoS ONE. 12(1):e0169774.*

NEBNext® Library Quant Kits

NEBNext Library Quant Kit for Illumina
#E7630S 100 reactions
#E7630L 500 reactions

NEW
NEBNext Library Quant Kit for Ultima Genomics
#E3410S 100 reactions
#E3410L 500 reactions

Companion Product:

NEBNext Library Dilution Buffer
#B6118S 15 ml

- *Provides more accurate and reproducible quant values than alternative methods and kits*
- *Compatible with libraries with a broad range of insert sizes and GC content, made by a variety of methods*
- *Utilizes a single extension time for all libraries, regardless of insert size*
- *Supplied with a convenient Library Dilution Buffer*
- *The NEBNext Library Quant Master Mix requires only the addition of primers*
- *Library quant values can be easily calculated using NEB's online tool, at NEBioCalculator.neb.com*

Accurate quantitation of next-generation sequencing (NGS) libraries is essential for optimizing data yield and quality from each sequencing run. Precise quantitation ensures that the appropriate amount of library is loaded onto the NGS platform, facilitating reliable predictions of sequencing output. Quantitative PCR (qPCR) is recognized as the most accurate and effective method for library quantitation, offering significantly higher consistency and reproducibility compared to alternative techniques. Amplification-based methods, such as qPCR, target adaptor-containing sequences, providing a more accurate estimate of the concentration of library molecules available for sequencing.

NEBNext library quant kits are optimized with platform-specific reagents to ensure efficient amplification of Illumina® or Ultima® genomic NGS libraries. The streamlined protocol minimizes pipetting steps and uses a single extension time for all libraries, enhancing efficiency while generating reproducible, high-quality results.

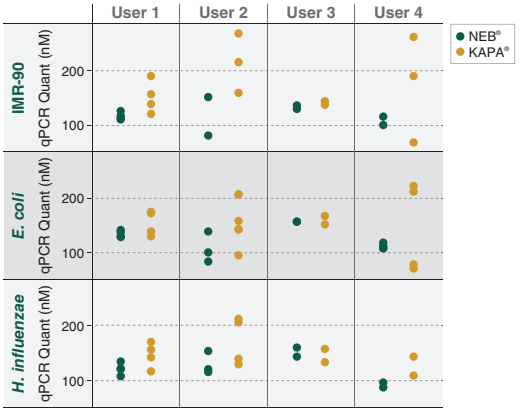
The NEBNext Library Quant Kit for Illumina is designed for precise qPCR-based quantitation of NGS libraries prepared for Illumina sequencing platforms. This kit includes primers targeting the P5 and P7 Illumina adaptor sequences and pre-diluted standards for reliable quantitation of DNA libraries ranging from 150 to 1,000 bp. It offers flexibility with the option to use either four or six standards.

The NEBNext® Library Quant Kit for Ultima Genomics® delivers precise and reliable qPCR-based quantitation of NGS libraries prepared for the Ultima Genomics UG 100™ sequencing platform. The kit features forward and reverse primers targeting the Ultima adaptor sequence, and includes five high-quality, pre-diluted DNA standards for reliable quantitation of diluted DNA libraries. Additionally, it incorporates the Luna® Universal qPCR Master Mix with a blue, non-interfering, visible tracking dye, which can also serve as a passive reference dye, eliminating the need for additional ROX in real-time instruments requiring ROX normalization.

NEBNext library quant kits ensure unparalleled accuracy and reliability in NGS library quantitation, giving users confidence in their results.

	Reagent Preparation	Library Dilution	Set Up	qPCR	Data Analysis	Total Workflow
Hands-On	5 min.	10 min.	25 min.	1 min.	10 min.	**51 min.**
Total	5 min.	10 min.	25 min.	60 min.	10 min.	**1 hr. 50 min.**

The NEBNext Library Quant workflow.



Greater reproducibility of library quantitation with the NEBNext Library Quant Kit for Illumina. *Three 340–400 bp libraries were quantitated by 4 different users 2–4 times using either the NEBNext or Kapa Library Quantification Kit (Universal). A notable improvement in quantitation consistency was observed for concentrations determined by the NEBNext Kit (green) versus those from the Kapa kit (gold).*

Reagents for Ion Torrent

NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent
#E6285L 50 reactions

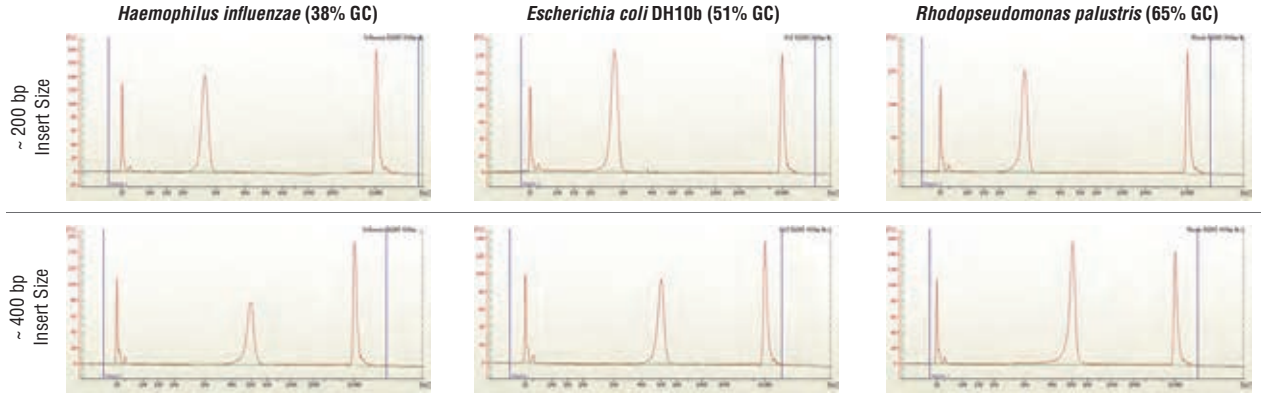
NEBNext Fast DNA Library Prep Set or Ion Torrent
#E6270L 50 reactions

Description: NEBNext kits are available for DNA library preparation for Ion Torrent, with or without enzymatic DNA fragmentation. In addition to stringent QCs on individual components, the NEBNext DNA kits are functionally validated by library preparation of a genomic DNA library, followed by Ion Torrent sequencing. Reagent lots are reserved specifically for inclusion in NEBNext kits. Most of these reagents are provided in master mix format, reducing the number of vials provided in the kits, and reducing pipetting steps. Adaptors and primers for singleplex libraries are supplied in the kits. For multiplexed libraries, the Ion XPress™ Barcode Adaptors from Thermo Fisher Scientific can be used.

Input 10 ng – 1 µg*

Fragmentation	End Repair	Adaptor Ligation/Fill-In	PCR Enrichment	Total Workflow
NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent (NEB #E6285)				
• DNA Fragmentation Master Mix • DNA Fragmentation Reaction Buffer	• End Repair Enzyme Mix • End Repair Reaction Buffer (10X)	• Adaptors for Ion Torrent • T4 DNA Ligase • T4 DNA Ligase Buffer for Ion Torrent (10X) • *Bst* 2.0 WarmStart® DNA Polymerase	• Primers for Ion Torrent • NEBNext Q5 Hot Start HiFi PCR Master Mix	**Hands-On Time** 12 min. **Total Time** 110 min. – 133 min.
NEBNext Fast DNA Library Prep Set for Ion Torrent (NEB #E6270)				
	• End Repair Enzyme Mix • End Repair Reaction Buffer (10X)	• Adaptors for Ion Torrent • T4 DNA Ligase • T4 DNA Ligase Buffer for Ion Torrent (10X) • *Bst* 2.0 WarmStart DNA Polymerase	• Primers for Ion Torrent • NEBNext Q5 Hot Start HiFi PCR Master Mix	**Hands-On Time** 12 min. **Total Time** 110 min. – 133 min.

**Note that a minimum of 100 ng is recommended when used in conjunction with Ion Express Barcode Adaptors.*



Varying GC Content Libraries. *0.5 µg of DNA from 3 different genomes with varying GC content were used to construct 200 bp and 400 bp libraries using the NEBNext Fast DNA Fragmentation and Library Prep Set for Ion Torrent, analyzed by the Agilent Bioanalyzer.*

Kits for Illumina DNA Library Preparation

Product	NEB #	Size
NEBNext UltraExpress DNA Library Prep Kit	E3325S E3325L	24 reactions 96 reactions
NEBNext UltraExpress FS DNA Library Prep Kit	E3340S E3340L	24 reactions 96 reactions
NEBNext Ultra II DNA Library Prep Kit for Illumina	E7645S E7645L	24 reactions 96 reactions
NEBNext Ultra II DNA Library Prep with Sample Purification Beads	E7103S E7103L	24 reactions 96 reactions
NEBNext Ultra II FS DNA Library Prep Kit for Illumina	E7805S E7805L	24 reactions 96 reactions
NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads	E6177S E6177L	24 reactions 96 reactions
NEBNext Ultra II DNA PCR-free Library Prep Kit for Illumina	E7410S E7410L	24 reactions 96 reactions
NEBNext Ultra II FS DNA PCR-free Library Prep Kit for Illumina	E7430S E7430L	24 reactions 96 reactions
NEBNext Ultra II FS DNA PCR-free Library Prep with Sample Purification Beads	E7435L	96 reactions
NEBNext Enzymatic Methyl-seq v2 Kit	E8015S E8015L	24 reactions 96 reactions
NEBNext Enzymatic 5hmC-seq Kit	E3350S E3350L	24 reactions 96 reactions
NEBNext FFPE DNA Library Prep Kit	E6650S E6650L	24 reactions 96 reactions
NEBNext UltraShear FFPE DNA Library Prep Kit	E6655S E6655L	24 reactions 96 reactions
NEBNext Ultra DNA Library Prep Kit for Illumina	E7370L	96 reactions

Modules & Enzymes for DNA Library Prep

Product	NEB #	Size
NEBNext Enzymatic Methyl-seq v2 Conversion Module	E8020S E8020L	24 reactions 96 reactions
NEBNext Enzymatic 5hmC-seq Conversion Module	E3365S E3365L	24 reactions 96 reactions
NEBNext FFPE DNA Repair v2 Module	E7360S E7360L	24 reactions 96 reactions
NEBNext FFPE DNA Repair Mix	M6630S M6630L	24 reactions 96 reactions
NEBNext Microbiome DNA Enrichment Kit	E2612S E2612L	6 reactions 24 reactions
NEBNext UltraShear	M7634S M7634L	24 reactions 96 reactions
NEBNext Ultra II FS DNA Module	E7810S E7810L	24 reactions 96 reactions
NEBNext Ultra II End Repair/dA-Tailing Module	E7546S E7546L	24 reactions 96 reactions
NEBNext Ultra II Ligation Module	E7595S E7595L	24 reactions 96 reactions
NEBNext Ultra II Q5 Master Mix	M0544S M0544L M0544X	50 reactions 250 reactions 500 reactions
NEBNext dsDNA Fragmentase	M0348S M0348L	50 reactions 250 reactions
NEBNext End Repair Module	E6050S E6050L	20 reactions 100 reactions
NEBNext dA-Tailing Module	E6053S E6053L	20 reactions 100 reactions
NEBNext Quick Ligation Module	E6056S E6056L	20 reactions 100 reactions
NEBNext Q5 Hot Start HiFi PCR Master Mix	M0543S M0543L	50 reactions 250 reactions
NEBNext High-Fidelity 2X PCR Master Mix	M0541S M0541L	50 reactions 250 reactions
NEBNext Q5U Master Mix	M0597S M0597L	50 reactions 250 reactions
NEBNext dsDNA Fragmentase Reaction Buffer v2	B0349S	6 ml

Pat joined NEB in 2017 and is currently the Facilities and Engineering Director. At NEB, he is part of the Mountain Biking and Bee Keeping Clubs. Learn more about Pat in his video reel on Instagram.

#NEBiographies

Adaptors & Primers for DNA Library Prep

Product	NEB #	Size
NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1)	E7395S E7395L	96 reactions 384 reactions
NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 2)	E7874S E7874L	96 reactions 384 reactions
NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 3)	E7876S E7876L	96 reactions 384 reactions
NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 4)	E7878S E7878L	96 reactions 384 reactions
NEBNext LV Unique Dual Index Primers Set 2A	E3390S	24 reactions
NEBNext LV Unique Dual Index Primers Set 2B	E3392S	24 reactions
NEBNext LV Unique Dual Index Primers Set 1	E3400S	96 reactions
NEBNext LV Unique Dual Index Primers Set 2	E3402S	96 reactions
NEBNext LV Unique Dual Index Primers Set 3	E3404S	96 reactions
NEBNext LV Unique Dual Index Primers Set 4	E3406S	96 reactions
NEBNext LV Unique Dual Index Primers Set 5	E3408S	96 reactions
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	E6440S E6440L	96 reactions 384 reactions
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 2)	E6442S E6442L	96 reactions 384 reactions
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 3)	E6444S E6444L	96 reactions 384 reactions
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 4)	E6446S E6446L	96 reactions 384 reactions
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 5)	E6448S E6448L	96 reactions 384 reactions
NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)	E7140S E7140L	24 reactions 96 reactions
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 reactions
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2)	E7780S	96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	E7335S E7335L	24 reactions 96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	E7500S E7500L	24 reactions 96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)	E7710S E7710L	24 reactions 96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)	E7730S E7730L	24 reactions 96 reactions
NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S E6609L	96 reactions 384 reactions
NEBNext Adaptor Dilution Buffer	B1430S	9.6 ml

Magnetic Separation

Product	NEB #	Size
NEBNext Magnetic Separation Rack	S1515S	24 tubes

Target Enrichment

Product	NEB #	Size
NEBNext Direct Genotyping Solution	E9530B-S E9500B-S	8 reactions 96 reactions
NEBNext Direct GS Baits	N9530B	8 reactions
NEBNext Immune Sequencing Kit (Human)	E6320S E6320L	24 reactions 96 reactions
NEBNext Immune Sequencing Kit (Mouse)	E6330S E6330L	24 reactions 96 reactions

Library Quantitation

Product	NEB #	Size
NEBNext Library Quant Kit for Illumina	E7630S E7630L	100 reactions 500 reactions
NEBNext Library Quant Kit for Ultima Genomics	E3410S E3410L	100 reactions 500 reactions
NEBNext Library Dilution Buffer	B6118S	15 ml
NEBNext Library Quant DNA Standards	E7642S	500 reactions

DNA Enrichment

Product	NEB #	Size
NEBNext Microbiome DNA Enrichment Kit	E2612S E2612L	6 reactions 24 reactions

Products for Oxford Nanopore DNA Library Prep

Product	NEB #	Size
NEBNext Companion Module for Oxford Nanopore Technologies Ligation Sequencing	E7180S E7180L	24 reactions 96 reactions
NEBNext Companion Module v2 for Oxford Nanopore Technologies Ligation Sequencing	E7672S E7672L	24 reactions 96 reactions
NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies)	E7660L	96 reactions

Products for Ion Torrent DNA Library Prep

Product	NEB #	Size
NEBNext Fast DNA Library Prep Set for Ion Torrent	E6270L	50 reactions
NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent	E6285L	50 reactions

Kits for Illumina RNA Library Preparation

Product	NEB #	Size
NEBNext UltraExpress RNA Library Prep Kit	E3330S E3330L	24 reactions 96 reactions
NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	E7760S E7760L	24 reactions 96 reactions
NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	E7765S E7765L	24 reactions 96 reactions
NEBNext Ultra II RNA Library Prep Kit for Illumina	E7770S E7770L	24 reactions 96 reactions
NEBNext Ultra II RNA Library Prep with Sample Purification Beads	E7775S E7775L	24 reactions 96 reactions
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1)	E7300S E7300L	24 reactions 96 reactions
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2)	E7580S E7580L	24 reactions 96 reactions
NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48)	E7560S	96 reactions
NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible)	E7330S E7330L	24 reactions 96 reactions
NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina	E6420S E6420L	24 reactions 96 reactions
NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina)	E7658L	96 reactions

NEBNext Selector

Use this tool to guide you through selection of NEBNext reagents for next generation sequencing sample preparation. Try it out at **NEBNextSelector.neb.com**

NEBNext Custom RNA Depletion Design Tool

This tool designs probes to be used with the NEBNext RNA Depletion Core Reagent Set or to supplement an existing NEBNext depletion kit for the depletion of unwanted RNA species. Try it out at **Depletion-Design.neb.com**

Modules & Enzymes for RNA Library Prep

Product	NEB #	Size
NEBNext ARTIC SARS-CoV-2 RT-PCR Module	E7626L	96 reactions
NEBNext RSV Primer Module	E9642S E9642L	24 reactions 96 reactions
NEBNext RNA Depletion Core Reagent Set	E7865S E7865L E7865X	6 reactions 24 reactions 96 reactions
NEBNext RNA Depletion Core Reagent Set with RNA Sample Purification Beads	E7870S E7870L E7870X	6 reactions 24 reactions 96 reactions
NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)	E7750S E7750L E7750X	6 reactions 24 reactions 96 reactions
NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E7755S E7755L E7755X	6 reactions 24 reactions 96 reactions
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)	E7400S E7400L E7400X	6 reactions 24 reactions 96 reactions
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads	E7405S E7405L E7405X	6 reactions 24 reactions 96 reactions
NEBNext rRNA Depletion Kit (Bacteria)	E7850S E7850L E7850X	6 reactions 24 reactions 96 reactions
NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads	E7860S E7860L E7860X	6 reactions 24 reactions 96 reactions
NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S E7490L	24 reactions 96 reactions
NEBNext High Input Poly(A) mRNA Isolation Module	E3370S	24 reactions
NEBNext Magnesium RNA Fragmentation Module	E6150S	200 reactions
NEBNext Ultra II RNA First Strand Synthesis Module	E7771S E7771L	24 reactions 96 reactions
NEBNext Ultra II Directional RNA Second Strand Synthesis Module	E7550S E7550L	24 reactions 96 reactions
NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module	E6111S E6111L	20 reactions 100 reactions
NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module	E6421S E6421L	24 reactions 96 reactions
NEBNext Single Cell Lysis Module	E5530S	96 reactions
NEBNext Ultra II End Repair/dA-Tailing Module	E7546S E7546L	24 reactions 96 reactions
NEBNext Ultra II Ligation Module	E7595S E7595L	24 reactions 96 reactions
NEBNext Ultra Ligation Module	E7445L	96 reactions
NEBNext End Repair Module	E6050S E6050L	20 reactions 100 reactions
NEBNext dA-Tailing Module	E6053S E6053L	20 reactions 100 reactions
NEBNext Quick Ligation Module	E6056S E6056L	20 reactions 100 reactions

Adaptors & Primers for RNA Library Prep

Product	NEB #	Size
NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors RNA Set 1)	E7416S E7416L	96 reactions 384 reactions
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	E6440S E6440L	96 reactions 384 reactions
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 2)	E6442S E6442L	96 reactions 384 reactions
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 3)	E6444S E6444L	96 reactions 384 reactions
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 4)	E6446S E6446L	96 reactions 384 reactions
NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 5)	E6448S E6448L	96 reactions 384 reactions
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 reactions
NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2)	E7780S	96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	E7335S E7335L	24 reactions 96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	E7500S E7500L	24 reactions 96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)	E7710S E7710L	24 reactions 96 reactions
NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)	E7730S E7730L	24 reactions 96 reactions
NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S E6609L	96 reactions 384 reactions
NEBNext Adaptor Dilution Buffer	B1430S	9.6 ml

Library Quantitation

Product	NEB #	Size
NEBNext Library Quant Kit for Illumina	E7630S E7630L	100 reactions 500 reactions
NEBNext Library Quant Kit for Ultima Genomics	E3410S E3410L	100 reactions 500 reactions
NEBNext Library Dilution Buffer	B6118S	15 ml

Products for Oxford Nanopore RNA Library Prep

Product	NEB #	Size
NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies)	E7660L	96 reactions

Magnetic Separation

Product	NEB #	Size
NEBNext Magnetic Separation Rack	S1515S	24 tubes

Deyra joined NEB in 2013 as a Product Development Scientist and is now a Product Marketing Manager for our NEBNext library preparation portfolio. Deyra has also enjoyed mentoring scientists in training.

Learn about Dylan's work
on the detection of malaria.

WATCH

Doctor checking pregnant woman
Credit: NanSan, Adobe Stock

Advancing Malaria Diagnostics to Protect Mothers and Newborns in Ethiopia

Malaria remains a significant global health challenge, particularly for pregnant women and their unborn children in sub-Saharan Africa. Dylan Pillai, a medical practitioner and researcher who has spent more than two decades researching malaria, is dedicated to improving diagnostics and outcomes for this vulnerable population.

Pillai led the LAMPREG project, an international collaboration between institutes in Canada and Ethiopia, aimed at enhancing malaria detection in pregnant women through Loop-mediated Isothermal Amplification (LAMP). This technique amplifies DNA at a constant temperature, making it a diagnostic tool that can be used in low-resource settings. Unlike traditional methods such as microscopy and rapid diagnostic tests (RDTs), which often miss asymptomatic cases, LAMP can detect lower levels of the malaria parasite. This makes it particularly valuable in many endemic regions where asymptomatic malaria is common among populations with partial immunity due to repeated exposure. By detecting both symptomatic and asymptomatic infections, LAMP addresses a crucial gap in malaria diagnostics, reducing the number of untreated cases and helping to curb ongoing transmission.

The project enrolled 2,425 pregnant women from three regions in Ethiopia. Participants were randomized into two groups: one received the standard of care using traditional diagnostics, and the other underwent additional testing with LAMP. By using LAMP to identify malaria cases that would otherwise go undetected, the project was able to treat women earlier in their pregnancies, ultimately leading to better health outcomes for both mothers and babies. One of the key findings of the study was that babies born to women in the LAMP intervention group showed improved weight gain between birth and 28 days of life, a critical marker of early childhood health. The study also examined other important health metrics, such as anemia, placental malaria, and preterm delivery, though the weight gain outcome stood out as the most impactful result.

The LAMPREG project strengthened local healthcare infrastructure by training over 100 workers and donating essential equipment, including portable ultrasound machines. This capacity-building effort has resulted in a sustainable model for malaria diagnosis that can be replicated in other regions and low-resource settings.

Looking ahead, Pillai and his team are in the process of sharing their findings with policymakers and NGOs. *“Our goal is to better understand the role technologies like LAMP will play in routine malaria diagnostics.”*, he said. With the success of the LAMPREG Project, Pillai and his team are now focused on expanding their research to deepen our understanding of the complex relationship between malaria and maternal health. Next generation sequencing and metabolomics studies are underway to explore drug resistance and other critical aspects of malaria in pregnancy.

By improving diagnostics for malaria in pregnancy, Pillai and his team are contributing to a future where fewer mothers and newborns suffer from a preventable and treatable disease. They have demonstrated how scientific advancements, coupled with international collaboration and on-the-ground implementation, can lead to meaningful improvements in global health.

Professor Dylan Pillai
University of Calgary, Alberta, Canada
2024 Passion in Science
Humanitarian Duty Award

Markers & Ladders (DNA, RNA & Protein)

A wide range of ladders and markers to meet your macromolecule quantification needs.

New England Biolabs provides a wide range of ladders and markers, featuring exceptional quality, uniform band intensities, convenient band spacing and easy-to-identify reference bands.

Double-stranded DNA markers are available for conventional electrophoresis. Conventional electrophoresis markers (size range: ~10 to 2.3 x 10^4 bp) include: HindIII and BstEII digests of Lambda DNA, BstNI and MspI digests of pBR322 DNA, and a HaeIII digest of ΦX174 DNA.

We also supply a series of DNA ladders ranging from 10 bp to 48.5 kb. Our 100 bp DNA ladder, 1 kb DNA Ladder and 1 kb Plus DNA Ladder are available in several formats: Conventional, Quick-Load® using either non-fluorescing purple dye or bromophenol blue as a tracking dye, and TriDye™ containing three dyes to track gel migration. Our Quick-Load Purple DNA ladders utilize our purple loading dye that improves band visibility by casting no UV shadow and are supplied with a vial of purple dye.

Our RNA ladders and markers have a size range of 17 to 9,000 bases. Both ssRNA ladders are supplied with 2X sample buffer and feature a higher intensity fragment to serve as a reference band. The dsRNA ladders are suitable for use as a size standard in dsRNA and RNAi analysis on both denaturing polyacrylamide and agarose gels.

For your protein analysis needs, NEB offers a selection of highly pure protein standards. Sizes range from 10–250 kDa, which is ideal for accurate molecular weight determination for a wide range of expressed proteins.

Featured Products

190 Quick-Load Purple 1 kb Plus DNA Ladder

190 1 kb Plus DNA Ladder for Safe Stains

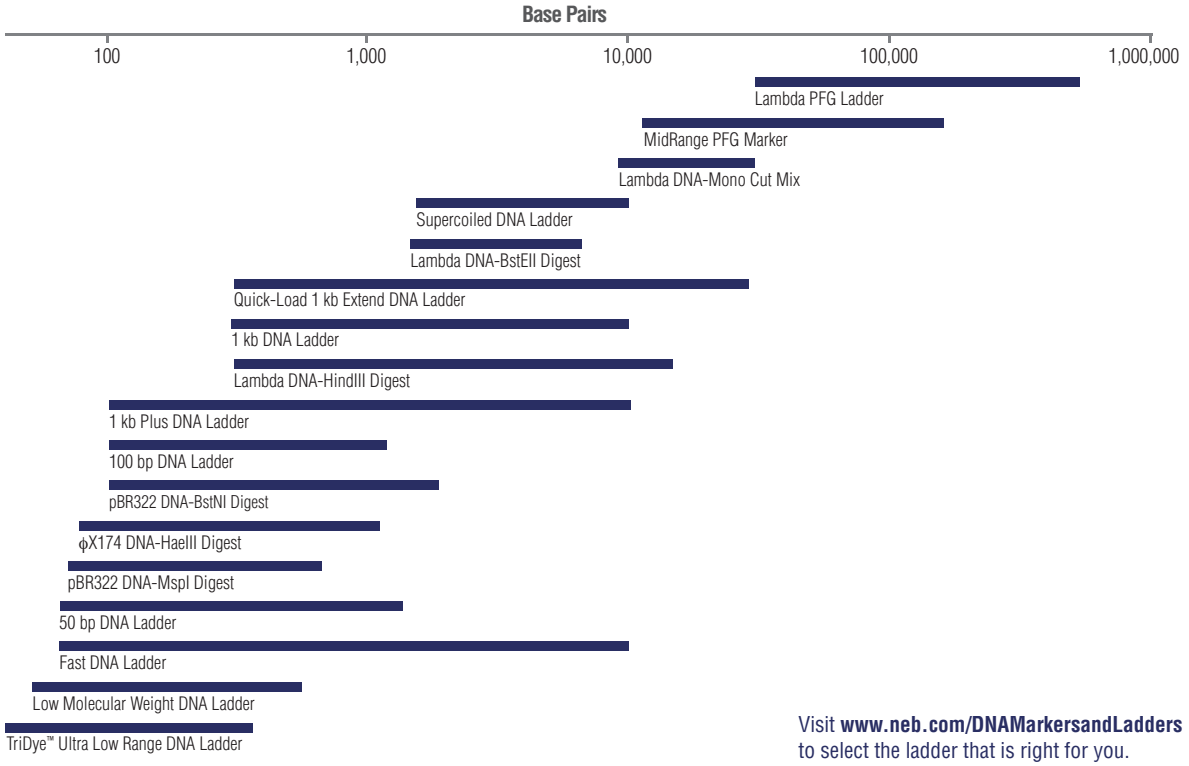
190 TriDye Ultra Low Range DNA Ladder

193 Color Prestained Protein Standard, Broad Range (10–250 kDa)

Featured Tools & Resources

Visit **www.neb.com/DNAladders** to find a selection tool for NEB's DNA markers and ladders.

Size Ranges of DNA Ladders



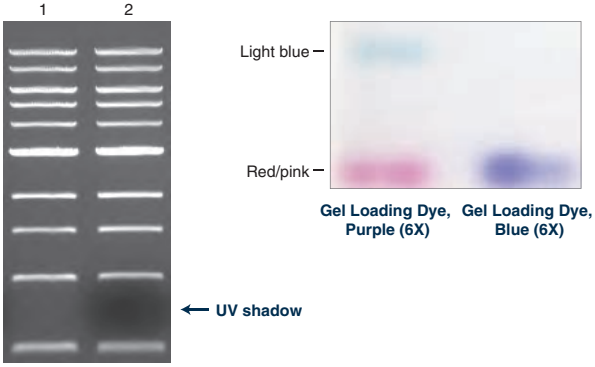
Visit **www.neb.com/DNAMarkersandLadders** to select the ladder that is right for you.

Purple Loading Dye

Gel Loading Dye, Purple (6X)
#B7024S 4 ml

Gel Loading Dye, Purple (6X), no SDS
#B7025S 4 ml

Our Gel Loading Dye, Purple (6X) (with and without SDS) is supplied with all unstained DNA Ladders, sharpens bands and eliminates the UV shadow seen with other dyes. These pre-mixed loading buffers contain a combination of two dyes, Dye 1 (pink/red) and Dye 2 (blue). The red dye serves as the tracking dye for both agarose and non-denaturing polyacrylamide gel electrophoresis. The two dyes separate upon electrophoresis; the red band is the major indicator and migrates similarly to Bromophenol Blue on agarose gels. Specifically chosen, this dye does not leave a shadow under UV light. EDTA is also included to chelate magnesium (up to 10 mM) in enzymatic reactions, thereby stopping the reaction. The dyes also contain Ficoll, which creates brighter and tighter bands when compared to glycerol loading dyes. Gel Loading Dye, Purple (6X) contains SDS, which often results in sharper bands, as some restriction enzymes are known to remain bound to DNA following cleavage.



The Gel Loading Dye, Purple (6X) (Lane 1) included in the Quick-Load Purple 1 kb DNA Ladder does not cast a UV shadow over the underlying bands, unlike the Gel Loading Dye, Blue (6X) (Lane 2).

DNA Ladders

1 kb DNA Ladder
#N3232S 200 gel lanes
#N3232L 1,000 gel lanes

100 bp DNA Ladder
#N3231S 100 gel lanes
#N3231L 500 gel lanes

1 kb Plus DNA Ladder
#N3200S 200 gel lanes
#N3200L 1,000 gel lanes

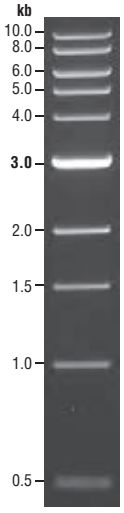
50 bp DNA Ladder
#N3236S 200 gel lanes
#N3236L 1,000 gel lanes

Low Molecular Weight DNA Ladder
#N3233S 100 gel lanes
#N3233L 500 gel lanes

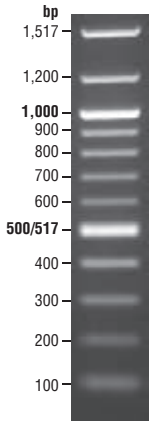
PCR Marker
#N3234S 100 gel lanes
#N3234L 500 gel lanes

NEB offers a variety of DNA Ladders with sizes ranging from 10 bp to 48.5 kb for use in agarose gel electrophoresis.

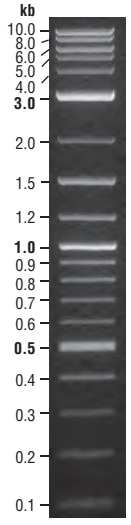
- Stable at room temperature
- Sharp, uniform bands
- Easy-to-identify reference bands
- Supplied with 1 vial of Gel Loading Dye, Purple (6X), no SDS
- Can be used for sample quantification (see www.neb.com for mass values)



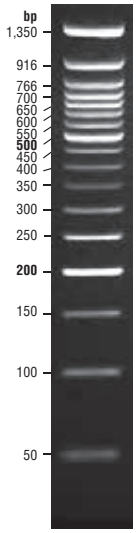
1 kb DNA Ladder
1 kb DNA Ladder visualized by ethidium bromide staining on a 0.8% TAE agarose gel.



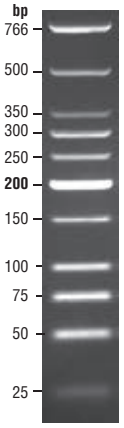
100 bp DNA Ladder
100 bp DNA Ladder visualized by ethidium bromide staining on a 1.3% TAE agarose gel.



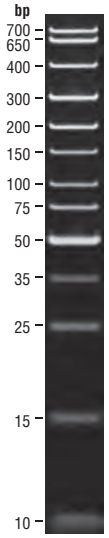
1 kb Plus DNA Ladder
1 kb Plus DNA Ladder visualized by ethidium bromide staining on a 1.0% TBE agarose gel.



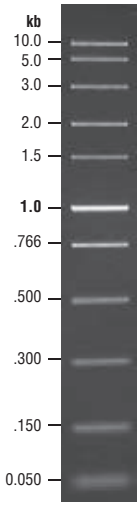
50 bp DNA Ladder
1.0 µg of 50 bp DNA Ladder visualized by ethidium bromide staining on a 3% TBE agarose gel.



Low Molecular Weight DNA Ladder
0.5 µg of LMW DNA Ladder visualized by ethidium bromide staining on a 3% TBE agarose gel.

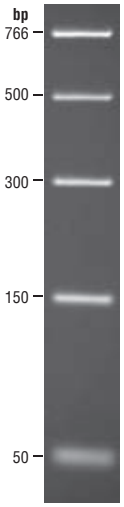


TriDye Ultra Low Range DNA Ladder
0.5 µg of Ultra Low Range DNA Ladder visualized by ethidium bromide staining on a 20% polyacrylamide gel.

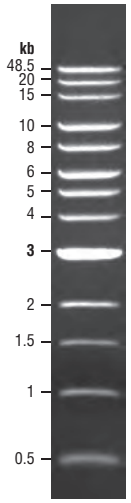


Fast DNA Ladder*
1.2% TBE agarose gel.

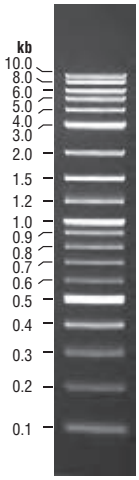
** Fast DNA Ladder can be used for fast electrophoresis systems as well as standard electrophoresis. It is in a ready-to-load format with a xylene cyanol dye.*



PCR Marker
0.3 µg of PCR Marker visualized by ethidium bromide staining on a 1.8% TBE agarose gel.



Quick-Load 1 kb Extend DNA Ladder
0.5 µg of Quick-Load 1 kb Extend DNA Ladder visualized by ethidium bromide staining on a 0.5% TBE agarose gel.



1 kb Plus DNA Ladder for Safe Stains**
1.0% TBE agarose gel.

*** 1 kb Plus DNA Ladder for Safe Stains, optimized for use with GelRed®, GelGreen® and SYBR® precast gels.*

Usage Notes: Dilution of these markers is recommended in TE or other buffer of minimal ionic strength. DNA may denature if diluted in dH_2O.

Individual DNA fragments, as seen in the 1 kb DNA Ladder, are available on request. Contact **info@neb.com** for more information.

DNA Ladders in Convenient Pre-mixed Formats

Quick-Load Purple 1 kb Plus DNA Ladder
#N0550S 250 gel lanes
#N0550L 750 gel lanes

Quick-Load Purple 1 kb DNA Ladder
#N0552S 125 gel lanes
#N0552L 375 gel lanes

Quick-Load Purple 100 bp DNA Ladder
#N0551S 125 gel lanes
#N0551L 375 gel lanes

Quick-Load Purple 50 bp DNA Ladder
#N0556S 250 gel lanes

Quick-Load Purple Low Molecular Weight DNA Ladder
#N0557S 125 gel lanes

1 kb Plus DNA Ladder for Safe Stains
#N0559S 1.25 ml

Fast DNA Ladder
#N3238S 1 ml

TriDye 1 kb Plus DNA Ladder
#N3270S 250 gel lanes

TriDye 1 kb DNA Ladder
#N3272S 125 gel lanes

TriDye 100 bp DNA Ladder
#N3271S 125 gel lanes

TriDye Ultra Low Range DNA Ladder
#N0558S 1.25 ml

Quick-Load 1 kb Plus DNA Ladder
#N0469S 250 gel lanes

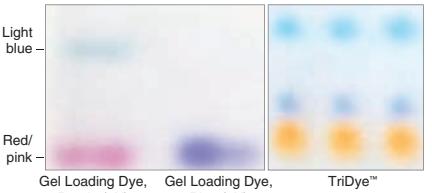
Quick-Load 1 kb DNA Ladder
#N0468S 125 gel lanes
#N0468L 375 gel lanes

Quick-Load 1 kb Extend DNA Ladder
#N3239S 125 gel lanes

Quick-Load 100 bp DNA Ladder
#N0467S 125 gel lanes
#N0467L 375 gel lanes

- Ready-to-load
- Uniform band intensities
- Easy-to-identify reference bands
- Defined mass profile for sample quantification.

Our 1 kb Plus, 1 kb and 100 bp DNA Ladders are offered in four formats. Conventional ladders are supplied with 1 vial of Gel Loading Dye, Purple (6X), no SDS. Quick-Load ladders use either non-fluorescing, purple dye or bromophenol blue as a tracking dye. TriDye ladders contain three dyes to facilitate monitoring of gel migration. Note that the TriDye Ultra Low Range DNA Ladder is suitable for both native polyacrylamide and agarose gels.



PFG Ladders

Lambda PFG Ladder
#N0341S 50 gel lanes

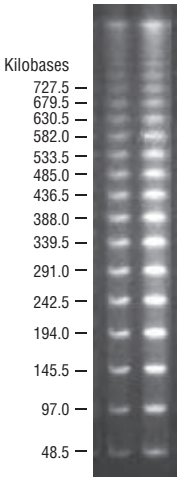
MidRange PFG Marker
#N0342S 50 gel lanes

λ DNA-Mono Cut Mix
#N3019S 100 gel lanes

The Lambda PFG Ladder consists of one GelSyringe dispenser, sufficient for 50 gel lanes. Successively larger concatemers of lambda DNA (*cI*857 *ind* 1 *Sam*7) are embedded in 1% LMP agarose. Size range: 48.5–1,018 kb.

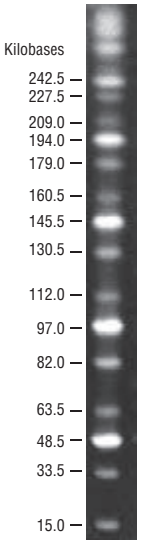
MidRange PFG Marker consists of concatemers of λ DNA isolated from the bacteriophage λ (*cI*857 *ind*1 *Sam*7) mixed with XhoI digested λ DNA embedded in 1% LMP agarose and supplied in a GelSyringe dispenser. XhoI produces fragments of 15.0 and 33.5 kb. These fragments anneal to and form concatemers with intact λ DNA. Size range: 15–291 kb.

The Lambda DNA-Mono Cut Mix is best separated by pulsed field gel electrophoresis, but can be alternatively used with standard electrophoresis systems. It is supplied in a liquid format. Size range: 1.5–48.5 kb.



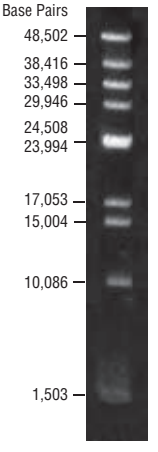
Lambda PFG Ladder

1% agarose gel, 4.5 V/cm, 15°C for 48 hours. Switch times ramped from 5–120 seconds.



MidRange PFG Marker

1% agarose gel, 6 V/cm, 15°C for 24 hours. Switch times ramped from 1–25 seconds.



Lambda DNA-Mono Cut Mix

PFGE separation of 0.5 µg of Lambda DNA-Mono Cut Mix. 1% agarose gel, 0.5X TBE, 6 V/cm, 15°C for 20 hours. Switch times ramped from 0.5–1.5 seconds.

Conventional DNA Markers

Lambda DNA HindIII Digest
#N3012S 150 gel lanes
#N3012L 750 gel lanes

λ DNA-BstEII Digest
#N3014S 150 gel lanes

ΦX174 DNA-HaeIII Digest
#N3026S 50 gel lanes
#N3026L 250 gel lanes

pBR322 DNA-BstNI Digest
#N3031L 250 gel lanes

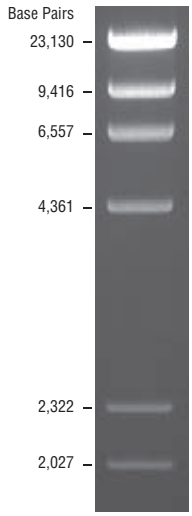
pBR322 DNA-MspI Digest
#N3032S 50 gel lanes

NEB offers a wide range of double-stranded DNA molecular weight markers for conventional agarose gel electrophoresis. These standards have a size range of approximately 10–23,000 base pairs.

The typical pattern generated by each of the conventional markers is shown below. The number of fragments generated by each marker, as well as the specific fragment sizes for each of the conventional markers can be found online.

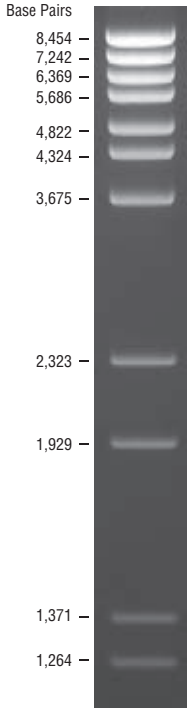
Usage Recommendation: Dilution of these markers is recommended in TE or other buffer of minimal ionic strength. DNA may denature if diluted in dH_2O.

The cohesive ends of fragments 1 and 4 of the Lambda DNA-HindIII and Lambda DNA-BstEII Digests can be separated by heating to 60°C for 3 minutes.



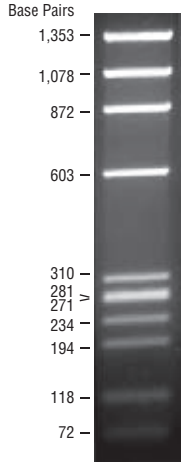
Lambda DNA-HindIII Digest

0.5 µg of Lambda DNA-HindIII Digest visualized by ethidium bromide staining. 1.0% agarose gel.



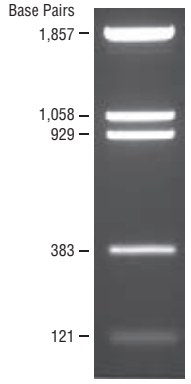
Lambda DNA-BstEII Digest

0.5 µg of Lambda DNA-BstEII Digest visualized by ethidium bromide staining. 1.0% agarose gel.



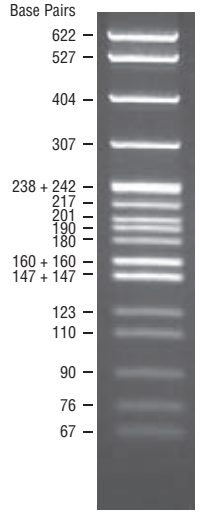
ϕX174 DNA-HaeIII Digest

0.5 µg of ϕX174 DNA-HaeIII Digest visualized by ethidium bromide staining. 1.7% agarose gel.



pBR322 DNA-BstNI Digest

0.5 µg of pBR322 DNA-BstNI Digest visualized by ethidium bromide staining. 1.4% agarose gel.



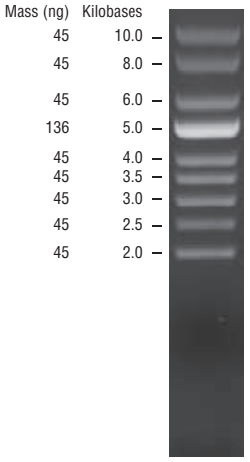
pBR322 DNA-MspI Digest

1.0 µg of pBR322 DNA-MspI Digest visualized by ethidium bromide staining. 3% agarose gel.

Matt joined NEB in 2023 as the Product Marketing Manager for the DNA Cloning and Assembly application areas.

Supercoiled DNA Ladder

#N0472S 100 gel lanes



Supercoiled DNA Ladder
0.5 µg/lane.
0.8% TAE agarose gel.

The Supercoiled DNA ladder contains 9 proprietary supercoiled plasmids, ranging in size from 2 to 10 kb, that are suitable for use as supercoiled molecular weight standards for agarose electrophoresis. The 5 kb plasmid has an increased intensity to serve as a reference band.

Concentration: 500 µg/ml

Note: This ladder may contain some traces of nicked DNA and dimers above the 10 kb plasmid. To minimize nicking of the supercoiled DNA, always use sterile pipette tips and avoid multiple freeze-thaw cycles. The migration of supercoiled plasmids in agarose gels can change depending on agarose concentration, buffer and electrophoresis conditions. Dilute in TE or other buffer of minimal ionic strength. DNA may denature if diluted in dH_2O. Centrifuge briefly and mix gently before use. We recommend loading 0.5 µg (1 µl) of the Supercoiled DNA Ladder diluted in sample buffer. This ladder was not designed for precise quantification of DNA mass, but can be used for approximating the mass of DNA in comparably intense samples of similar size.

The approximate mass of DNA in each of the bands in our Supercoiled DNA ladder is as follows (assuming a 0.5 µg loading):

Band	Base Pairs	DNA Mass
1	10,000	45 ng
2	8,000	45 ng
3	6,000	45 ng
4	**5,000**	**136 ng**
5	4,000	45 ng
6	3,500	45 ng
7	3,000	45 ng
8	2,500	45 ng
9	2,017	45 ng

RNA Markers & Ladders

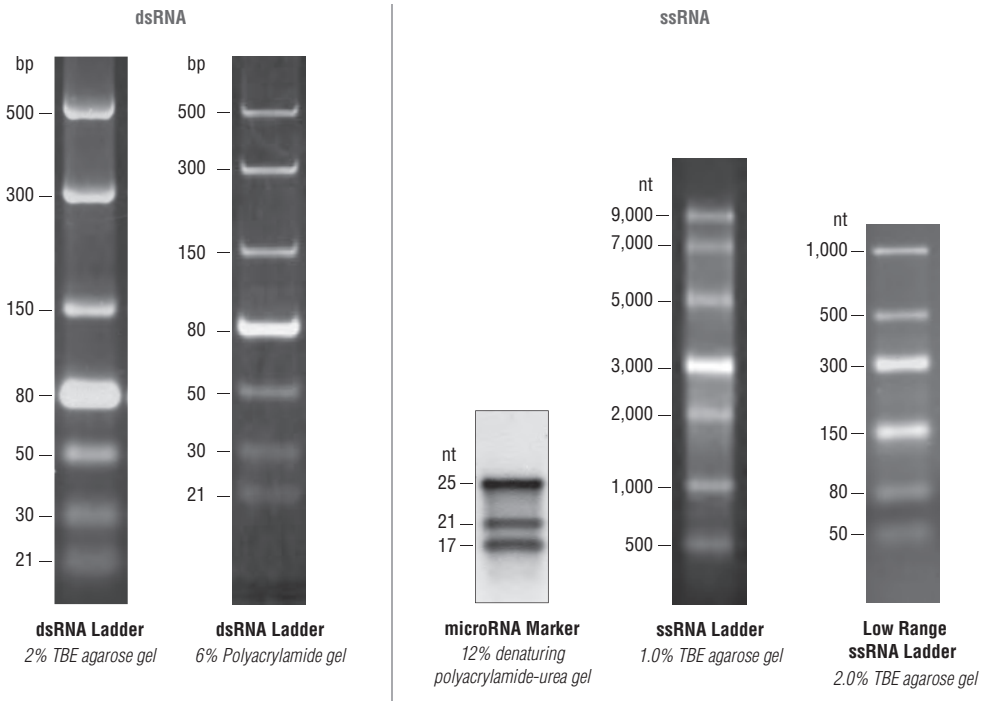
dsRNA Ladder
#N0363S 25 gel lanes

microRNA Marker
#N2102S 100 gel lanes

ssRNA Ladder
#N0362S 25 gel lanes

Low Range ssRNA Ladder
#N0364S 100 gel lanes

NEB offers several RNA Markers and Ladders with a size range from 17 to 9,000 bases. The Low Range ssRNA Ladder and the ssRNA Ladder are suitable for use as RNA size standards on denaturing or native gels. Both are supplied with RNA Loading Dye (2X) (NEB #B0363) and feature a higher intensity fragment to serve as a reference band. The microRNA Marker, provided in a ready-to-load denaturing solution, is ideally used as a size marker on denaturing polyacrylamide gels or northern blots and is best visualized stained with SYBR®-Gold. It is supplied with a 3´-biotinylated 21-mer oligonucleotide probe that can be labeled with g32-P-ATP and T4 PNK (NEB #M0201). The dsRNA Ladder is suitable for use as a size standard in dsRNA and RNAi analysis on both polyacrylamide and agarose gels.



dsRNA Ladder
2% TBE agarose gel

dsRNA Ladder
6% Polyacrylamide gel

microRNA Marker
12% denaturing polyacrylamide-urea gel

ssRNA Ladder
1.0% TBE agarose gel

Low Range ssRNA Ladder
2.0% TBE agarose gel

Protein Standards

Unstained Protein Standard, Broad Range (10-200 kDa)

#P7717S	150 gel lanes
#P7717L	750 gel lanes

Color Prestained Protein Standard, Broad Range (10-250 kDa)

#P7719S	150 gel lanes
#P7719L	750 gel lanes

Blue Prestained Protein Standard, Broad Range (11-250 kDa)

#P7718S	150 gel lanes
#P7718L	750 gel lanes

Companion Product:

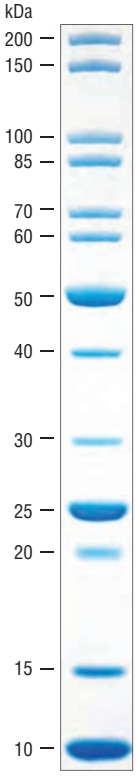
Blue Protein Loading Dye

#B7703S	8 ml

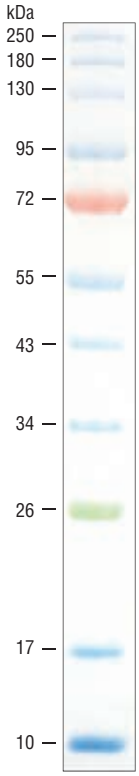
NEB offers a selection of highly pure protein standards available as unstained, blue prestained or color prestained (containing two colored reference bands for easy identification). Sizes range from 10 to 250 kDa which is ideal for calculating molecular weight determination for a wide range of expressed proteins. NEB protein standards provide uniform band intensities, convenient band spacing and easy-to-identify reference bands.

Recommended Load Volume: 3 µl

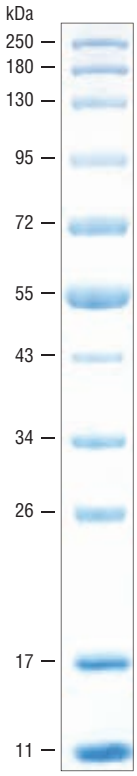
Note: For calculating molecular weight determinations, use NEB's Unstained Protein Standard, Broad Range.



Unstained Protein Standard, Broad Range (10–200 kDa)
10–20% Tris-glycine SDS-PAGE Gel



Color Prestained Protein Standard, Broad Range (10–250 kDa)
10–20% Tris-glycine SDS-PAGE Gel

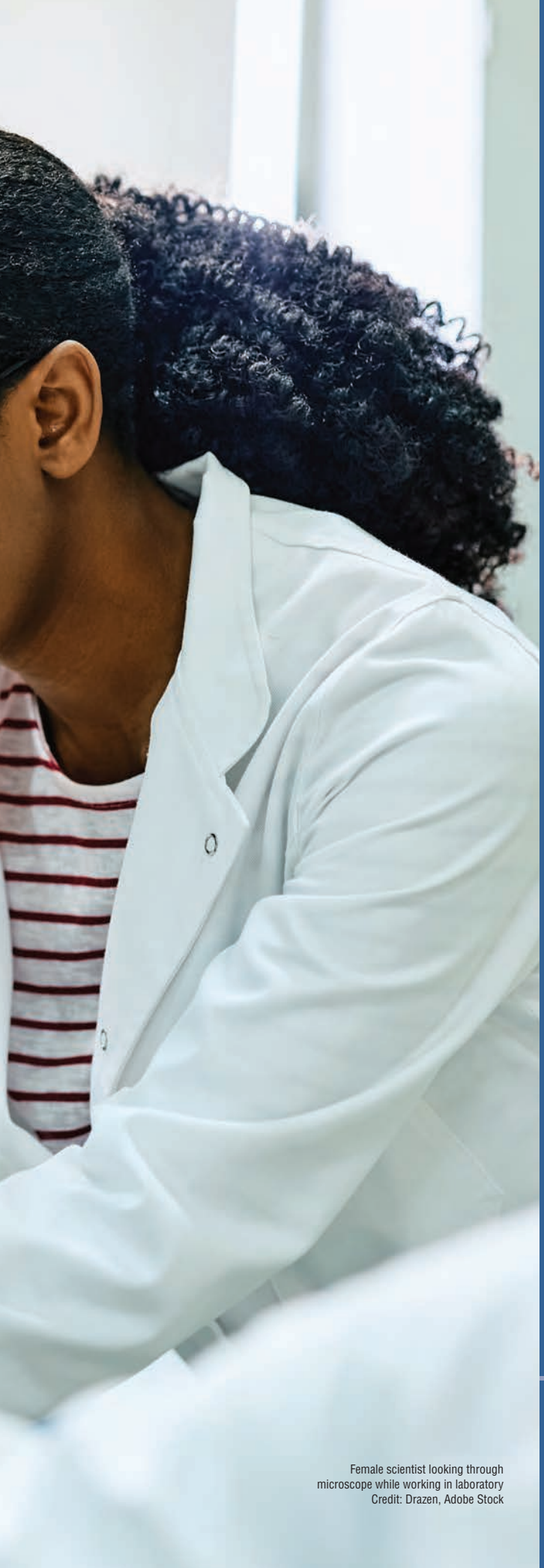


Blue Prestained Protein Standard, Broad Range (11–250 kDa)
10–20% Tris-glycine SDS-PAGE Gel

Gemma joined NEB in 2024 as the Lyophilization Associate Director for NEB Lyophilization Sciences.

Learn more about
SWIS Africa.

VIEW



Female scientist looking through microscope while working in laboratory
Credit: Drazen, Adobe Stock

Championing African Women in STEM through Storytelling and Mentorship

In Africa, only about 20% of scientists and engineers are women, highlighting a significant gender disparity in STEM fields. This issue became evident to Samuel Ogunsola during his undergraduate studies at the Federal University of Technology in Akure, Nigeria, where he saw talented female classmates abandon their STEM aspirations due to cultural and societal barriers, such as stereotypes, a lack of role models and inadequate support systems.

Ogunsola's determination to address this issue was further galvanized after reading *"The Moment of Lift"* by Melinda Gates, a book that sheds light on the systemic challenges women face globally. It was particularly resonant given Africa's traditional expectations, which often confine women to domestic roles like staying at home, raising children and cooking. Inspired by stories of women who had overcome immense barriers, Ogunsola realized that storytelling could be an influential way to encourage young women to pursue bigger dreams, such as careers in STEM fields.

In June 2020, Ogunsola launched the Shaping African Women in STEM (SWIS Africa) initiative with a clear mission: to use storytelling to celebrate, inspire, and shape young women aged 16 to 35 across Africa. Through its popular *"STEM Women Crush"* series, SWIS Africa celebrates the success stories of over 50 African women making significant impacts in STEM, sharing these narratives across social media to provide relatable role models for aspiring scientists and engineers.

The inspiration aspect delves deeper by interviewing women in global organizations like NVIDIA, Google and Microsoft. These conversations explore their journeys, challenges and successes, offering insights and encouragement to aspiring scientists and engineers. One of the initiative's notable successes was collaborating with Carnegie Mellon University Africa to celebrate 40 African women in tech. This partnership amplified these inspiring stories to a wider audience and validated the global relevance of SWIS Africa.

The shaping component focuses on practical support through training and workshops. To date, the organization has held over 10 training sessions impacting more than 1,000 women. Topics have ranged from data analytics and bioinformatics to research writing and scholarship applications. These programs equip participants with essential skills and knowledge, helping them navigate their educational and professional paths. A spin-off organization called BioinformHER was established in January 2024, focusing on teaching bioinformatics to young women.

The SWIS Africa initiative aligns with the United Nations Sustainable Development Goals on quality education, gender equality and economic growth, highlighting its broader significance in promoting social equity and economic development. Together with Abosede Salami, Ogunsola has officially registered the organization in Nigeria as a nonprofit. Looking ahead, he plans to develop an online mentorship platform and launch a scholarship fund for young women in STEM across Africa. He emphasizes the need for partnerships with corporate and academic institutions to overcome funding challenges and sustain the organization's long-term impact.

Ogunsola and the SWIS Africa team's dedication to empowering African women in STEM is transforming gender perceptions across the continent. Through initiatives like SWIS Africa, he fosters social change and opens doors for those who might be overlooked. By nurturing a new generation of women in STEM, his work drives economic growth, spurs innovation, and enriches the STEM community with diverse perspectives, paving the way for a more inclusive future.

Samuel Ogunsola
University of Manitoba, Winnipeg, MB, Canada
2024 Passion in Science
Science Mentorship and Advocacy Award

Genome Editing

Programmable nucleases for your applications.

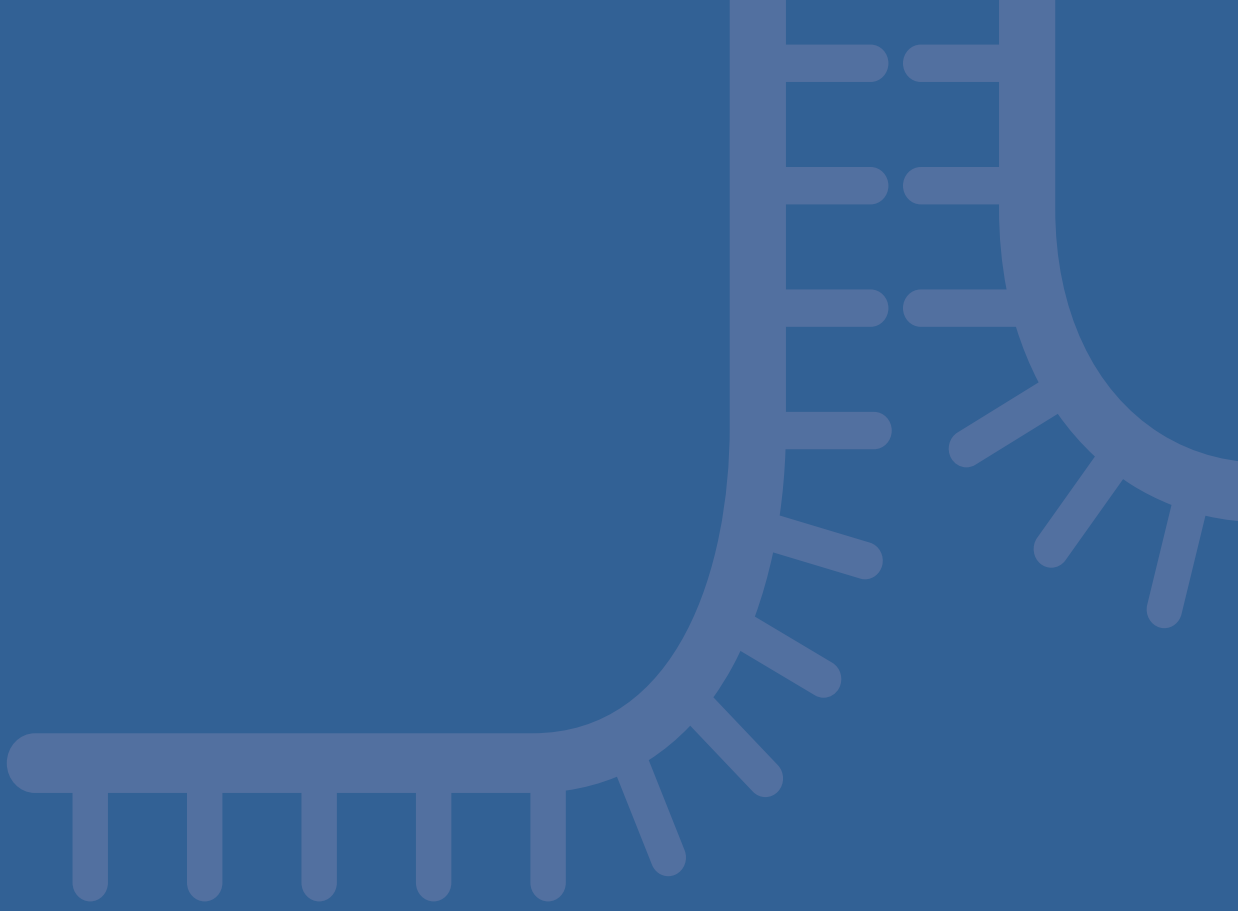
Easily changing the sequence specificity of a DNA binding protein enables many new possibilities for the detection and manipulation of DNA, including for genome editing – creating targeted changes in the DNA of living cells. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) -associated (Cas) nucleases have been adapted from bacterial immune systems into useful tools for biotechnology. Cas nucleases including Cas9 and Cas12a, are attractive for genome editing because they are easily programmable with a single guide RNA or crRNA/tracrRNA to introduce a double-stranded break at a specific target. The resultant breaks are repaired by cellular machinery, in some applications using an exogenous repair template.

The ease of programming Cas nucleases, and their conversion into nicking endonucleases, or DNA binding proteins without nuclease activity has expanded their use to include delivering a specific cargo to a locus for applications including visualization, activation, repression and base editing. Furthermore, Cas nuclease and their variants are useful tools for the detection and manipulation of DNA *in vitro*.

Featured Products

Featured Tools & Resources

Visit **www.neb.com/GenomeEditing** for more information, including our feature article and latest brochure.



Featured NEB Products Supporting CRISPR Workflows

Programmable Nucleases

RI Recombinant Enzyme

Featured NEB Products Supporting CRISPR Workflows

New England Biolabs provides reagents to support a broad variety of CRISPR/Cas genome editing approaches. From introduction of Cas and single guide RNA (sgRNA) on plasmids, to direct introduction of Cas ribonucleoprotein (RNP) and detection of edits using next generation sequencing or enzymatic mutation detection, NEB provides reagents that simplify and shorten genome editing workflows.

Product	NEB #	Size	Applications
EnGen Seq1 Cas9	M0668T	500 pmol	• *in vitro* cleavage of dsDNA • Recognizes 5´- NAGA -3´ PAM • Genome engineering by direct introduction of Cas9/sgRNA complexes
NEW **EnGen SpRY Cas9**	M0669T M0669M	500 pmol 2,500 pmol	• *in vitro* cleavage of dsDNA • Recognizes non-specific PAM (5´-NNN-3´ PAM)
NEW **EnGen Spy Cas9 HF1**	M0667T M0667M	500 pmol 2,500 pmol	• High-fidelity *in vitro* cleavage of dsDNA • Genome engineering by direct introduction of active ribonucleotides • Recognizes 5´-NGG-3´ PAM
EnGen Spy Cas9 NLS	M0646T M0646M	500 pmol 2,500 pmol	• *in vitro* cleavage of dsDNA • Genome engineering by direct introduction of active ribonucleoproteins • Recognizes 5´-NGG-3´ PAM
EnGen Mutation Detection Kit	E3321S	25 reactions	• Determination of the targeting efficiency of genome editing protocols
EnGen sgRNA Synthesis Kit, *S. pyogenes*	E3322V E3322S	10 reactions 20 reactions	• Generation of microgram quantities of custom sgRNA • Recognized 5´-NGG-3´ PAM
EnGen Spy Cas9 Nickase	M0650S M0650T	90 pmol 500 pmol	• *in vitro* nicking of dsDNA • Genome engineering by direct introduction of active nickase complexes • Recognizes 5´- NGG-3´ PAM
EnGen Spy dCas9 (SNAP-tag)	M0652S M0652T	90 pmol 500 pmol	• Programmable binding of DNA • Compatible with SNAP-tag substrates for visualization and enrichment • Recognizes 5´-NGG-3´ PAM
EnGen Lba Cas12a (Cpf1)	M0653S M0653T	70 pmol 2,000 pmol	• *in vitro* cleavage of dsDNA • Genome engineering by direct introduction of active nuclease complexes • Signal generation for nucleic acid sequence detection • Recognizes 5´-TTTN- 3´
EnGen Sau Cas9	M0654T	500 pmol	• *in vitro* cleavage of dsDNA • Genome engineering by direct introduction of active nuclease complexes • Recognizes 5´-NNGRRT-3´ PAM
Cas9 Nuclease, *S. pyogenes*	M0386S M0386T M0386M	90 pmol 500 pmol 2,500 pmol	• *in vitro* cleavage of dsDNA • Genome engineering by direct introduction of active ribonucleoproteins • Recognizes 5´-NGG-3´ PAM
Monarch® Spin RNA Isolation Kit (Mini)	T2110S	50 preps	• Purification of total RNA, with a binding capacity up to 100 µg
Monarch Spin RNA Cleanup Kit (50 µg)	T2040S T2040L	10 preps 100 preps	• Purification of sgRNA, with a capacity of up to 50 µg
Q5® Site-Directed Mutagenesis Kit	E0554S	10 reactions	• Insertion of target sequence into a Cas9-sgRNA construct and modification of HDR templates
Q5 Site-Directed Mutagenesis Kit (Without Competent Cells)	E0552S	10 reactions	• Insertion of target sequence into a Cas9-sgRNA construct and modification of HDR templates
NEBuilder® HiFi DNA Assembly Master Mix	E2621S E2621L E2621X	10 reactions 50 reactions 250 reactions	• Single-tube, isothermal generation of the Cas9-sgRNA construct and HDR templates
NEBuilder HiFi DNA Assembly Cloning Kit	E5520S	10 reactions	• Single-tube, isothermal generation of the Cas9-sgRNA construct and HDR templates
HiScribe® T7 mRNA Kit with CleanCap Reagent AG	E2080S E2080L	20 reactions 100 reactions	• Generation of Cas9 mRNA with CleanCap Reagent AG cap
HiScribe T7 ARCA mRNA Kit (with tailing)	E2060S	20 reactions	• Generation of Cas9 mRNA with ARCA cap
HiScribe T7 ARCA mRNA Kit	E2065S	20 reactions	• Generation of Cas9 mRNA with ARCA cap
HiScribe T7 High Yield RNA Synthesis Kit	E2040S E2040L	50 reactions 250 reactions	• Generation of sgRNA and Cas9 mRNA
HiScribe T7 Quick High Yield RNA Synthesis Kit	E2050S E2050L	50 reactions 250 reactions	• Generation of sgRNA and Cas9 mRNA
T7 Endonuclease I	M0302S M0302L	250 units 1,250 units	• Determination of the editing efficiency of genome editing experiments
Authenticase	M0689S M0689L	25 reactions 125 reactions	• Determination of the editing efficiency of genome editing experiments

Programmable Nucleases

The highest efficiency strategy for genome editing with CRISPR/Cas nucleases is direct introduction of Cas/guide RNA complexes. This method further simplifies CRISPR/Cas workflows and has been reported to increase on-target editing activity and reduce off-target events. NEB provides purified Cas9 nucleases from *S. pyogenes, S. aureus,* and *S. equinus,* and Cas12a (Cpf1) nuclease from *Lachnospiraceae* bacterium ND2006. In addition, NEB provides variants of Cas9 from *S. pyogenes*, including nicking endonuclease and endonuclease deficient versions. NEB also provides *Thermus thermophilus argonaute* (TtAgo), a programmable DNA endonuclease which requires a short 5´-phosphorylated single-stranded DNA guide to target its activity to a specific corresponding sequence on a substrate.

Product	NEB #	Size	Features
EnGen Seq1 Cas9	M0668T	500 pmol	• 5´- NAGA -3´ PAM sequence allows targeting of additional genomic regions • Ideal for direct introduction of Cas9/sgRNA complexes • Compatible with the EnGen Mutation Detection Kit (NEB #E3321)
NEW **EnGen SpRY Cas9**	M0669T M0669M	500 pmol 2,500 pmol	• Eliminate sequence constraints for dsDNA targeting with non-specific PAM (5´-NNN-3´ PAM) • Digest large plasmids in cloning workflows successfully • Use in conjunction with the EnGen sgRNA Synthesis Kit, S. pyogenes (NEB #E3322), EnGen Mutation Detection Kit (NEB #E3321), and NEBuilder HiFi DNA Assembly Master Mix (NEB #E2621)
NEW **EnGen Spy Cas9 HF1**	M0667T M0667M	500 pmol 2,500 pmol	• Reduced off-target cleavage • Ideal for direct introduction of Cas9/sgRNA complexes • Dual NLS for improved transport to the nucleus • Compatible with EnGen sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322S) and the EnGen Mutation Detection Kit (NEB #E3321S)
EnGen Spy Cas9 NLS	M0646T M0646M	500 pmol 2,500 pmol	• Ideal for direct introduction of Cas9/sgRNA complexes • Dual NLS for improved transport to the nucleus • Compatible with EnGen® sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322) and the EnGen Mutation Detection Kit (NEB #E3321)
EnGen Spy Cas9 Nickase	M0650S M0650T	90 pmol 500 pmol	• Variant of Cas9 nuclease differing by a point mutation (D10A) in the RuvC nuclease domain • Capable of generating nicks, but not cleaving DNA • DNA double strand breaks can be generated, with reduced off-target cleavage, by targeting two sites with EnGen Cas9 Nickase in close proximity • Compatible with the EnGen sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322)
Cas9 Nuclease, *S. pyogenes*	M0386S M0386T M0386M	90 pmol 500 pmol 2,500 pmol	• Ideal for *in vitro* digestion of dsDNA • Compatible with EnGen® sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322) and the EnGen Mutation Detection Kit (NEB #E3321) • For help with oligo design, try EnGen sgRNA Template Oligo Designer
EnGen Spy dCas9 (SNAP-tag)	M0652S M0652T	90 pmol 500 pmol	• An inactive mutant of Cas9 nuclease that retains programmable DNA binding activity • The N-terminal SNAP-tag allows for covalent attachment of fluorophores, biotin, and a number of other conjugates useful for visualization and target enrichment • Compatible with the EnGen sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322)
EnGen Lba Cas12a (Cpf1)	M0653S M0653T	70 pmol 2,000 pmol	• T-rich 5´-TTTV-3´ PAM sequence opens up additional genomic regions for targeting • Shorter, 40-44 base guide RNA • Two nuclear localization signals for improved transport to the nucleus • 5´ overhanging termini on cleavage products • Active from 16 to 48°C • Maintains activity at lower temperatures than the *Acidaminococcus* orthologs, permitting editing in ectothermic organisms such as zebra fish and xenopus • High concentration liquid format can be used for microinjection, electroporation and lipofection.
EnGen Sau Cas9	M0654T	500 pmol	• 5´-NNGRRT-3´ PAM • Dual NLS for improved transport to nucleus • High concentration liquid format option can be used for microinjection, electroporation and lipofection • Cleaves 3 bases upstream of PAM, blunt-ended cleavage
***Tth* Argonaute (TtAgo)**	M0665S	50 pmol	• Short 16–18 nucleotide 5´-phosphorylated ssDNA guides are cost effective and can be phosphorylated with NEB T4 Polynucleotide Kinase • Guide/target sequence selection is not limited by the requirement of an adjacent sequence motif • Highly active on ssDNA and most dsDNA substrates (generates a nick in dsDNA substrates), with mild activity on ssRNA substrates • Recommended for *in vitro* applications

Learn more about genome editing.

EnGen Mutation Detection Kit

#E3321S 25 reactions

Companion Products:

Q5 Hot Start High-Fidelity 2X Master Mix
#M0494S 100 reactions
#M0494L 500 reactions
#M0494X 500 reactions

Quick-Load Purple 1 kb Plus DNA Ladder
#N0550S 250 gel lanes
#N0550L 750 gel lanes

Monarch PCR & DNA Cleanup Kit (5 µg)
#T1030S 50 preps
#T1030L 250 preps

EnGen Spy Cas9 NLS
#M0646T 500 pmol
#M0646M 2,500 pmol

Cas9 Nuclease, *S. pyogenes*
for high (20X) concentration
#M0386T 500 pmol
#M0386M 2,500 pmol

#M0386S 90 pmol

EnGen Sau Cas9
#M0654S 90 pmol

for high (20X) concentration
#M0654T 500 pmol

T7 Endonuclease I
#M0302S 250 units
#M0302L 1,250 units

- *T7 Endonuclease-based detection of genome editing events*

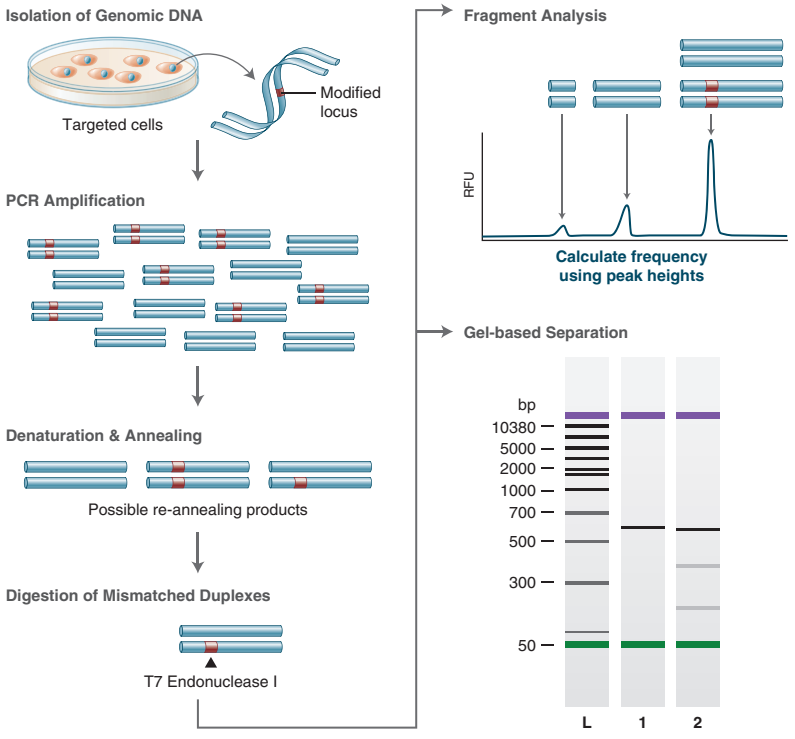
Description: The EnGen Mutation Detection Kit provides reagents for detection of on-target genome editing events. In the first step, regions targeted for genome editing (i.e. CRISPR/Cas9, TALENs, Zinc-finger Nucleases) are amplified using Q5 Hot Start High-Fidelity 2X Master Mix. Upon denaturation and re-annealing, heteroduplexes are formed when mutations from insertions and deletions (indels) are present in the amplicon pool. In the second step, annealed PCR products are digested with EnGen T7 Endonuclease I, a structure-specific enzyme that will recognize mismatches larger than 1 base. Both strands of the DNA are cut when a mismatch is present, which results in the formation of smaller fragments. Analysis of the resulting fragments provides an estimate of the efficiency of the genome editing experiments.

The EnGen Mutation Detection Kit includes a Control Template and Primer Mix that can be used as a control for the PCR reaction and T7 Endonuclease I digestion. The Control Template and Primer Mix provided contains two plasmids and primers that when amplified, denatured and re-annealed will form heteroduplexes that contain a 10-base insertion. This structure is a substrate for T7 Endonuclease I. The digestion of the 600 bp heteroduplex containing amplicon yields products of 200 bp and 400 bp. 600 bp parental homoduplexes are uncleaved, and are easily distinguished from cleaved heteroduplexes when separated and visualized by agarose gel electrophoresis or fragment analysis instrument.

The protocol has been optimized so that PCR products generated by the Q5 Hot Start High-Fidelity 2X Master Mix can be introduced directly into the T7 Endonuclease I digestion without the need for purification. Digestion of the heteroduplex is complete in only 15 minutes, and Proteinase K is included to stop the reaction efficiently. Additional Q5 Hot Start High-Fidelity 2X Master Mix is also included to allow for optimization of target site amplification before digestion.

Kit Includes:

- Q5 Hot Start High-Fidelity 2X Master Mix
- NEBuffer 2
- Control Template and Primer Mix
- Proteinase K, Molecular Biology Grade
- Quick-Load Purple 1 kb Plus DNA Ladder
- Gel Loading Dye, Purple (6X), no SDS
- EnGen T7 Endonuclease I



Genomic DNA is amplified with primers bracketing the modified locus. *PCR products are then denatured and re-annealed yielding three classes of possible structures. Duplexes containing a mismatch greater than one base are digested by T7 Endonuclease I. The DNA is then electrophoretically separated and fragment analysis is used to estimate targeting efficiency.*

EnGen sgRNA Synthesis Kit, *S. pyogenes*

#E3322V	10 reactions
#E3322S	20 reactions

Companion Products:

EnGen Spy Cas9 NLS
#M0646T 500 pmol
#M0646M 2,500 pmol

EnGen Spy Cas9 HF1
#M0667T 500 pmol
#M0667M 2,500 pmol

Monarch RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

EnGen Spy Cas9 Nickase
#M0650S 90 pmol

for high (20X) concentration
#M0650T 500 pmol

EnGen Spy dCas9 (SNAP-tag)
#M0652S 90 pmol

for high (20X) concentration
#M0652T 500 pmol

EnGen Mutation Detection Kit
#E3321S 25 reactions

DNase I (RNase-free)
#M0303S 1,000 units
#M0303L 5,000 units

- *Rapid generation of microgram quantities of sgRNAs in less than one hour*

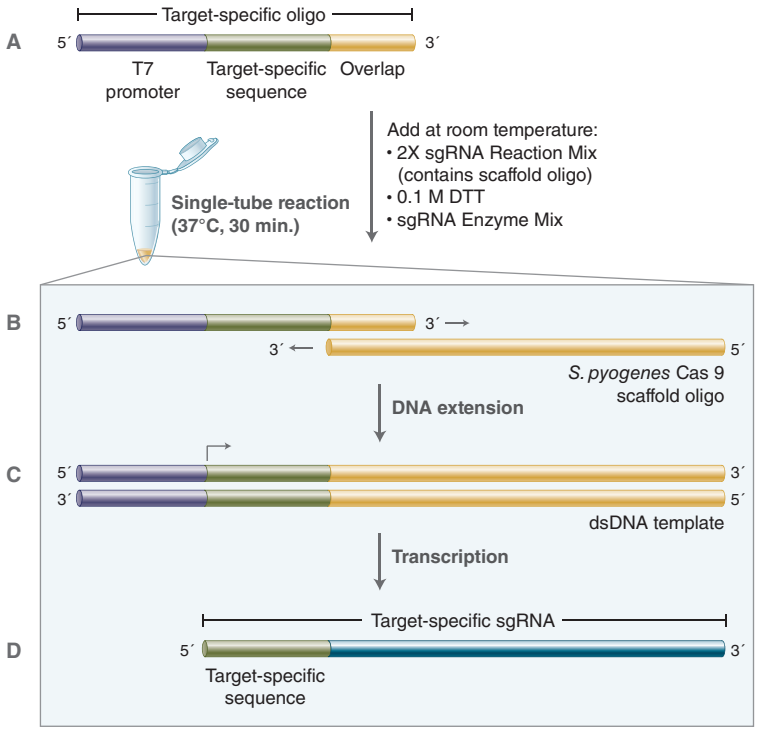
Description: The EnGen sgRNA Synthesis Kit, *S. pyogenes* provides a simple and quick method for transcribing high yields of sgRNA in a single 30-minute reaction, using the supplied reagents and target-specific DNA oligos designed by the user.

In nature, *S. pyogenes* Cas9 is programmed with two separate RNAs, the crRNA and tracrRNA. The crRNA, or CRISPR RNA sequence contains approximately 20 nucleotides of homology complementary to the strand of DNA opposite and upstream of a PAM (Protospacer Adjacent Motif) (NGG) sequence. The tracrRNA, or transactivating crRNA, contains partial complementary sequence to the crRNA as well as the sequence and secondary structure that is recognized by Cas9. These sequences have been adapted for use in the lab by combining the tracrRNA and crRNA into one long single guide RNA (sgRNA) species capable of complexing with Cas9 to recognize and cleave the target DNA.

The EnGen sgRNA Synthesis Kit, *S. pyogenes* combines an *S. pyogenes* Cas9-specific Scaffold Oligo (included in the EnGen 2X sgRNA Reaction Mix) that is partially complementary to the target-specific oligos designed by the user. The two oligos anneal at the overlapping region and are filled in by the DNA polymerase, creating a double-stranded DNA (dsDNA) template for transcription. Synthesis of the dsDNA template and transcription of RNA occur in a single reaction, resulting in the generation of a functional sgRNA.

Kit Includes:

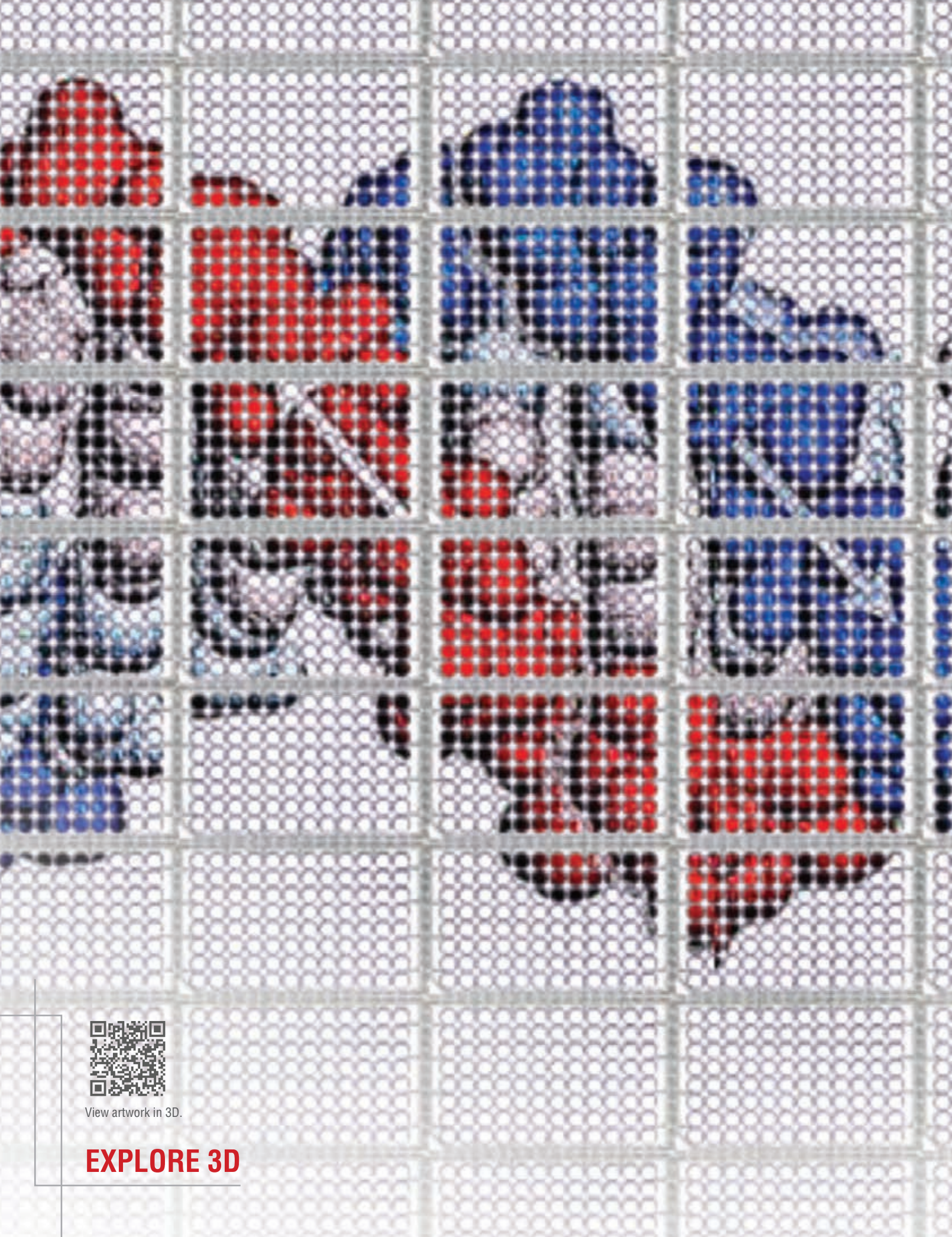
- EnGen sgRNA Enzyme Mix
- EnGen 2X sgRNA Reaction Mix, *S. pyogenes*
- DNase I (RNase-free)
- EnGen sgRNA Control Oligo, *S. pyogenes*
- Dithiothreitol (DTT)



A. *The target-specific oligo contains the T7 promoter sequence, ~20 nucleotides of target-specific sequence and a 14 nucleotide overlap sequence complementary to the S. pyogenes Cas9 Scaffold Oligo supplied in the reaction mix. Target-specific oligos (or EnGen sgRNA Control Oligo, S. pyogenes) are mixed with the EnGen 2X sgRNA Reaction Mix (NTPs, dNTPs, S. pyogenes Cas9 Scaffold Oligo), 0.1 M DTT and the EnGen sgRNA Enzyme Mix (DNA and RNA polymerases) at room temperature. B. At 37°C the two oligos anneal at the 14 nucleotide overlap region of complementarity. C. The DNA polymerase extends both oligos from their 3´ ends creating a double-stranded DNA template. D. The RNA polymerase recognizes the double-stranded DNA of the T7 promoter and initiates transcription. The resulting sgRNA contains the target-specific/crRNA sequence as well as the tracrRNA. All steps occur in a single reaction during a 30 minute incubation at 37°C.*

EnGen sgRNA Template
Oligo Designer

Configure target-specific DNA oligos design for use with the EnGen sgRNA Synthesis Kit, *S. pyogenes* with our oligo design tool accessible at **sgrna.neb.com**



View artwork in 3D.

EXPLORE 3D

Pipette tip DNA helix art
Credit: Michael Weiner

Transforming Discarded Lab Materials into Artistic Masterpieces

Michael Weiner, a molecular biologist by training and an artist at heart, transforms discarded laboratory materials into captivating works of art, seamlessly blending scientific precision with artistic imagination. His most ambitious project — a 22-foot-long "stained glass" DNA double helix crafted from over 400 recycled 96-well microtiter plates — beautifies a scientific space and introduces a novel way to think about sustainability in the lab.

Weiner's journey began with a fascination for stained glass and ceramics, hobbies that nurtured his artistic inclinations while he pursued his scientific career. During a visit to New England Biolabs in the 1980s, while he was collaborating on a Ph.D. project to clone BamHI, he was inspired by the art collection of Dr. Don Comb, NEB's founder. The integration and appreciation of art within a scientific environment left a lasting impression on him. Later, as a biotech startup founder without the funds to purchase art, he decided to craft his own using materials readily available in the laboratory.

His first major project was a portrait of Albert Einstein crafted from 144 pipette tip boxes. He photographed the pipette tip boxes and overlaid them onto a grayscale image of Einstein. Meticulously determining which pipette tip holes to fill or leave empty — and considering the spacing between boxes akin to the solder lines in stained glass — he achieved the desired visual effect. This project was as much an exercise in artistic ingenuity as it was in logistical planning. *"The creativity is really in thinking about how to do it. That's the intersection — using scientific understanding to solve an artistic problem,"* he explained.

Determined to depict the DNA molecule in vibrant color, Weiner embarked on his largest endeavor. After a year of experimentation, he discovered that mixing epoxy resin with food coloring produced the ideal translucency and hue for his "stained glass" effect. Using a high-resolution image of the DNA double helix, he overlaid a template corresponding to the wells of the microtiter plates. Each well was hand-filled with colored epoxy, requiring hand pipetting and a keen eye for detail. To enhance its durability and stained-glass effect, Weiner sandwiched the filled microtiter plates between large sheets of UV-resistant plexiglass, chemically welding them with an organic solvent-based adhesive. The final piece accurately represents 11 turns of the DNA helix. Framed with aluminum U-channel and designed to be suspended from the ceiling, the artwork allows light to illuminate the vibrant colors, transforming the space it occupies.

Building on the success of his DNA piece, Weiner is now focusing on large-scale portraits of notable women in science, such as Rosalind Franklin and Barbara McClintock. Using UV-resistant outdoor paint mixed into epoxy to create opaque colorants, these pieces honor their contributions.

Weiner's artwork not only beautifies scientific spaces but also demonstrates how discarded lab materials can be creatively reimagined to promote sustainability. His transformation of everyday lab items into masterpieces highlights that the creativity, passion and planning that drive scientific discovery and artistic expression are surprisingly alike. By inviting us to reconsider our environments, Weiner reveals the boundless possibilities that emerge when science and art come together.

Dr. Michael Weiner
Abbratech, Branford, CT, USA
2024 Passion in Science Arts and Creativity Award

RNA Reagents

A broad portfolio of reagents to support RNA research.

RNA molecules play a multitude of cellular roles in all kingdoms of life, perhaps reflecting the hypothetical, prehistoric RNA world. RNA is an essential carrier of genetic information, can scaffold molecular interactions, catalyze chemical reactions, and influence gene expression.

In the last several years, our understanding of RNAs as regulatory molecules in the cell has dramatically changed. Many new classes of small and large non-coding RNAs, with largely unexplored functions, have been reported, ushering in a renaissance of RNA-focused research in biology.

Small RNAs play a major role in the post-transcriptional regulation of gene expression in eukaryotic and prokaryotic organisms. Small RNAs play central roles in CRISPR pathways of adaptive immunity. Researchers have capitalized on these pathways to enable analysis and manipulation of gene function not previously possible.

Deploying RNA, and in particular mRNA, into cells is an exciting new platform for inducing phenotypic changes in cells with implications for therapy, vaccines, and research applications.

New England Biolabs continues its strong tradition of providing high quality reagents to support RNA research. Our expanding range of products includes tools for the synthesis, processing, cleanup, isolation, analysis, amplification, copying and cloning of RNA molecules. Further, all NEB products pass stringent quality control assays to ensure the highest level of functionality and purity.

Featured Products

Featured Tools & Resources

View our **video tutorial** describing **high yield *in vitro* synthesis** of both **capped and uncapped mRNA**.

View our **video for avoiding ribonuclease contamination**.

Learn more about RNA modifications.

RM Recombinant Enzyme

Avoiding Ribonuclease Contamination

Maintaining the integrity of RNA is a critical aspect of nearly all applications that use it. RNA is more susceptible to degradation than DNA, due to the ability of the 2´ hydroxyl group to act as nucleophiles. Many ribonucleases (RNases) bypass the need for metal ions by taking advantage of the 2´ hydroxyl group as a reactive species. These RNases are resistant to metal chelating agents and, some of them, like RNase A family enzymes, can survive prolonged boiling or autoclaving. RNase A-type enzymes rely on active site histidine residues for catalytic activity (1) and can be inactivated by the histidine-specific alkylating agent diethyl pyrocarbonate (DEPC).

Sources of RNase Contamination:

RNases are found in all cell types and organisms from prokaryotes to eukaryotes. RNases generally have very high specific activity, meaning miniscule amounts of contamination in an RNA sample is sufficient to destroy the RNA. The major sources of RNase contamination include:

- Aqueous solutions, reagents used in experiments
- Exposure to RNase from environmental sources (lab surfaces, aerosols from pipetting, ungloved hands, etc.)

Laboratory Precautions (2,3):

NEB's enzymes have been purified free of ribonucleases. However, it is possible to reintroduce RNases during the course of experimentation from various sources. RNase contamination can be prevented with a few common sense laboratory procedures:

- Always wear gloves during an experiment and change them often, especially after contact with skin, hair or other potentially RNase-contaminated surfaces, such as doorknobs, keyboards and animals.
- Use RNase-free solutions. Use RNase-free certified, disposable plasticware and filter tips whenever possible.
- Maintain a separate area for RNA work. Carefully clean the surfaces.
- Decontaminate glassware by baking at 180°C or higher for several hours or by soaking in freshly prepared 0.1% (v/v) DEPC in water or ethanol for 1 hour, followed by draining and autoclaving. Autoclaving will destroy any unreacted DEPC which can otherwise react with other proteins and RNA.
- Decontaminate polycarbonate or polystyrene materials (e.g., electrophoresis tanks) by soaking in 3% hydrogen peroxide for 10 minutes. Remove peroxide by extensively rinsing with RNase-free water prior to use.

Preparation of Solutions (2,3):

Preparation of solutions using the following suggestions can help prevent RNase contamination:

- As an alternative to the historic use of DEPC, which can inhibit enzymatic reactions if not completely removed, we have found that Milli-Q® (Millipore) purified water is sufficiently free of RNases for most RNA work. NEB also offers Nuclease-free Water (NEB #B1500).
- DEPC treatment of solutions is accomplished by adding 1 ml DEPC (Sigma) per liter of solution, stirring for 1 hour, and autoclaving for 1 hour to remove any remaining DEPC. [Note: Compounds with primary amine groups (e.g., Tris) which will react with DEPC, cannot be DEPC-treated. Other compounds, which are not stable during autoclaving, cannot be DEPC-treated].
- Solutions and buffers (e.g. DTT, nucleotides, manganese salts) should be prepared by dissolving the solid (highest available purity) in autoclaved DEPC-treated Milli-Q water and passing the solution through a 0.22 µm filter to sterilize.

Inhibitors of Ribonucleases:

RNA can also be protected from RNase activity by using one of the following RNase inhibitors:

- RNase Inhibitor, Human Placenta, (NEB #M0307), a recombinant protein of human placenta, is a specific inhibitor for RNases A, B and C. Similar to the Murine RNase Inhibitor, it is compatible with many enzymatic reactions involving RNA (e.g., *in vitro* transcription, RT-PCR, ligation, etc.).
- RNase Inhibitor, Murine, (NEB #M0314) is the murine version of RNase inhibitor. It has the same inhibition profile as human or porcine inhibitors, but is more stable due to improved resistance to oxidation (4). The inhibitor requires low concentrations of DTT (< 1 mM) to maintain activity, making it ideal for reactions where low DTT concentration is required (e.g., real-time RT-PCR).
- Ribonucleoside Vanadyl Complex (NEB #S1402) is a transition-state analog inhibitor of RNase A-type enzymes with $K_i = 1 \times 10^{-5}$ M. This complex is compatible with many RNA isolation procedures, but it should not be used in the presence of EDTA. The complex also inhibits many other enzymes used in RNA work (5).

Find tips for avoiding ribonuclease contamination.

References:

(1) Fersht, A.R. (1977) *Enzyme Structure and Mechanism* Freeman, Reading, PA, 325–329.

(2) Blumberg, D.D. (1987) *Methods Enzymol.*, 152, 20–24.

(3) Sambrook, J., et al. (1989). *Molecular Cloning: A Laboratory Manual*, (2nd ed.). Cold Spring Harbor: Cold Spring Harbor Laboratory Press., 7.3–7.5.

(4) Kim, B.M. et al. (1999) *Protein Science*, 8, 430–434.

(5) Berger, S.L. (1987) *Methods Enzymol.*, 152, 227–234.

GMP-grade Products for RNA Synthesis – Tools to Take you from Template to Transcript

For almost 50 years, NEB® has been a world leader in the discovery and production of reagents for the life science industry. Our enzymology expertise effectively positions us to supply reagents for the synthesis of high-quality RNA – from template generation and transcription, to capping, tailing and cleanup after synthesis. These products are designed and manufactured based on decades of molecular biology experience, so that you can be confident they will work for your application.

From research to therapeutic production, NEB's *in vitro* transcription portfolio will meet your needs

NEB's portfolio of research-grade and GMP-grade* products enables bench-scale to commercial-scale mRNA manufacturing. Our optimized HiScribe® kits enable convenient *in vitro* transcription (IVT) workflows. When it is time to scale up and optimize reaction components, our standalone reagents are readily available in formats matching our GMP-grade offering, enabling a seamless transition to large-scale therapeutic mRNA manufacturing.

mRNA synthesis workflow example & available NEB products

TEMPLATE GENERATION	*IN VITRO* TRANSCRIPTION	RNA CAPPING	POLY(A) TAILING	RNA PURIFICATION
GMP* COMING SOON Q5® Hot Start High-Fidelity DNA Polymerase	HiScribe® T7 mRNA Kit with CleanCap® Reagent AG		*E. coli* Poly(A) Polymerase	Monarch® Spin RNA Cleanup Kit (10 µg)
	HiScribe T7 ARCA mRNA Synthesis Kit (with tailing)			
GMP* phi29 DNA Polymerase	HiScribe T7 ARCA mRNA Synthesis Kit			Monarch Spin RNA Cleanup Kit (50 µg)
GMP* TelN Protelomerase	GMP* HiScribe T7 High Yield RNA Synthesis Components	GMP* Faustovirus Capping Enzyme		Monarch Spin RNA Cleanup Kit (500 µg)
GMP* dNTP solution mixes		GMP* Vaccinia Capping System		
GMP* BspQI*	HiScribe T7 Quick High Yield RNA Synthesis Kit	GMP* mRNA Cap 2´-O-Methyltranferase		Lithium Chloride
GMP* NEBuffer™ 4	HiScribe SP6 High Yield RNA Synthesis Kit	ARCA and other mRNA cap analogs		
DNA Assembly: • NEBuilder HiFi DNA Assembly • Golden Gate Assembly	T3 & SP6 RNA Polymerases	GMP* S-Adenosylmethionine (SAM)		
	GMP* T7 RNA Polymerase			
	Hi-T7 RNA Polymerase			
	Companion Products			**Companion Products**
	GMP* RNase inhibitor (Murine)			Monarch Buffer BX
	RNase Inhibitor (Human Placental)			Monarch Buffer WX
	GMP* Pyrophosphatase, Inorganic (*E. coli*)			Nuclease-free Water
	Pyrophosphatase, Inorganic (Yeast)			
	GMP* DNase I (RNase-free)			
	DNase I-XT			
	GMP* NTPs			
	Modified NTPs			

GMP* = available in GMP-grade

* NEB can offer large-scale preparations of restriction enzymes using Recombinant Albumin (BSA-free)

HiScribe® T7 mRNA Kit with CleanCap® Reagent AG

#E2080S 20 reactions

Companion Products:

Q5 Site-Directed Mutagenesis Kit
#E0554S 10 reactions

Monarch Spin RNA Cleanup Kit (500 µg)
#T2050S 10 preps
#T2050L 100 preps

Monarch Spin RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

- *Streamlined workflow with single-step co-transcriptional capping*
- *CleanCap Reagent AG trinucleotide cap technology results in a natural Cap-1 structure, maximizing translatability and minimizing immune response from synthetic mRNA*
- *High capping efficiency*
- *Optimized for high yields*
- *Suitable for full or partial modified-nucleotide substitution*

The HiScribe T7 mRNA Kit with CleanCap Reagent AG utilizes an optimized RNA synthesis formulation and trinucleotide cap analog technology for co-transcriptionally capping mRNAs with a natural Cap-1 structure, in a single simplified reaction without compromising RNA yield. The kit contains individual vials of NTPs and CleanCap Reagent AG to allow for partial or complete substitution of modified NTPs, with a total kit yield of 1.8 mg of mRNA. Synthetic Cap-1 mRNA can be used in many downstream applications, including transfections, microinjections, *in vitro* translation, preclinicial therapeutic mRNA studies, as well as RNA structure and function analysis.

Reagents Supplied:

- T7 RNA Polymerase Mix
- LiCl Solution
- DNase I (RNase-free)
- CLuc AG Control Template
- 10X T7 CleanCap Reagent AG Reaction Buffer
- CleanCap Reagent AG
- ATP
- GTP
- CTP
- UTP
- Dithiothreitol (DTT)

HiScribe® T7 High Yield RNA Synthesis Kits

HiScribe T7 High Yield RNA Synthesis Kit
#E2040S 50 reactions
#E2040L 250 reactions

HiScribe T7 Quick High Yield RNA Synthesis Kit
#E2050S 50 reactions
#E2050L 250 reactions

- *Synthesis of long and short RNA transcripts*
- *Incorporation of modified nucleotides*
- *Incorporation of labeled nucleotides*
- *Generation of capped RNA using cap analogs*
- *Synthesis of radioactively-labeled probes with high or low specific activity*
- *Compatible with trinucleotide cap analogs*

GMP-grade reagent now available (HiScribe T7 High-Yield RNA Synthesis Kit only). See page 6 for details.

Description: NEB's HiScribe T7 High Yield RNA Synthesis Kits offer robust *in vitro* RNA transcription of many kinds of RNA, including internally labeled and co-transcriptionally capped RNA. Utilizing T7 RNA Polymerase, these kits achieve efficient transcription with small amounts of template, and can generate up to 180 µg per reaction, or up to 30–40 µg of capped RNA using a cap analog. RNA generated can be used in a variety of applications, including RNA structure/function studies, ribozyme biochemistry, probes for RNase protection assays and hybridization-based blots, anti-sense RNA and RNAi experiments, microarray analysis, microinjection, and *in vitro* translation and RNA vaccines.

The HiScribe T7 High Yield RNA Synthesis Kit is an extremely flexible system, with separate NTPs included for flexible reaction setup. The HiScribe T7 Quick High Yield RNA Synthesis Kit is provided in master mix format for fast reaction setup. Each kit can yield up to 9 mg of RNA.

The HiScribe T7 High Yield RNA Synthesis Kit Includes:

- T7 RNA Polymerase Mix
- 10X T7 Reaction Buffer
- ATP, GTP, UTP, CTP (100 mM)
- FLuc Control Template
- DTT

The HiScribe T7 Quick High Yield RNA Synthesis Kit Includes:

- T7 RNA Polymerase Mix
- LiCl Solution
- NTP Buffer Mix
- FLuc Control Template
- DNase I (RNase-free)
- DTT

Watch our webinar on tools for RNA synthesis.

HiScribe® SP6 RNA Synthesis Kit

#E2070S 50 reactions

Companion Products:

Monarch Spin RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

DNase I-XT
#M0570S 1,000 units
#M0570L 5,000 units

DNase I (RNase-free)
#M0303S 1,000 units
#M0303L 5,000 units

RNA Loading Dye, (2X)
#B0363S 4 ml

Faustovirus Capping Enzyme
#M2081S 500 units
#M2081L 2,500 units

Vaccinia Capping System
#M2080S 400 units

Monarch Spin RNA Cleanup Kit (10 µg)
#T2030S 10 preps
#T2030L 100 preps

Monarch Spin RNA Cleanup Kit (500 µg)
#T2050S 10 preps
#T2050L 100 preps

E. coli Poly(A) Polymerase
#M0276S 100 units
#M0276L 500 units

mRNA Cap 2´-O-Methyltransferase
#M0366S 2,000 units
#M0366L 10,000 units

3´-O-Me-m^{7}G(5')ppp(5')G RNA Cap Structure Analog
#S1411S 1 µmol
#S1411L 5 µmol

G(5')ppp(5')A RNA Cap Structure Analog
#S1406S 1 µmol
#S1406L 5 µmol

G(5')ppp(5')G RNA Cap Structure Analog
#S1407S 1 µmol
#S1407L 5 µmol

m^{7}G(5')ppp(5')G RNA Cap Structure Analog
#S1404S 1 µmol
#S1404L 5 µmol

m^{7}G(5')ppp(5')A RNA Cap Structure Analog
#S1405S 1 µmol
#S1405L 5 µmol

Use Monarch Spin RNA Cleanup Kits to purify your synthesized RNA.

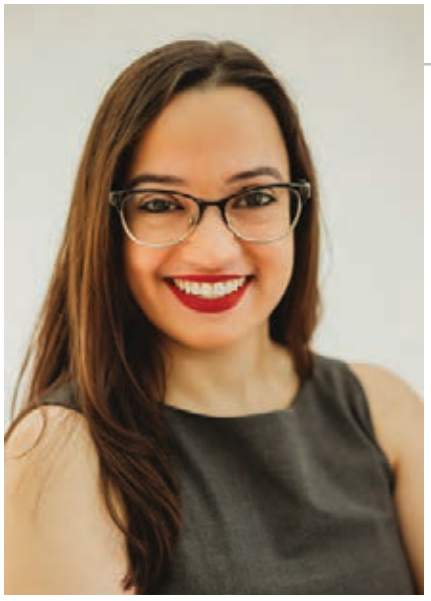
Description: The HiScribe SP6 RNA Synthesis Kit is designed for the *in vitro* transcription of RNA using SP6 RNA Polymerase. This kit is suitable for synthesis of high yield RNA transcripts and allows for incorporation of cap analogs (not included) or modified nucleotides (not included) to obtain capped-biotin-labeled or dye-labeled RNA. The kit is also capable of synthesizing radiolabeled RNA for use as probes or targets.

RNA synthesized from this kit is suitable for many applications, including RNA structure and function studies, ribozyme biochemistry, probes for RNase protection or gel shift assays, hybridization-based blots, anti-sense RNA or RNAi experiments, microarray analysis, microinjection and *in vitro* translation studies.

This kit contains sufficient reagents for 50 reactions of 25 µl each. Each standard reaction yields ≥ 80 µg of RNA from 1 µg SP6 Control Template DNA. Each kit can yield ≥ 4 mg of RNA.

Kit Includes:

- SP6 Reaction Buffer
- SP6 RNA Polymerase Mix
- SP6 Control Template
- ATP (Tris)
- GTP (Tris)
- UTP (Tris)
- CTP (Tris)
- DNase I (RNase-free)
- LiCl Solution
- Dithiothreitol (DTT)



Michelle joined NEB in 2019 as a Technical Support Scientist and is now Associate Channel Program Coordinator in the Marketing Sales Operations Team. At NEB, she is part of the Toastmasters Club and the Gaming and Jam Sessions Groups. Learn more about Michelle in her video reel on Instagram.

#NEBiographies

HiScribe® T7 ARCA Kits

HiScribe T7 ARCA mRNA Kit
#E2065S 20 reactions

HiScribe T7 ARCA mRNA Kit (with tailing)
#E2060S 20 reactions

Companion Products:

DNase I (RNase-free)
#M0303S 1,000 units
#M0303L 5,000 units

RNA Loading Dye, (2X)
#B0363S 4 ml

N1-Methyl-Pseudouridine-5´-Triphosphate (N1-Methyl-Pseudo-UTP)
#N0431S 0.1 ml

5-Methyl-Cytidine-5´-Triphosphate (5-Methyl-CTP)
#N0432S 0.1 ml

5-Methoxy-Uridine-5´-Triphosphate (5-Methoxy-UTP)
#N0434S 0.1 ml

Pseudouridine-5´-Triphosphate (Pseudo-UTP)
#N0433S 0.1 ml

- *Synthesis of capped and tailed mRNA*
- *Incorporation of modified nucleotides*
- *Template removal and mRNA purification reagents included*

Description: Most eukaryotic mRNAs require a N7-methyl guanosine (m^7G) cap structure at the 5´ end and a poly(A) tail at the 3´ end to be efficiently translated. By using a DNA template encoding a poly(A) tail, the HiScribe T7 ARCA mRNA Kit can be used to synthesize capped and tailed mRNAs. The cap structure is added to the mRNA by co-transcriptional incorporation of Anti-Reverse Cap Analog (ARCA, NEB #S1411) using T7 RNA Polymerase. The transcription reaction can be set up easily by combining the ARCA/NTP Mix, T7 RNA Polymerase Mix and a suitable DNA template. The kit also allows for partial incorporation of 5mCTP, Pseudo-UTP, N1-Methyl-Pseudo-UTP, 5-Methoxy-UTP and other modified nucleotides into mRNA. mRNAs synthesized with the kit can be used for cell transfection, microinjection, *in vitro* translation and RNA vaccines. Poly(A) tail is incorporated during the transcription reaction. The kit also includes DNase I and LiCl for DNA template removal and quick mRNA purification.

The HiScribe T7 ARCA mRNA Kit (with tailing) is designed for quick production of ARCA capped and poly(A) tailed mRNA *in vitro* from templates without encoded poly(A) tails.

The HiScribe T7 ARCA mRNA Kit Includes:

- T7 RNA Polymerase Mix
- ARCA/NTP Mix
- DNase I (RNase-free)
- LiCl Solution
- CLuc Control Template
- Dithiothreitol (DTT)

The HiScribe T7 ARCA mRNA Kit (with tailing) Includes:

- T7 RNA Polymerase Mix
- ARCA/NTP Mix
- DNase I (RNase-free)
- *E. coli* Poly(A) Polymerase
- Poly(A) Polymerase Reaction Buffer
- LiCl Solution
- CLuc Control Template
- Dithiothreitol (DTT)

Advantages:

- Quick reaction setup and streamlined protocol
- Enables partial incorporation of 5mCTP, Pseudo-UTP, N1-Methyl-Pseudo-UTP, 5-Methoxy-UTP and other modified nucleotides
- High quality components ensure mRNA integrity

EnGen® sgRNA Synthesis Kit, *S. pyogenes*

#E3322V 10 reactions
#E3322S 20 reactions

Companion Products:

EnGen Spy Cas9 NLS
#M0646T 500 pmol
#M0646M 2,500 pmol

EnGen Spy Cas9 HF1
#M0667T 500 pmol
#M0667M 2,500 pmol

Monarch Spin RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

EnGen Spy Cas9 Nickase
#M0650S 90 pmol

for high (20X) concentration
#M0650T 500 pmol

EnGen Spy dCas9 (SNAP-tag)
#M0652S 90 pmol

for high (20X) concentration
#M0652T 500 pmol

EnGen Mutation Detection Kit
#E3321S 25 reactions

DNase I (RNase-free)
#M0303S 1,000 units
#M0303L 5,000 units

- *Rapid generation of microgram quantities of sgRNAs in less than one hour*

The EnGen sgRNA Synthesis Kit, *S. pyogenes* provides a simple and quick method for transcribing high yields of sgRNA in a single 30-minute reaction, using the supplied reagents and target-specific DNA oligos designed by the user.

In nature, S. pyogenes Cas9 is programmed with two separate RNAs, the crRNA and tracrRNA. The crRNA, or CRISPR RNA sequence contains approximately 20 nucleotides of homology complementary to the strand of DNA opposite and upstream of a PAM (Protospacer Adjacent Motif) (NGG) sequence. The tracrRNA, or transactivating crRNA, contains partial complementary sequence to the crRNA as well as the sequence and secondary structure that is recognized by Cas9. These sequences have been adapted for use in the lab by combining the tracrRNA and crRNA into one long single guide RNA (sgRNA) species capable of complexing with Cas9 to recognize and cleave the target DNA.

The EnGen sgRNA Synthesis Kit, S. pyogenes combines an S. pyogenes Cas9-specific Scaffold Oligo (included in the EnGen 2X sgRNA Reaction Mix) that is partially complementary to the target-specific oligos designed by the user. The two oligos anneal at the overlapping region and are filled in by the DNA polymerase, creating a double-stranded DNA (dsDNA) template for transcription. Synthesis of the dsDNA template and transcription of RNA occur in a single reaction, resulting in the generation of a functional sgRNA.

Kit Includes:

- EnGen sgRNA Enzyme Mix
- EnGen 2X sgRNA Reaction Mix, *S. pyogenes*
- DNase I (RNase-free)
- EnGen sgRNA Control Oligo, *S. pyogenes*
- Dithiothreitol (DTT)

Recommended HiScribe RNA Synthesis Kits by Application

Application		T7 Kits					SP6 Kits
		HiScribe T7 High Yield RNA Synthesis Kit NEB #E2040	**HiScribe T7 Quick High Yield RNA Synthesis Kit** NEB #E2050	**HiScribe T7 ARCA mRNA Kit** NEB #E2065	**HiScribe T7 ARCA mRNA Kit (with tailing)** NEB #E2060	**HiScribe T7 mRNA Kit with CleanCap Reagent AG** NEB #E2080	**HiScribe SP6 RNA Synthesis Kit** NEB #E2070
Probe labeling	Fluorescent labeling: FAM, Cyanine (Cy) dyes, etc. • Fluorescent *in situ* hybridization (FISH)	●	●				●
	Non-fluorescent labeling: Biotin, Digoxigenin • *In situ* hybridization • Blot hybridization with secondary detection • Microarray	●	●				●
	High specific activity radiolabeling • Blot hybridization • RNase protection	●					●
mRNA & RNA for transfection	Streamlined high yield CleanCap Reagent AG capped RNA synthesis • Template encoded poly(A) tails • Non-polyadenylated transcripts • Transfection • Microinjection • *In vitro* translation					●	
	Streamlined mRNA synthesis with ARCA co-transcriptional capping and enzymatic poly(A) tailing • Transfection • Microinjection • *In vitro* translation				●		
	Streamlined ARCA capped RNA synthesis • Template encoded poly(A) tails • Non-polyadenylated transcripts • Transfection • Microinjection • *In vitro* translation			●			
	Co-transcriptional capping with alternate cap analogs • Transfection • Microinjection • *In vitro* translation	●	●				●
	Post-transcriptional capping with Vaccinia Capping System • Transfection • Microinjection • *In vitro* translation	●	●				●
	Complete substitution of NTPs: 5-mC, pseudouridine, 5mCTP, Pseudo-UTP, N-Methyl-Pseudo-UTP, 5-Methyl-UTP, etc.	●				●	●
	Partial substitution of NTPs: 5-mC, pseudouridine, 5mCTP, Pseudo-UTP, N-Methyl-Pseudo-UTP, 5-Methyl-UTP, etc.	●	●	●	●	●	●
	Unmodified RNA	●	●			●	●
	Hairpins, short RNA, dsRNA • Gene knockdown	●	●				●
Structure, function, & binding studies	Complete substitution of NTPs • Aptamer selection • Isotopic labeling	●					●
	Partial substitution of one or more NTPs • Aptamer selection • Structure determination	●	●				●
	Unmodified RNA • SELEX • Structure determination	●	●				●

RNA Polymerases

T3 RNA Polymerase
#M0378S 5,000 units

T7 RNA Polymerase
#M0251S 5,000 units
#M0251L 25,000 units

T7 RNA Polymerase (High Concentration)
#M0460T 50,000 units

SP6 RNA Polymerase
#M0207S 2,000 units

Hi-T7 RNA Polymerase
#M0658S 5,000 units

Hi-T7 RNA Polymerase (High Concentration)
#M0470T 50,000 units

- *Radiolabeled RNA probe preparation*
- *RNA generation for in vitro translation*
- *RNA generation for studies of RNA structure, processing and catalysis*

T7 RNA Polymerase (NEB #M0254) is available as a **GMP-grade reagent**. See page 6 for details.

Description: Initiation of transcription with T3, T7 and SP6 RNA Polymerases is highly specific for the T3, T7 and SP6 phage promoters, respectively. Cloning vectors have been developed which direct transcription from the T3, T7 or SP6 promoter through polylinker cloning sites. These vectors allow *in vitro* synthesis of defined RNA transcripts from a cloned DNA sequence. T7 RNA Polymerase (High Concentration) is offered at a 20-fold higher concentration than our standard T7 RNA Polymerase and is ideal for experienced users interested in building and optimizing their own *in vitro* transcription reactions.

Hi-T7 RNA Polymerase is an engineered, thermoactive T7 RNA Polymerase. Hi-T7 uses T7 RNA Polymerase promoters. It can increase capping efficiency and eliminate dsRNA by-product formation during synthesis. Hi-T7 RNA Polymerase (High Concentration) is offered at a 20-fold higher concentration than our standard Hi-T7 RNA Polymerase and is ideal for experienced users interested in building and optimizing their own *in vitro* transcription reactions.

Reaction Conditions: 1X RNAPol Reaction Buffer. Supplement with 0.5 mM each ATP, UTP, GTP, CTP (not included) and DNA template containing the appropriate promoter. Incubate at 37°C (T3, T7 and SP6) or 50°C (Hi-T7). Protocols involving high concentration T7 and Hi-T7 RNA Polymerases are to be designed and optimized by the user.

Unit Definition: One unit is defined as the amount of enzyme required to incorporate 1 nmol ATP into an acidinsoluble material in 1 hour at 37°C or 50°C for Hi-T7. Unit assay conditions can be found at www.neb.com.

Concentration: T3 RNA Polymerase: 50,000 units/ml. T7 RNA Polymerase: 50,000 units/ml. T7 RNA Polymerase (High Concentration): 1,000,000 units/ml. SP6 RNA Polymerase: 20,000 units/ml. Hi-T7 RNA Polymerase: 50,000 units/ml. Hi-T7 RNA Polymerase (High Concentration): 1,000,000 units/ml.

E. coli Poly(A) Polymerase

#M0276S 100 units
#M0276L 500 units

Companion Products:

Adenosine 5'-Triphosphate (ATP)
#P0756S 1 ml
#P0756L 5 ml

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

- *Labeling of RNA with ATP or cordycepin 5´-triphosphate*
- *Poly(A) tailing of RNA for cloning or affinity purification*
- *Enhances translation of RNA transferred into eukaryotic cells*

Description: *E. coli* Poly(A) Polymerase catalyzes the template independent addition of AMP from ATP to the 3´ end of RNA.

Source: An *E. coli* strain that carries the cloned Poly(A) Polymerase gene from *E. coli* (1).

Reaction Conditions: Poly(A) Polymerase Reaction Buffer, 37°C. Supplement with 1 mM ATP.

Reagents Supplied:

- Poly(A) Polymerase Reaction Buffer
- Adenosine-5'-Triphosphate (ATP)

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 1 nmol of AMP into RNA in a 20 µl volume in 10 minutes at 37°C.

Concentration: 5,000 units/ml

Poly(U) Polymerase

#M0337S 60 units

Companion Products:

Ribonucleotide Solution Set
#N0450S 10 µmol
#N0450L 50 µmol

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

- *Labeling of RNA with UTP*
- *Poly(U) tailing of RNA for cloning*

Description: Poly(U) Polymerase catalyzes the template-independent addition of UMP from UTP or AMP from ATP to the 3´ end of RNA.

Source: An *E. coli* strain that carries the cloned poly(U) polymerase gene of *Schizosaccharomyces pombe* Cid1.

Reaction Conditions: NEBuffer 2, 37°C. Supplement with 0.5 mM UTP . Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that incorporates 1 nmol of UMP into RNA in a 50 µl volume in 10 minutes at 37°C.

Concentration: 2,000 units/ml

Note: Poly(U) Polymerase in NEBuffer 2 will incorporate NMP from NTP into RNA. Tailing length of poly(U) varies with UTP. Poly(U) Polymerase is highly processive under low primer concentrations (<100 pmol).

E. coli RNA Polymerase, Core Enzyme & Holoenzyme

E. coli RNA Polymerase, Core Enzyme
#M0550S 100 units

E. coli RNA Polymerase, Holoenzyme
#M0551S 50 units

- *RNA synthesis from E. coli promoter*
- *Transcription initiation studies*
- *In vitro translation with PURExpress*

Description: *E. coli* RNA Polymerase Core Enzyme consists of 5 subunits designated α, α, β´, β, and ω. The enzyme is free of sigma factor and does not initiate specific transcription from bacterial and phage DNA promoters. The enzyme retains the ability to transcribe RNA from nonspecific initiation sequences. Addition of sigma factors will allow the enzyme to initiate RNA synthesis from specific bacterial and phage promoters. The core enzyme has a molecular weight of ~ 400 kDa.

E. coli RNA Polymerase Holoenzyme is the core enzyme saturated with sigma factor 70. The Holoenzyme initiates RNA synthesis from sigma 70 specific bacterial and phage promoters.

Reaction Conditions: 1X *E. coli* RNA Polymerase Reaction Buffer, 0.5 mM of each rNTP and DNA template. Incubate at 37°C.

Unit Definition: One unit is the amount of enzyme required to incorporate 1 nmol of NTP into RNA in 10 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 1,000 units/ml

Pyrophosphatases

Pyrophosphatase, Inorganic (*E. coli*)
#M0361S 10 units
#M0361L 50 units

Pyrophosphatase, Inorganic (yeast)
#M2403S 10 units
#M2403L 50 units

Thermostable Inorganic Pyrophosphatase
#M0296S 250 units
#M0296L 1,250 units

- *Increasing RNA yield in transcription reactions*
- *Enhancing DNA replication*

Pyrophosphatase, Inorganic (*E. coli*) (NEB #M0361) is available as a **GMP-grade reagent**. See page 6 for details.

Description: Inorganic pyrophosphatase (PPase) catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate.

$P2O_7^{-4} + H_2O \rightarrow 2HPO_4^{-2}$

Source: Pyrophosphatase, Inorganic (*E. coli*) is prepared from a clone of the *E. coli* inorganic pyrophosphatase gene.

Pyrophosphatase, Inorganic (yeast) is from an *E. coli* strain containing a genetic fusion of the *Saccharomyces cerevisiae ppa* gene and the gene coding for *Mycobacterium xenopi* GyrA intein. Developed by BioHelix Corporation, now a wholly owned subsidiary of Quidel Corporation, and produced at New England Biolabs.

Thermostable Inorganic Pyrophosphatase is from an *E. coli* strain carrying a plasmid encoding a pyrophosphatase from the extreme thermophile *Thermococcus litoralis*.

Unit Definition: One unit is defined as the amount of enzyme that will generate 1 µmol of phosphate per minute from inorganic pyrophosphate under standard reaction conditions.

Concentration: Pyrophosphatase, Inorganic (*E. coli*) and Pyrophosphatase, Inorganic (yeast): 100 units/ml. Thermostable Inorganic Pyrophosphatase: 2,000 units/ml. NudC Pyrophosphatase: 10 µM

NudC Pyrophosphatase

#M0607S 250 pmol

Description: NudC is a NUDIX pyrophosphatase that efficiently hydrolyzes NAD^+- and NADH-capped RNA, generating a ligatible 5´ monophosphate on the RNA (NAD^+ decapping or deNADding). Deletion of the nudC gene has been shown to increase the fraction of NAD^+-capped RNA in *E. coli*.

Unit Definition: 1 µM of NudC hydrolyzes 200 µM or more NAD^+ into NMN^+ and AMP in 1X NEBuffer 3.1 and 5 mM DTT at 37°C for 30 min.

Concentration: 10 µM

Ribonucleotides

Ribonucleotide Solution Set
#N0450S 10 µmol
#N0450L 50 µmol

Ribonucleotide Solution Mix
#N0466S 10 µmol
#N0466L 50 µmol

NEW
N1-Methyl-Pseudouridine-5´-Triphosphate (N1-Methyl-Pseudo-UTP)
#N0431S 0.1 ml

NEW
5-Methyl-Cytidine-5´-Triphosphate (5-Methyl-CTP)
#N0432S 0.1 ml

NEW
Pseudouridine-5´-Triphosphate (Pseudo-UTP)
#N0433S 0.1 ml

NEW
5-Methoxy-Uridine-5´-Triphosphate (5-Methoxy-UTP)
#N0434S 0.1 ml

Description: Ribonucleotide Solution Set consists of four separate solutions of ATP, GTP, CTP and UTP, pH 7.5, as sodium salts. Each nucleotide is supplied at 100 mM.

Ribonucleotide Solution Mix consists of a buffered equimolar solution of ribonucleotide triphosphates (ATP, CTP, GTP and UTP), pH 7.5, as sodium salts. Each nucleotide is supplied at a concentration of 25 mM (total NTP concentration equals 100 mM).

Modified NTPs are commonly used for reduction of immunogenicity for *in vitro* transcription RNA. 5-Methyl-Cytidine-5'-Triphosphoate (5-Methyl-CTP) is supplied as sodium salt, pH 7.3. Pseudouridine-5'-Triphosphate (Pseudo-UTP) is supplied as sodium salt, pH 7.0. N1-Methyl-Pseudouridine-5'-Triphosphate (N1-Methyl-Pseudo-UTP) is supplied as sodium salt, pH 7.5. 5-Methoxy-Uridine-5'-Triphosphate (5-Methoxy-UTP) is supplied as sodium salt, pH 7.1. All modified NTPs are supplied at a concentration of 100 mM.

Note: To ensure maximum activity upon long-term storage, aliquot and store at –80°C

Sce PUS1

NEB r1.1 | RR | 30° | 55°

#M0526S 5,000 pmol

- *Sequence-specific pseudouridine modification is an alternative to randomly incorporated modified nucleosides by RNA polymerases*

Description: Sce Pseudouridine Synthase I (Sce PUS1) converts Uridine to Pseudouridine in single-stranded RNA, with a preference for Uridines in single-stranded RNA regions over Uridines in double-stranded RNA. The optimal substrate is an unstructured RNA that is 15 nt long or longer.

Reaction Conditions: NEBuffer r1.1, 30°C. Heat inactivation: 55°C.

Concentration: 100 pmol/µl

NudC and Sce PUS1 are **Enzymes for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

Faustovirus Capping Enzyme

NEB U | 37° | 70° | SAM

#M2081S 500 units
#M2081L 2,500 units

- *Experience improved capping efficiency, even on difficult substrates*
- *Achieve robust capping with less enzyme*
- *Set up reactions under a broad temperature range, for added flexibility*
- *Choose as an alternative to Vaccinia Capping System, with minimal optimization required*
- *Enable one-pot Cap-1 synthesis, as FCE is compatible with mRNA Cap 2´-O-Methyltransferase*
- *Benefit from no licensing fees from NEB for the use of FCE*

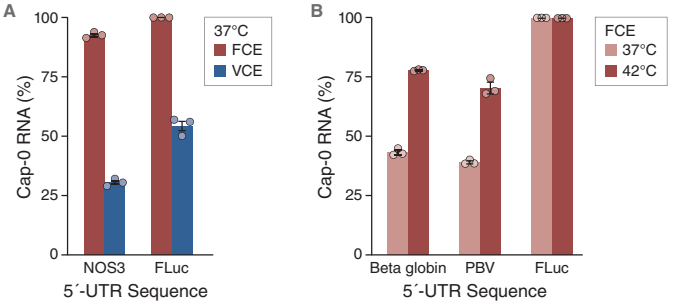
GMP-grade reagent now available.
See page 6 for details.

Faustovirus Capping Enzyme (FCE) catalyzes the addition of N7-methylguanosine cap (m^7G) to the 5´ end of the triphosphorylated and diphosphorylated transcripts, producing Cap-0 RNA. FCE is a single-subunit enzyme that combines the three activities necessary to produce the Cap-0 structure – triphosphatase, guanylyltransferase, and (guanine-N7)-methyltransferase. FCE retains significant capping activity at low temperatures and tolerates reaction temperatures up to 55°C. In many cases, 1 µl of FCE (25 units) can cap over 100 µg of RNA in 1 hour at 37°C.

Reaction Conditions: Capping Buffer, 37°C. Supplement with 0.5 mM GTP and 0.1 mM S-adenosylmethionine (SAM). Heat inactivation: 70°C for 10 minutesAddition of EDTA to 5 mM is recommended to avoid RNA hydrolysis..

Unit Definition: One unit of Faustovirus Capping Enzyme is defined as the amount of enzyme required to convert 75 pmol of a 20-mer ppp-RNA to Cap-0 RNA in 30 minutes at 37°C.

Concentration: 25,000 units/ml



Faustovirus Capping Enzyme (FCE) offers increased capping efficiency and workflow optimization. *200 µg (~350 picomoles) of a 1.77 kb transcript having 5´-UTR sequences as indicated were treated with A) a limiting amount of FCE (25 units, 1 picomole, 20 nM in 50 µl) or VCE (10 units, 1 picomole, 20 nM in 50 µl) for 1 hour at 37°C or B) or for FCE only for 1 hour at 37°C or 42°C. Note that this is less than our recommended amount of enzyme highlighting the increased capping efficiency of FCE vs VCE. 50 µl reactions contained 0.1 mM SAM, and 0.5 mM GTP, 1X FCE Capping Buffer for FCE reactions or 1X Capping Buffer for VCE reactions. mRNA capping was measured using targeted RNase H cleavage and LC-MS.*

Vaccinia Capping System

NEB U | RR | 37° | SAM

#M2080S 400 units

Companion Product:

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

- *Capping mRNA prior to in vivo or in vitro translation*
- *Labeling 5´ end of mRNA*

GMP-grade reagent now available.
See page 6 for details.

Description: Based on the Vaccinia Virus Capping Enzyme, the Vaccinia Capping System provides the necessary components to add N7-methylguanylate cap structures (Cap-0) to the 5´ end of RNA. In eukaryotes, these terminal cap structures are involved in stabilization, transport and translation of mRNAs. Enzymatic production of capped RNA is an easy way to improve the stability and translational competence of RNA used for *in vitro* translation, transfection and microinjection. Alternatively, use of labeled GTP in a reaction provides a convenient way to label any RNA containing a 5´ terminal triphosphate.

This single enzyme is composed of two subunits (D1 and D12) and has three enzymatic activities (RNA triphosphatase and guanylyltransferase by the D1 subunit and guanine methyltransferase by the D12 subunit), all necessary for addition of a complete Cap-0 structure, m^7Gppp(5´)N. All capped structures are added in the proper orientation, unlike co-transcriptional addition of some cap analogs.

Source: An *E. coli* strain that carries an engineered His-tagged Vaccine capping gene.

Reaction Conditions: Capping Buffer, 37°C. Supplement with 0.5 mM GTP and 0.1 mM S-adenosylmethionine (SAM).

Reagents Supplied:

- Capping Buffer
- S-adenosylmethionine (SAM)
- GTP

Unit Definition: One unit of Vaccinia Capping Enzyme is defined as the amount of enzyme required to incorporate 10 pmol of (α^{32}P) GTP into an 80 nt transcript in 1 hour at 37°C.

Concentration: 10,000 units/ml

Cloned at NEB | RR Recombinant Enzyme | PCR PCR Enzyme | Hot Start/WarmStart | SAM Requires SAM | 37° Incubation Temperature | 65° Heat Inactivation

mRNA Cap 2'-O-Methyltransferase

NEB U RR 37° SAM

#M0366S 2,000 units
#M0366L 10,000 units

- *Enhances translation of RNA*
- *Improves mRNA expression during microinjection and transfection*

GMP-grade reagent now available.
See page 6 for details.

Description: mRNA Cap 2´-O-Methyltransferase adds a methyl group at the 2´-O position of the first nucleotide adjacent to the cap structure at the 5´ end of the RNA. The enzyme utilizes S-adenosylmethionine (SAM) as a methyl donor to methylate capped RNA (Cap-0) resulting in a Cap-1 structure.

Source: An *E. coli* strain carrying a gene encoding for his-tagged variant of Vaccinia mRNA Cap 2´-O-Methyltransferase.

Reaction Conditions: Capping Buffer, 37°C. Supplement with 0.2 mM S-adenosylmethionine (SAM).

Reagents Supplied:

• Capping Buffer

• S-adenosylmethionine (SAM)

Unit Definition: One unit is defined as the amount of enzyme required to methylate 10 pmoles of 80 nt long capped RNA transcript in 1 hour at 37°C.

Concentration: 50,000 units/ml

RNA Cap Analog Selection Chart

The 5´ terminal m^{7}G cap present on most eukaryotic mRNAs is required for translation, *in vitro*, at the initiation level. For most RNAs, the cap structure increases stability, decreases susceptibility to exonuclease degradation, and promotes the formation of mRNA initiation complexes. Certain prokaryotic mRNAs with 5´ terminal cap structures are translated as efficiently as eukaryotic mRNA in a eukaryotic cell-free protein synthesizing system. Splicing of certain eukaryotic substrate RNAs has also been observed to require a cap structure.

Product	NEB #	Size	Applications
3´-O-Me-m^{7}G(5')ppp(5')G RNA Cap Structure Analog	S1411S S1411L	1 µmol 5 µmol	• Ensures incorporation in the correct orientation • Co-transcriptional capping with T7 (NEB #M0251), SP6 (NEB #M0207) and T3 RNA Polymerases • Synthesis of m^{7}G capped RNA for *in vitro* splicing assays • Synthesis of m^{7}G capped RNA for transfection or microinjection
m^{7}G(5')ppp(5')G RNA Cap Structure Analog	S1404S S1404L	1 µmol 5 µmol	• Co-transcriptional capping with T7, SP6 and T3 RNA polymerases • Synthesis of m^{7}G capped RNA for *in vitro* splicing assays • Synthesis of m^{7}G capped RNA for transfection or microinjection
G(5')ppp(5')G RNA Cap Structure Analog	S1407S S1407L	1 µmol 5 µmol	• Co-transcriptional capping with T7, SP6 and T3 RNA polymerases • Synthesis of unmethylated G-capped RNA
m^{7}G(5')ppp(5')A RNA Cap Structure Analog	S1405S S1405L	1 µmol 5 µmol	• Co-transcriptional capping with T7 RNA polymerase from the phi2.5 promoter that contains an A at the transcription initiation site • Synthesis of m^{7}G capped RNA for *in vitro* splicing assays • Synthesis of m^{7}G capped RNA for transfection or microinjection
G(5')ppp(5')A RNA Cap Structure Analog	S1406S S1406L	1 µmol 5 µmol	• Co-transcriptional capping with T7 RNA polymerase from the phi2.5 promoter that contains an A at the transcription initiation site • Synthesis of unmethylated G capped RNA • Synthesis of A capped RNA

3´-Desthiobiotin-GTP & 3´-Biotin-GTP

3´-Desthiobiotin-GTP
#N0761S 0.5 µmol

3´-Biotin-GTP
#N0760S 0.5 µmol

Description: 3´-Desthiobiotin-GTP or 3´-Biotin GTP are guanosine triphosphate (GTP) analogs which are modified at their 3´ position with desthiobiotin or biotin, respectively. When used with the Vaccinia Capping System, (NEB #M2080) these reagents enable affinity tagging of RNA triphosphate ends. Tagged RNAs are enriched by binding to Hydrophilic Streptavidin Magnetic Beads (NEB #S1421). Desthiobiotin-tagged RNAs can be eluted with free biotin. This approach is used in Cappable-seq, a method developed at NEB for directly enriching the 5´ ends of primary transcripts (1).

Reference:
(1) Ettwiller, L. et al. (2016) *BMC Genomics*, 17,199.

yDcpS

NEB U RR 37° 70°

#M0463S 4,000 units

- *mRNA decapping, enabling recapping with tagged-GTP analogs*
- *Biotinylation of 5´ ends of primary transcripts*
- *Recappable-seq*

Description: yDcpS decapping enzyme from S. cerevisiae hydrolyzes the phosphodiester bond between the gamma and beta phosphates of m^{7}G capped mRNA, leaving behind a diphosphorylated 5´ end and m^{7}GMP. yDcpS is capable of decapping full length mRNAs and the diphosphorylated 5´ end it leaves behind is suitable for recapping using Vaccinia Capping Enzyme.

Source: An E. coli strain carrying the S. cerevisiae gene DCS1 (encoding yDcpS) on a plasmid.

Reaction Conditions: yDcpS Reaction Buffer, 37°C. Heat inactivation: 70°C.

Reagents Supplied:

• yDcpS Reaction Buffer

Unit Definition: One unit is defined as the amount of yDcpS required to convert 50% of a 500 nM solution of the following 25-mer m7G-capped RNA to a 5'-diphosphorylated form in a total reaction volume of 20 µl in 1 hour at 37°C: 5'-[m7Gppp]rGrUrArGrArArCrU rUrCrGrUrCrGrArGrUrArCrGrCrUrCrArA[3-6FAM]-3'

Concentration: 200,000 units/ml

mRNA Decapping Enzyme

NEBU 37° M0

#M0608S 2,000 units

- *Efficient replacement for Tobacco Acid Pyrophosphatase*
- *Cap-0 and Cap-1 are removed with equal efficiency*
- *Suitable for 5´ RLM-RACE and RNA-seq*

Description: mRNA Decapping Enzyme catalyzes the removal of N7-methylguanosine cap (m^7G) from the 5´ end of mRNA, producing 5´ monophosphate and releasing m^7GDP. mRNA Decapping Enzyme is capable of decapping mRNAs of various lengths and removes both Cap-0 and Cap-1 structures with similar efficiency. mRNA Decapping Enzyme also converts 5´ triphosphate ends to 5´ monophosphate, albeit with reduced efficiency.

Source: mRNA Decapping Enzyme from *S.pombe* is expressed as a His-tagged fusion in *E. coli.*

Reaction Conditions: mRNA Decapping Enzyme Reaction Buffer, 37°C.

Reagents Supplied:

• mRNA Decapping Enzyme Reaction Buffer

Unit Definition: One unit is defined as the amount of mRNA Decapping Enzyme required to convert 50% of a 500 nM m^7G-capped substrate to a 5´-monophosphorylated form in a total reaction volume of 20 µl in 1 hour at 37°C.

Concentration: 100,000 units/ml

cDNA Synthesis Selection Chart

Product	NEB #	Size	Features
LunaScript RT SuperMix Kit	#E3010S #E3010L	25 reactions 100 reactions	• Ideal for cDNA synthesis of targets up to 3 kb (two-step RT-qPCR, amplicon sequencing) • Single tube supermix contains random hexamer and oligo-dT primers, dNTPs, Murine RNase Inhibitor, and Luna Reverse Transcriptase • Visible blue tracking dye for easy reaction setup • Fast 13-minute protocol
LunaScript RT Master Mix Kit (Primer-free)	#E3025S #E3025L	25 reactions 100 reactions	• Ideal for first strand cDNA synthesis • Compatible with random primers, oligo dT primers and gene-specific primers • 5X master mix contains dNTPs, Murine RNase Inhibitor and Luna Reverse Transcriptase • Visible blue tracking dye for easy reaction setup • Fast 13-minute protocol
ProtoScript II First Strand cDNA Synthesis Kit	#E6560S #E6560L	30 reactions 150 reactions	• Generates cDNA up to 10 kb in length • Contains ProtoScript II Reverse Transcriptase, an enzyme with increased thermostability and reduced RNase H activity • Convenient 2-tube kit • Includes dNTPs, Oligo-dT primer and Random Primer Mix
ProtoScript First Strand cDNA Synthesis Kit	#E6300S #E6300L	30 reactions 150 reactions	• Generates cDNA at least 5 kb in length • Contains M-MuLV Reverse Transcriptase • Convenient 2-tube kit • Includes dNTPs, Oligo-dT primer and Random Primer Mix
Template Switching RT Enzyme Mix	#M0466S #M0466L	20 reactions 100 reactions	• Incorporates a universal adaptor sequence at the 3´ end of cDNA during the RT reaction • Enzyme mix and buffer are optimized for efficient template switching • RT enzyme mix includes RNase Inhibitor • High sensitivity for cDNA amplification – enables transcriptome analysis by RNA-seq from single cells or as low as 2 pg of human total RNA • Robust and simple workflow for 5´ Rapid Amplification of cDNA Ends (RACE) • Retains the complete 5´ end of transcripts for 2nd Strand cDNA Synthesis
Induro Reverse Transcriptase	#M0681S #M0681L	4,000 units 10,000 units	• Fast and processive intron-encoded RT for generating long transcripts (> 12 kb in under 10 mn.) • Increased reaction temperatures (50–60°C) • Increased inhibitor tolerance
ProtoScript II Reverse Transcriptase	#M0368S #M0368L #M0368X	4,000 units 10,000 units 40,000 units	• RNase H– mutant of M-MuLV Reverse Transcriptase with increased thermostability and reduced RNase H activity • Increased reaction temperatures (37–50°C)
M-MuLV Reverse Transcriptase	#M0253S #M0253L	10,000 units 50,000 units	• Robust reverse transcriptase for a variety of templates • Standard reaction temperatures (37–45°C)
AMV Reverse Transcriptase	#M0277S #M0277L	200 units 1,000 units	• Robust reverse transcriptase for a broad temperature range (37–52°C) • Can be used for templates requiring higher reaction temperatures
WarmStart RTx Reverse Transcriptase	#M0380S #M0380L	400 units 2,000 units	• Permits room temperature reaction setup • Increased reaction temperatures (50–65°C) • Optimized for RT-LAMP isothermal detection
WarmStart RTx Reverse Transcriptase (Glycerol-free)	#M0439L	2,000 units	• Permits room temperature reaction setup • Increased reaction temperatures (50–65°C) • Optimized for RT-LAMP isothermal detection

For RT-PCR, RT-qPCR and glycerol-free kits, see DNA Polymerases and Amplification Technologies.

ProtoScript® II Reverse Transcriptase

NEBU RR 42° 65°

#M0368S 4,000 units
#M0368L 10,000 units
#M0368X 40,000 units

Companion Products:

RNase H
#M0297S 250 units
#M0297L 1,250 units

Monarch Spin RNA Isolation Kit (Mini)
#T2110S 50 preps

- *Efficient reverse transcription from different starting RNA amounts*
- *Increased thermostability*
- *Generates cDNA up to 10 kb or more*

Description: ProtoScript II Reverse Transcriptase is a recombinant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. It can be used to synthesize first strand cDNA at higher temperatures than the wild type M-MuLV. The enzyme is active up to 50°C, providing higher specificity, higher yield of cDNA and more full length cDNA product, up to 12 kb in length. This product was formerly known as M-MuLV Reverse Transcriptase RNase H^-.

Source: The gene encoding a mutant M-MuLV Reverse Transcriptase (RNase H^-) is expressed in *E. coli* and purified to near homogeneity.

Reaction Conditions: ProtoScript II Reverse Transcriptase Reaction Buffer, 42°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in a total reaction volume of 50 µl in 10 minutes at 37°C using poly(rA)•oligo$(dT)_{18}$ as template.

Concentration: 200,000 units/ml

LunaScript® RT SuperMix Kit & LunaScript RT Master Mix Kit (Primer-free)

LunaScript RT SuperMix
#M3010L 100 reactions
#M3010X 500 reactions
#M3010E 2,500 reactions

LunaScript RT SuperMix Kit
#E3010S 25 reactions
#E3010L 100 reactions

LunaScript RT Master Mix Kit (Primer-free)
#E3025S 25 reactions
#E3025L 100 reactions

LunaScript RT SuperMix Kit is an optimized master mix containing all the necessary components for first strand cDNA synthesis in the context of a two-step RT-qPCR workflow. It features the thermostable Luna Reverse Transcriptase, which supports cDNA synthesis at elevated temperatures. Murine RNase Inhibitor is also included to protect template RNA from degradation. The LunaScript RT SuperMix Kit contains random hexamer and poly-dT primers, allowing for even coverage across the length of the RNA targets.

LunaScript RT Master Mix Kit (Primer-free) is the same formulation as the LunaScript RT SuperMix Kit but does not contain primers. It includes all components needed for carrying out first strand cDNA synthesis except for RNA template and user-supplied primers. It provides flexible options for using different primers (dT primers, random primers, gene-specific primers, modified primers, etc.) for first strand cDNA synthesis.

M-MuLV Reverse Transcriptase

NEBU RR 42° 65°

#M0253S 10,000 units
#M0253L 50,000 units

Companion Product:

Monarch Spin RNA Isolation Kit (Mini)
#T2110S 50 preps

- *cDNA synthesis*
- *RNA Sequencing*
- *RT-PCR*

Description: Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase is an RNA-directed DNA polymerase. This enzyme can synthesize a complementary DNA strand initiating from a primer using either RNA (cDNA synthesis) or single-stranded DNA as a template. M-MuLV Reverse Transcriptase lacks 3´→ 5´ exonuclease activity.

Source: The gene encoding M-MuLV Reverse Transcriptase is expressed in *E. coli* in a vector that results in 16 additional amino acids at the N-terminus and 13 amino acids at the C-terminus. This construct results in a fully functional Reverse Transcriptase protein with a functional RNase H domain.

Reaction Conditions: M-MuLV Reverse Transcriptase Reaction Buffer, 42°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in a total reaction volume of 50 µl in 10 minutes at 37°C using poly(rA)-oligo(dT) as template primer with 50 mM Tris-HCl (pH 8.3), 6 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM [^{3}H]-dTTP and 0.4 mM poly(rA)-oligo(dT)12-18.

Concentration: 200,000 units/ml

AMV Reverse Transcriptase

NEBU 42° 85°

#M0277S 200 units
#M0277L 1,000 units

Companion Product:

Monarch Spin RNA Isolation Kit (Mini)
#T2110S 50 preps

- *cDNA synthesis*
- *RNA Sequencing*
- *RT-PCR*

Description: Avian Myeloblastosis Virus (AMV) Reverse Transcriptase is an RNA-directed DNA polymerase. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA (cDNA synthesis) or single-stranded DNA as a template.

Source: Insect cells (Sf21) infected with *baculovirus* containing the pol gene of AMV.

Reaction Conditions: AMV Reverse Transcriptase Reaction Buffer, 42°C. Heat inactivation: 85°C for 5 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into an acid-insoluble form in 10 minutes at 37°C using poly(rA)-oligo(dT) as template primer.

Concentration: 10,000 units/ml

Note: Storage: Once thawed, store at -20°C. Repeated freeze thaw cycles will inactivate the enzyme. Aliquots can be stored for longer periods at -70°C.

WarmStart® RTx Reverse Transcriptase

WarmStart RTx Reverse Transcriptase
#M0380S 400 units
#M0380L 2,000 units

NEW
WarmStart RTx Reverse Transcriptase (Glycerol-free)
#M0439L 2,000 units

Companion Products:

WarmStart RTx Reverse Transcriptase (Glycerol-free)
#M0439L 2,000 units

Monarch Spin RNA Isolation Kit (Mini)
#T2110S 50 preps

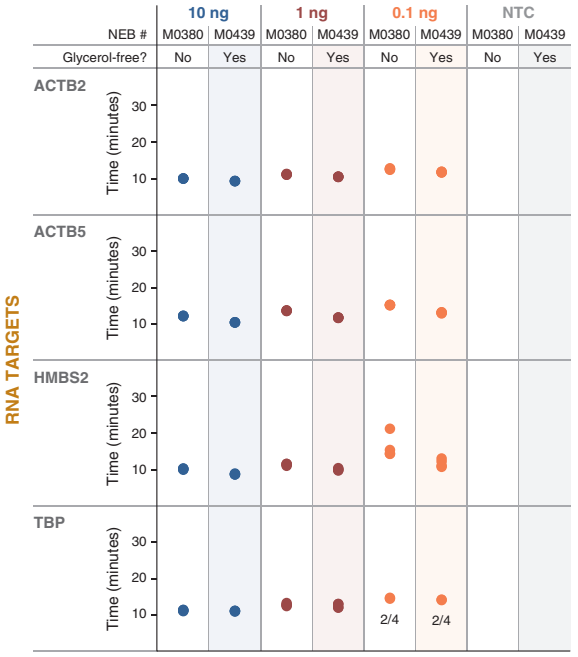
- *RT-LAMP*
- *cDNA Synthesis*
- *RT reactions requiring room temperature setup*

Description: WarmStart RTx Reverse Transcriptase is a unique *in silico*-designed, RNA-directed DNA polymerase coupled with a reversibly-bound aptamer that inhibits RTx activity below 40°C. This enzyme can synthesize a complementary DNA strand initiating prom a primer using RNA (cDNA synthesis) or single-stranded DNA as a template. RTx is a robust enzyme for RNA detection in amplification reactions and is particularly well suited for use in Loop-mediated Isothermal Amplification (LAMP). The WarmStart property enables high-throughput applications, room-temperature setup, and increases consistency and specificity of amplification reactions. RTx contains intact RNase H activity. The glycerol-free version of WarmStart RTx Reverse Transcriptase supports lyophilization and automated workflows.

Reaction Conditions: 1X Isothermal Amplification Buffer (or 1X Isothermal Amplification Buffer (Lyo-compatible)), template, primer, dNTPs and 0.25-0.5 µl of WarmStart RTx Reverse Transcriptase in a reaction volume of 25 µl. Incubate at 50-55°C for cDNA synthesis or directly at 65°C for RT-LAMP. Heat inactivation: 80°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 1nmol of dTTP into acid-insoluble material in a total reaction volume of 50 µl in 20 minutes at 50°C using poly(rA) - oligo(dT)18 as a template.

Concentration: WarmStart RTx Reverse Transcriptase: 15,000 units/ml. WarmStart RTx Reverse Transcriptase (Glycerol-free): 75,000 units/ml.



WarmStart RTx Reverse Transcriptase (Glycerol-free) offers the same robust detection of human RNA targets in RT-LAMP assays as the glycerol-containing enzyme. *RT-LAMP (RNA targets) experiments were performed with WarmStart RTx Reverse Transcriptase (NEB #M0380) and Bst 2.0 WarmStart® DNA Polymerase (NEB #M0538), or WarmStart RTx Reverse Transcriptase (Glycerol-free) (NEB #M0439) and Bst 2.0 WarmStart DNA Polymerase (Glycerol-free) (NEB #M0402). Reactions containing 1X LAMP primers and 0.5X LAMP Fluorescent dye were set up in quadruplicate over three logs of total Jurkat RNA (10 ng to 0.1 ng) in 96-well, 25 µl reactions. Control reactions without template (NTC) were also evaluated. Reactions were incubated at 65°C for 40 minutes and fluorescence was monitored every 15 seconds in the SYBR/FAM channel of a real-time thermocycler (Bio-Rad® CFX96). Each dot represents the time at which the fluorescence signal for a single reaction crosses the instrument-defined threshold. All four replicates were detected at each template input unless otherwise indicated (note that dots frequently overlap given similar detection time for the replicates). Overall, similar performance was observed for both glycerol-containing (NEB #M0538 and NEB #M0380) and glycerol-free (NEB #M0402 and NEB #M0439) enzymes at each template input. No amplification was observed in any of the no template control reactions.*

Cloned at NEB | RR Recombinant Enzyme | PCR PCR Enzyme | Hot Start/WarmStart | SAM Requires SAM | 37° Incubation Temperature | 65° Heat Inactivation

Induro® Reverse Transcriptase

#M0681S 4,000 units
#M0681L 10,000 units

Companion Products:

Oligo $d(T)_{23}$ VN
#S1327S 1 A260 units

Random Primer Mix
#S1330S 100 µl

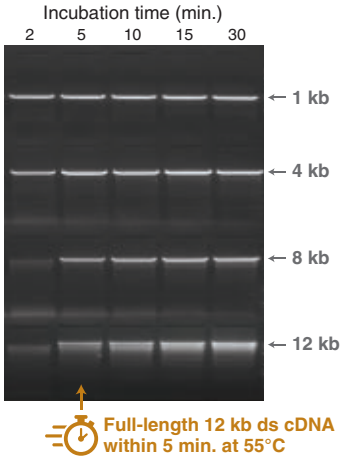
Deoxynucleotide (dNTP) Solution Mix
#N0447S 8 µmol
#N0447S 8 µmol

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314S 3,000 units

- *Rapidly generate high yields of long cDNA*
- *Strong inhibitor tolerance enables robust cDNA synthesis performance*
- *Support direct RNA sequencing and long read cDNA sequencing workflows*
- *Generate cDNA at higher temps, which is ideal for challenging sample types*
- *Experience comparable fidelity to retroviral RTs*

Description: Induro Reverse Transcriptase is a group II intron-encoded RT that exhibits high processivity, increased thermostability, and increased tolerance of inhibitors for the synthesis of cDNA from long transcripts (>8 kb), RNAs with strong secondary structures, and RNA samples with inhibitors. With improved 5´ sequencing coverage of long transcripts, Induro Reverse Transcriptase can enable RNA-seq applications such as direct RNA and long read cDNA sequencing workflows.

Concentration: 200,000 units/ml



Induro Reverse Transcriptase exhibits high processivity, permitting rapid cDNA synthesis. *Induro Reverse Transcriptase can synthesize a full-length 12 kb cDNA product within 5 min. at 55°C. In vitro transcribed poly(A) RNA templates (1 kb, 4 kb, 8 kb or 12 kb) were used to investigate full-length cDNA synthesis. After first-strand cDNA synthesis, RNA was hydrolyzed immediately by NaOH. Subsequently, an aliquot of the cDNA products was used to make full-length ds cDNA in the presence of a 5´ specific primer. Equal volume of ds cDNA was analyzed on an agarose gel.*

Template Switching RT Enzyme Mix

#M0466S 20 reactions
#M0466L 100 reactions

Companion Products:

Q5 Hot Start High-Fidelity 2X Master Mix
#M0494S 100 reactions
#M0494L 500 reactions
#M0494X 500 reactions

NEBNext High-Fidelity 2X PCR Master Mix
#M0541S 50 reactions
#M0541L 250 reactions

LongAmp Hot Start *Taq* 2X Master Mix
#M0533S 100 reactions
#M0533L 500 reactions

- *Incorporates a universal adaptor sequence at the 3´ end of cDNA during the RT reaction*
- *High sensitivity for cDNA amplification – enables transcriptome analysis by RNA-seq from single cells or as low as 2 pg of human total RNA*
- *Robust and simple workflow for 5´-Rapid Amplification of cDNA Ends (RACE)*
- *Retains the complete 5´ end of transcripts for 2nd Strand cDNA Synthesis*

Description: Template switching reverse transcription (RT) incorporates a universal adaptor sequence to the 3´ end of cDNA. This convenient feature can be utilized in several downstream applications:

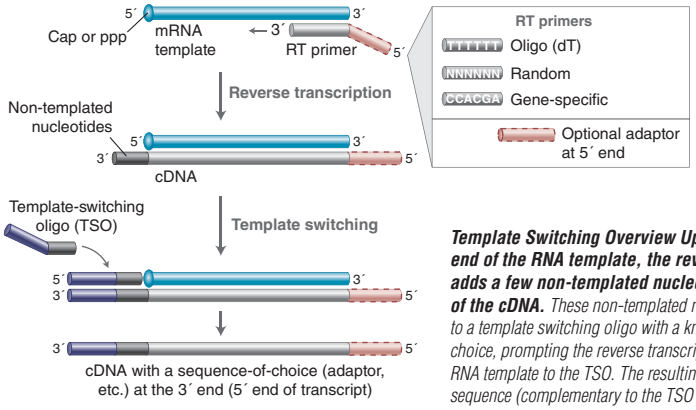
- cDNA synthesis and amplification in a one-tube reaction
- 5´ Rapid Amplification of cDNA Ends (RACE)
- 2nd strand cDNA synthesis that keeps the 5´ end of the transcripts intact

The Template Switching RT Enzyme Mix is optimized for efficient template switching during the RT reaction. The enzyme mix contains RNase Inhibitor in a specially formulated buffer, making reactions easy to setup with no additives needed. It is highly sensitive and specific and can generate RNA-seq libraries from as little as 2 pg of human total RNA or 5´-RACE from 10 ng of total RNA, both with minimal background.

Reagents Supplied:

- Template Switching RT Buffer

Concentration: 10 X



Template Switching Overview Upon reaching the 5´ end of the RNA template, the reverse transcriptase adds a few non-templated nucleotides to the 3´ end of the cDNA. *These non-templated nucleotides can anneal to a template switching oligo with a known sequence handle of choice, prompting the reverse transcriptase to switch from the RNA template to the TSO. The resulting cDNA contains a universal sequence (complementary to the TSO sequence) at the 3´ end.*

Watch our webinar on Induro Reverse Transcriptase.

Primers for cDNA Synthesis

Oligo d(N)n primers are used for the priming and sequencing of mRNA adjacent to the 3´-poly A tail or tailed cDNA. Note: #S1316 does not contain a 5´-phosphate.

Product	NEB #	Size
Random Primer 6	#S1230S	1 A_{260} units
Random Primer 9	#S1254S	1 A_{260} units
Oligo $d(T)_{23}$ VN	#S1327S	1 A_{260} units
Random Primer Mix	#S1330S	100 µl
Oligo $d(T)_{18}$ mRNA Primer	#S1316S	5 A_{260} units

ProtoScript® II First Strand cDNA Synthesis Kit

#E6560S 30 reactions
#E6560L 150 reactions

Companion Products:

Magnetic mRNA Isolation Kit
#S1550S 25 isolations

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

Random Primer Mix
#S1330S 100 µl

Oligo $d(T)_{23}$ VN
#S1327S 1 A260 units

Monarch Spin RNA Isolation Kit (Mini)
#T2110S 50 preps

- *Enzyme and reaction mixes add flexibility to reaction setup*
- *Suitable for any PCR format*
- *Efficient reverse transcription from different starting RNA amounts*
- *Generates cDNA at least 10 kb*

Description: ProtoScript II First Strand cDNA Synthesis Kit features two optimized mixes, ProtoScript II Enzyme Mix and ProtoScript II Reaction Mix. The enzyme mix combines ProtoScript II Reverse Transcriptase and Murine RNase Inhibitor, while the reaction mix contains dNTPs and an optimized buffer. ProtoScript II Reverse Transcriptase is a recombinant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. It can be used to synthesize first strand cDNA at higher temperatures than the wild type M-MuLV. The enzyme is active up to 50°C, providing higher specificity and higher yield of cDNA.

The kit also provides two optimized primers for reverse transcription and nuclease-free water. An anchored Oligo-dT primer [$d(T)_{23}$VN] forces the primer to anneal to the beginning of the poly(A) tail. The optimized Random Primer Mix provides random and consistent priming sites covering the entire RNA templates including both mRNAs and non-polyadenylated RNAs. The first strand cDNA product generated is up to 10 kb.

Kit Includes:

- ProtoScript II Reaction Mix
- ProtoScript II Enzyme Mix
- Random Primer Mix
- Nuclease-free Water
- Oligo $d(T)_{23}$ VN

For robust amplification of a wide range of DNA templates, we recommend One*Taq*® or Q5® High-Fidelity DNA Polymerases.

ProtoScript® First Strand cDNA Synthesis Kit

#E6300S 30 reactions
#E6300L 150 reactions

Companion Products:

Magnetic mRNA Isolation Kit
#S1550S 25 isolations

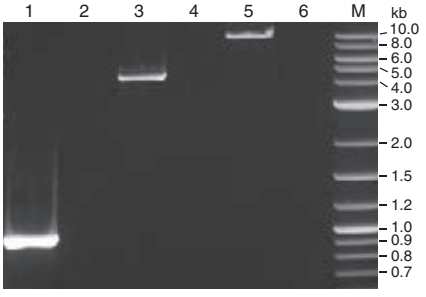
Monarch Spin RNA Isolation Kit (Mini)
#T2110S 50 preps

- *Enzyme and reaction mixes add flexibility to reaction setup*
- *Suitable for any PCR format*
- *Efficient reverse transcription from different starting RNA amounts*
- *Generates cDNA at least 5 kb*

Description: ProtoScript First Strand cDNA Synthesis Kit features two optimized mixes, ProtoScript Enzyme Mix and Protoscript Reaction Mix. Protoscript Enzyme Mix combines M-MuLV Reverse Transcriptase and RNase Inhibitor, Murine, while ProtoScript Reaction Mix contains dNTPs and an optimized buffer. The kit also contains two optimized primers for reverse transcription and nuclease-free water. An anchored oligo-dT primer [$d(T)_{23}$VN] forces the primer to anneal to the beginning of the polyA tail. The optimized Random Primer Mix provides random and consistent priming sites covering the entire RNA template including both mRNAs and non-polyadenylated RNAs. The first strand cDNA product generated is over 13.0 kb. This product was formerly known as M-MuLV First Strand cDNA Synthesis Kit.

Kit Includes:

- M-MuLV Enzyme Mix
- M-MuLV Reaction Mix
- Random Primer Mix
- Oligo $d(T)_{23}$ VN
- Nuclease-free Water



First Strand cDNA Synthesis with the ProtoScript Kit. *Reactions were performed at 42°C using 2 µg of human spleen total RNA. Negative control reactions (–RT) were carried out with 1X ProtoScript Reaction Mix. A fraction of the first strand cDNA product was used to amplify sequences specific for three different messenger RNAs using 1X LongAmp® Taq 2X Master Mix (NEB #M0287). Lane 1: 1.1 kb of beta-actin gene. Lane 2: –RT control of 1.1 kb of beta-actin gene. Lane 3: 4.7 kb of Xrn-1 gene. Lane 4: –RT control of 4.7 kb of Xrn-1 gene. Lane 5: 9.8 kb of guanine nucleotide exchange factor p532. Lane 6: -RT control of 9.8 kb of guanine nucleotide exchange factor p532. Marker M is 1 kb Plus DNA Ladder (NEB #N3200).*

RNA REAGENTS

Cloned at NEB | RR Recombinant Enzyme | PCR PCR Enzyme | Hot Start/WarmStart | SAM Requires SAM | 37° Incubation Temperature | 65° Heat Inactivation

RT-PCR & RT-qPCR Kits

Luna Universal One-Step RT-qPCR Kit
#E3005S 200 reactions
#E3005L 500 reactions
#E3005X 1,000 reactions
#E3005E 2,500 reactions

Luna Universal Probe One-Step RT-qPCR Kit
#E3006S 200 reactions
#E3006L 500 reactions
#E3006X 1,000 reactions
#E3006E 2,500 reactions

Luna Probe One-Step RT-qPCR Kit (No ROX)
#E3007E 2,500 reactions

Luna Cell Ready One-Step RT-qPCR Kit
#E3030S 100 reactions

Luna Cell Ready Probe One-Step RT-qPCR Kit
#E3031S 100 reactions

Luna Probe One-Step RT-qPCR 4X Mix with UDG
#M3019S 200 reactions
#M3019L 500 reactions
#M3019X 1,000 reactions
#M3019E 2,000 reactions

Luna Probe One-Step RT-qPCR 4X Mix with UDG (No ROX)
#M3029S 200 reactions
#M3029L 500 reactions
#M3029E 2,000 reactions

Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit
#E3019S 96 reactions

LunaScript Multiplex One-Step RT-PCR Kit
#E1555S 50 reactions
#E1555L 250 reactions

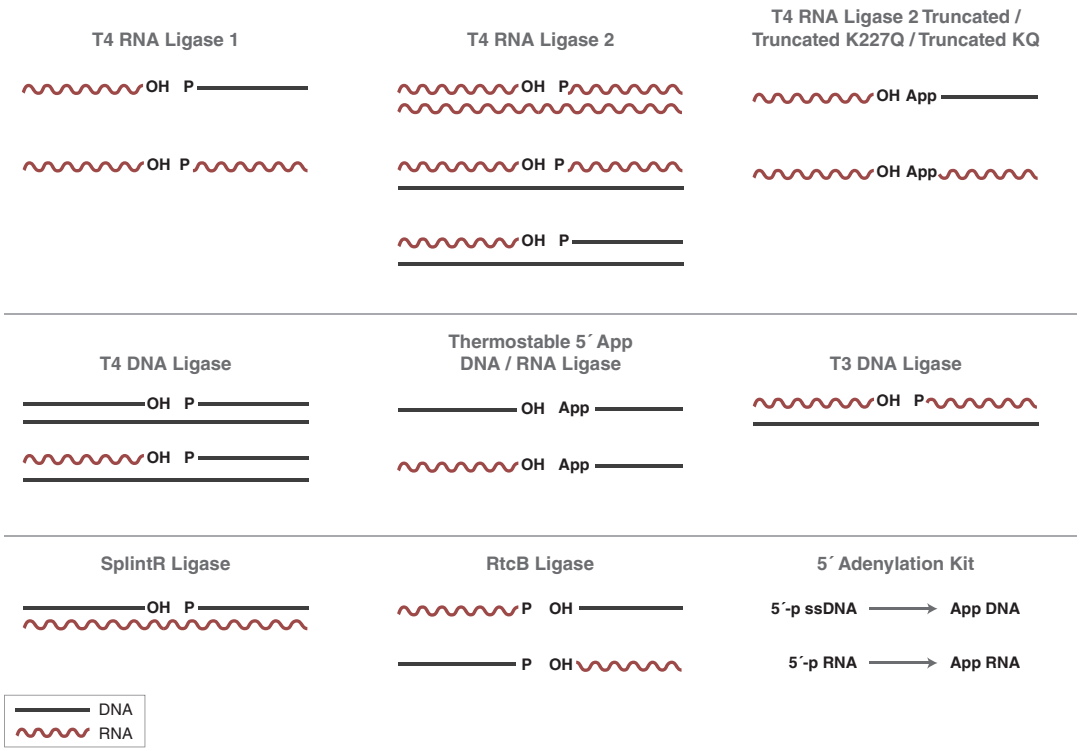
One*Taq* One-Step RT-PCR Kit
#E5315S 30 reactions

One*Taq* RT-PCR Kit
#E5310S 30 reactions

RNA Ligase Activity Chart

NEB offers a variety of ligases for DNA and RNA research. The chart below highlights reported activities of our T4 ligases. For more information, see the substrate-based ligase selection chart at www.neb.com.

Reported Activities and Applications for T4 Ligases



RNA Ligase Selection Chart

	T4 RNA Ligase 1	T4 RNA Ligase 2	T4 RNA Ligase 2 Truncated	T4 RNA Ligase 2, Truncated K227Q	T4 RNA Ligase 2, Truncated KQ	Thermostable 5' AppDNA/ RNA Ligase	5' Adenylation Kit	SplintR® Ligase	RtcB Ligase
RNA Applications									
Nicks in dsRNA		★★★							
Labeling of 3´ termini of RNA	★★★		★	★	★	★			
Ligation of ssRNA to ssRNA	★★★								
Ligation of preadenylated adaptors to RNA*	★★		★★	★★	★★★	★★			
5´ Adenylation							★★★		
Ligation of 3'P and 5'OH of ssRNA									★★★
DNA Applications									
Ligation of preadenylated adaptors to ssDNA						★★★			
DNA/RNA Applications									
Joining of RNA & DNA in a ds-structure		★★							
ssDNA Ligation with RNA splint								★★★	
Ligation of RNA and DNA with 3'P and 5'OH									★★
NGS Applications									
NGS Library Prep ssRNA-ssDNA (ligation)	▲		▲	▲	▲				
NGS Library Prep ssRNA-ds-Adaptor splinted ligation		▲							
Features									
Thermostable						●	●		
Recombinant	●	●	●	●	●	●	●	●	●

* Do not add ATP

KEY			
★★★	★★	★	▲
Optimal, recommended ligase for selected application	Works well for selected application	Will perform selected application, but is not recommended	Please consult the specific NGS protocol to determine the optimal enzyme for your needs

Cloned at NEB | Recombinant Enzyme | PCR Enzyme | Hot Start/WarmStart | Requires SAM | Incubation Temperature | Heat Inactivation

T4 RNA Ligase 1 (ssRNA Ligase)

T4 RNA Ligase 1 (ssRNA Ligase)
#M0204S 1,000 units
#M0204L 5,000 units

T4 RNA Ligase 1 (ssRNA Ligase), High Concentration
#M0437M 5,000 units

Companion Products:

Adenosine 5'-Triphosphate (ATP)
#P0756S 1 ml
#P0756L 5 ml

Universal miRNA Cloning Linker
#S1315S 5 µg

- *Ligation of ss-RNA and DNA*
- *Labeling of 3´-termini of RNA with 5´-[^{32}P] pCp*
- *Inter- and intramolecular joining of RNA and DNA molecules*

Description: Catalyzes ligation of a 5´ phosphorylterminated nucleic acid donor to a 3´ hydroxyl-terminated nucleic acid acceptor through the formation of a 3´→ 5´ phosphodiester bond with hydrolysis of ATP to AMP and PPi. Substrates include single-stranded RNA and DNA as well as dinucleoside pyrophosphates.

Source: An *E. coli* strain that carries the T4 RNA Ligase 1 gene

Reaction Conditions: 1X T4 RNA Ligase Reaction Buffer, 25°C. Supplement with 1 mM ATP (included). Heat Inactivation: 65°C for 15 minutes.

Notes on Use: Addition of DMSO to 10% (v/v) is required for pCp ligation.

Reagents Supplied:

10X T4 RNA Ligase Reaction Buffer

10 mM ATP (with NEB #M0204) or 100 mM ATP (with NEB #M0437)

50% PEG 8000

Unit Definition: One unit is defined as the amount of enzyme required to convert 1 nmol of 5´-[^{32}P] rA16 into a phosphate resistant form in 30 minutes at 37°C.

Concentration: 10,000 or 30,000 units/ml

T4 RNA Ligase 2 (dsRNA Ligase)

#M0239S 150 units
#M0239L 750 units

- *Cohesive-end adaptor ligation*
- *Best choice for ligating nicks in dsRNA*
- *Suitable for ligating 3´ OH of RNA to 5´ phosphate of DNA in a DNA/RNA hybrid*

Description: T4 RNA Ligase 2, also known as T4 Rnl2 (gp24.1), has both intermolecular and intramolecular RNA strand-joining activity. Unlike T4 RNA Ligase 1 (NEB #M0204), T4 RNA Ligase 2 is much more active joining nicks on double stranded RNA than on joining the ends of single stranded RNA. The enzyme requires an adjacent 5´ phosphate and 3´ OH for ligation. The enzyme can also ligate the 3´ OH of RNA to the 5´ phosphate of DNA in a double stranded structure.

Source: An *E. coli* strain that carries the T4 RNA Ligase 2 gene.

Reaction Conditions: T4 Rnl2 Reaction Buffer, 37°C. Heat inactivation: 80°C for 5 minutes.

Reagents Supplied:

• T4 Rnl2 Reaction Buffer

Unit Definition: One unit is defined as the amount of enzyme required to ligate 0.4 µg of an equimolar mix of a 23-mer and 17-mer RNAs in a total reaction volume of 20 µl in 30 minutes at 37°C.

Concentration: 10,000 units/ml

T4 RNA Ligase 2, truncated

#M0242S 2,000 units
#M0242L 10,000 units

Companion Product:

Universal miRNA Cloning Linker
#S1315S 5 µg

- *Ligate a pre-adenylated DNA or RNA sequence tag to any RNA 3´ end*
- *Join a single-stranded adenylated primer to small RNAs for cDNA library creation*

Description: T4 RNA Ligase 2, truncated (T4 Rnl2tr) specifically ligates the pre-adenylated 5´ end of DNA or RNA to the 3´ end of RNA. The enzyme does not require ATP, but does need the pre-adenylated substrate. T4 Rnl2tr is expressed from a plasmid in *E. coli* which encodes the first 249 amino acids of the full-length T4 RNA Ligase 2. Unlike the full-length ligase, T4 Rnl2tr cannot ligate the phosphorylated 5´ end of RNA or DNA to the 3´ end of RNA. This enzyme, also known as Rnl2 (1–249), has been used for optimized linker ligation for the cloning of microRNAs. This enzyme reduces background ligation, because it can only use pre-adenylated linkers.

Source: An *E. coli* strain that carries the cloned truncated T4 RNA Ligase 2 gene.

Reaction Conditions: T4 RNA Ligase Reaction Buffer , 25°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied:

• T4 RNA Ligase Reaction Buffer

• PEG 8000

Unit Definition:

200 units is defined as the amount of enzyme required to give 80% ligation of a 31-mer RNA to the pre-adenylated end of a 17-mer DNA Universal miRNA Cloning Linker (#S1315) in a total reaction volume of 20 µl in 1 hour at 25°C.

5´-FAM-rArGrUrCrGrUrArGrCrCrUrUrUrArUrCrCrGrArGrArUrUrCrArGrCrArArUrA-3´

5´-rAppCTGTAGGCACCATCAAT–NH2-3´

Molarity = 14 µM

Concentration: 200,000 units/ml

T4 RNA Ligase 2, truncated K227Q and truncated KQ

T4 RNA Ligase 2, truncated K227Q
#M0351S 2,000 units
#M0351L 10,000 units

T4 RNA Ligase 2, truncated KQ
#M0373S 2,000 units
#M0373L 10,000 units

Companion Product:

Universal miRNA Cloning Linker
#S1315S 5 µg

- *Ligate a pre-adenylated DNA or RNA sequence tag to any RNA 3´ end*
- *Join a single stranded adenylated primer to small RNAs for cDNA library creation*

Description: T4 RNA Ligase 2, K227Q and truncated KQ (T4 Rnl2tr KQ) specifically ligate the pre-adenylated 5´ end of DNA or RNA to the 3´ OH end of RNA. The enzymes do not use ATP for ligation, but requires preadenylated linkers.

Mutation of K227 in T4 RNA Ligase 2 reduces enzyme lysyl adenylation. K227Q reduces the formation of undesired ligation products (concatemers and circles) by T4 Rnl2tr. It does so by reducing the trace activity of T4 Rnl2tr in transfer of adenylyl groups from linkers to the 5´-phosphates of input RNAs. T4 Rnl2tr KQ is a double-point mutant of T4 RNA Ligase 2, truncated (NEB #M0242). Mutation of R55K in T4 Rnl2tr K227Q increases the ligation activity of the enzyme to levels similar to T4 Rnl2tr.

The exclusion of ATP, use of pre-adenylated linkers, and the reduced enzyme lysyl adenylation activity provide the lowest possible background in ligation reactions.These enzymes have been used for optimized linker ligation for high-throughput sequencing library construction of small RNAs.

Source: Expressed as an MBP fusion from a plasmid in *E. coli* which encodes the first 249 amino acids of the full length T4 RNA Ligase 2. T4 Rnl2tr K227Q has a lysine to glutamine mutation at position 227. T4 Rnl2tr KQ has an arginine to lysine and lysine to glutamine mutation at positions 55 and 227, respectively.

Reaction Conditions: 1X T4 RNA Ligase Reaction Buffer, 25°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied:

- 10X T4 RNA Ligase Reaction Buffer
- 50% PEG 8000

Unit Definition: 200 units is defined as the amount of enzyme required to give 80% ligation of a 31-mer RNA to the pre-adenylated end of a 17-mer DNA [Universal miRNA Cloning Linker (NEB #S1315)] in a total reaction volume of 20 µl in 1 hour at 25°C.

Concentration: 200,000 units/ml

RtcB Ligase

#M0458S 25 reactions

- *Ligate ssRNA or ssDNA with a 3´-phosphate or a 2´,3´-cyclic phosphate to the 5´-OH of ssRNA*
- *Circularization of ssRNA with compatible ends*

Description: RtcB Ligase from *E. coli* joins single stranded RNA with a 3´-phosphate or 2´,3´-cyclic phosphate to another RNA with a 5´ -hydroxyl. Ligation requires both GTP and $MnCl_2$ and proceeds through a 3´-guanylate intermediate. With substrates having a 2´,3´-cyclic phosphate end, hydrolysis to a 3´-phosphate precedes 3´ end activation with GMP and ligation.

Source: RtcB Ligase is expressed as a His-tagged fusion in *E. coli*.

Reaction Conditions: RtcB Reaction Buffer, 37°C. Supplement with 0.1 mM GTP and 1 mM $MnCl_2$.

Reagents Supplied:

- RtcB Reaction Buffer
- $MnCl_2$
- GTP

Concentration: 15 µM

This is an **Enzyme for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

Thermostable 5´ App DNA/RNA Ligase

#M0319S 10 reactions
#M0319L 50 reactions

Companion Product:

Universal miRNA Cloning Linker
#S1315S 5 µg

- *Ligation of ssDNA to an adenylated DNA linker for NGS library construction*
- *Ligation of an adenylated DNA linker to RNA at elevated temperatures for small RNA NGS library construction*

Description: Thermostable 5´ App DNA/RNA Ligase is a point mutant of catalytic lysine of RNA ligase from *Methanobacterium thermoautotrophicum*. This enzyme is ATP independent. It requires a 5´ pre-adenylated linker for ligation to the 3´-OH end of either RNA or single stranded DNA (ssDNA). The enzyme is also active in ligation of RNA with 2´-*O*-methylated 3´ end to 5´-adenylated linkers. The optimal temperature for ligation reaction is 60–65°C. The mutant ligase is unable to adenylate the 5´-phosphate of RNA or ssDNA, which reduces the formation of undesired ligation products (concatemers and circles).

The ability of the ligase to function at 65°C might reduce the constraints of RNA secondary structure in RNA ligation experiments.

Source: Thermostable 5´ App DNA/RNA Ligase is expressed as His-tag fusion in *E. coli*.

Reaction Conditions: NEBuffer 1, 65°C.

Reagents Supplied:

- NEBuffer 1
- $MnCl_2$

Concentration: 20 µM

Note: For optimal ligation of ssDNA to adenylated DNA linkers, we recommend using NEBuffer 1 supplemented with manganese. For ligation of ssRNA to adenylated DNA linkers, just use NEBuffer 1.

5´ DNA Adenylation Kit

#E2610S 10 reactions
#E2610L 50 reactions

- *Enzymatic 5´ adenylation of ssDNA linkers for next gen sequencing*
- *One-step reaction gives quantitative adenylation*
- *Simpler than existing chemical and enzymatic methods*
- *Reduces need for purification of reaction product*

Description: The 5´ DNA Adenylation Kit is a simple and efficient enzymatic method for generating 5´-adenylated DNA oligonucleotides using *Mth* RNA ligase, ATP and single stranded 5´-phosphorylated DNA. The kit is optimized to produce the adenylated DNA intermediate with or without 3´ terminator. The 5´ DNA adenylation kit routinely generates greater than 95% conversion of pDNA to AppDNA. This highly efficient process eliminates the need for gel isolation of the product and increases overall yield.

Kit Includes:

- Mth RNA Ligase
- 5' DNA Adenylation Reaction Buffer
- Adenosine 5' Triphosphate

Note: The low turnover of the enzyme requires an approximately equimolar concentration of the enzyme and the oligonucleotide substrate. Adenylated DNA linkers can be used for 3´ end ligation of RNA in cDNA library preparation for Next Generation sequencing protocols.

SplintR® Ligase

#M0375S 1,250 units
#M0375L 6,250 units

- *Ligation of adjacent, single-stranded DNA splinted by a complimentary RNA*
- *Characterization of miRNAs and mRNAs, including SNPs*

Description: SplintR Ligase, also known as PBCV-1 DNA Ligase or *Chlorella* virus DNA Ligase, efficiently catalyzes the ligation of adjacent, single-stranded DNA oligonucleotides splinted by a complementary RNA strand. This previously unreported activity may enable novel approaches for characterization of miRNAs and mRNAs, including splice variant detection and single-nucleotide polymorphism (SNPs). SplintR is ideally suited for many target enrichment workflows with applications in next-generation sequencing and molecular diagnostics. The robust activity of the enzyme and its affinity for RNA-splinted DNA substrates (apparent Km = 1 nM) enable sub-nanomolar detection of unique RNA species within a complex mixture, making SplintR ligase a superior choice for demanding RNA detection technologies.

Source: An *E. coli* strain that carries a recombinant gene encoding PBCV-1 DNA Ligase.

Reaction Conditions: SplintR Ligase Reaction Buffer, 25°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied:

- SplintR Ligase Reaction Buffer

Unit Definition: One unit is defined as the amount of enzyme needed to ligate (to 50% completion) 2 picomoles of a tripartite FAM-labeled DNA:RNA hybrid substrate in a 20 µl reaction at 25°C in 15 minutes in 1X SplintR Ligase Reaction Buffer.

Concentration: 25,000 units/ml

RNA 5' Pyrophosphohydrolase (RppH)

#M0356S 200 units

- *Conversion of 5´-triphosphate RNA to monophosphate RNA*
- *Preparation of 5´-phosphate RNA for ligation*
- *Characterization of RNA 5´ ends*

Description: The bacterial RNA 5´ Pyrophosphohydrolase (RppH) removes pyrophosphate from the 5´ end of triphosphorylated RNA to leave a 5´ monophosphate RNA. The RppH protein was also known as NudH/YgdP which can split diadenosine penta-phosphate to ADP and ATP.

Source: An *E. coli* strain containing a clone of the *E. coli* RppH gene.

Reaction Conditions: NEBuffer 2, 37°C.

Reagents Supplied:

- NEBuffer 2

Unit Definition: One unit is the amount of enzyme that converts 1 µg 300 mer RNA transcript into a XRN-1 digestible RNA in 30 minutes at 37°C.

Concentration: 5,000 units/ml

5´ Deadenylase

NEB1 RR 30° 70°

#M0331S 2,500 units

- *Deadenylation of 5´ end of DNA and RNA*
- *Aprataxin-dependent DNA repair assay*
- *Analysis of dinucleoside tetraphosphate*

Description: Yeast 5´ Deadenylase is a member of the HIT (histidine triad) family of proteins and specifically a member of the Hint branch. It is the yeast orthologue of aprataxin. Mutations in human aprataxin have been known to be involved in the neurological disorder known as ataxia oculomotor apraxia-1. The human protein has been shown to resolve abortive ligation intermediates by removing the AMP at the 5´ end of DNA (AMP-DNA hydrolase activity). It also repairs DNA damage at 3´ ends by removing 3´-phosphate and 3´-phosphoglycolate. Human aprataxin acts on small molecules, such as nucleotide polyphosphates diadenosine tetraphosphate (AppppA) and lysyl-AMP.

The 5´ Deadenylase is encoded by the *HNT3* gene of *S. cerevisiae*. NEB has shown this protein is capable of deadenylation from 5´ end of DNA and RNA, leaving the phosphate at 5´ end. It also cleaves AppppA into ATP and AMP. Its activity on lysyl-AMP is not detectable.

Source: Purified from an *E. coli* strain carrying a plasmid encoding 5´ Deadenylase from S. *cerevisiae*.

Reaction Conditions: NEBuffer 1, 30°C. Heat inactivation: 70°C for 20 minutes.

Reagents Supplied:

• NEBuffer 1

Unit Definition: One unit is defined as the amount enzyme required to remove 10 pmoles of AMP from a 5'-adenylated DNA oligo in 10 minutes at 30°C.

Concentration: 50,000 units/ml

NEW

RNase 4

NEB r1.1 RR 37° No

#M1284S 2,500 units
#M1284L 12,500 units

Companion Products:

RNase Inhibitor, Human Placenta
#M0307S 2,000 units
#M0307L 10,000 units

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

- *Generate a larger population of uniquely mappable oligonucleotides for improved RNA sequence characterization by LC-MS/MS*
- *Endoribonuclease activity tolerates common RNA chemical modifications*
- *Enhance analysis of mRNA 5´ cap identity and distribution*
- *Reaction can be stopped with addition of RNase Inhibitor, Murine (NEB #M0314) or Human Placenta (NEB #M0307)*

Description: RNase 4 is a single-stranded RNA endonuclease that cleaves 3´ of uridine in uridine-purine sequences (cut sites: U/A and U/G). RNase 4 allows more targeted digestion of substrate RNA compared to single-nucleotide specific RNases like T1 (cut site: after G), RNase U2 (cut sites: after A and G), or bovine pancreatic RNase A (cut sites: after C and U). RNase 4 endonuclease activity tolerates uridine base modifications such as pseudo-, N1-methyl-pseudo-, dihydro-, and 5-methoxy-uridine species (, m1 , D, and mo5U). Due to the chemical mechanism of RNase

RNase 4 cuts at U/A and U/G.

4 endonucleolytic cleavage, product oligonucleotides contain heterogenous 3´ ends, where most species contain a linear 3´-phosphate or cyclic 2´,3´-phosphate.

Source: An *Escherichia coli* strain that carries the cloned RNASE4 gene from *Homo sapiens*, with an N-terminal 6xHis tag.

Reaction Conditions: NEBuffer r1.1, 37°C.

Reagents Supplied:

• NEBuffer r1.1

Concentration: 50,000 units/ml

NEW

RNase 4 Digestion and 3´ End Repair Mix

NEB r1.1 37°

#M1288S 50 reactions
#M1288L 250 reactions

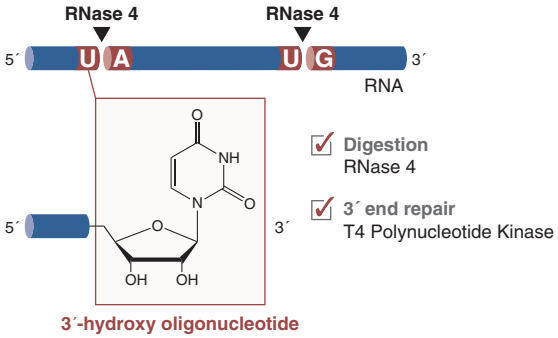
Companion Products:

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

RNase Inhibitor, Human Placenta
#M0307S 2,000 units
#M0307L 10,000 units

- *Convenient enzyme co-formulation to produce RNase 4 digests with homogeneous 3´-OH terminus*
- *Reduce data complexity and increase RNA mapping sensitivity*
- *For mRNA 5´ cap analysis, use stand-alone RNase 4 with our protocol for DNA Probe-Directed Analysis of mRNA 5´Cap Structures*
- *Consistent results across mRNA sequences and enzyme dilutions*

Description: RNase 4 is a single-stranded RNA endonuclease that cleaves 3´ of uridine in uridine-purine sequences (cut sites: U/A and U/G). Digestion of RNA with RNase 4 produces U-ending RNA oligonucleotides that contain cyclic 2´, 3´-phosphate and/or linear 3´-phosphate termini. RNase 4 Digestion and 3´ End Repair Mix is a coformulation of RNase 4 and the 3´ end repair activity of T4 Polynucleotide Kinase to produce a pool of U-ending RNA oligonucleotides with a 3´-hydroxy terminus.



3´-hydroxy oligonucleotide

The additional 3´ end repair of RNase 4 digestion products simplifies and improves RNA sequencing coverage analysis and modification mapping by liquid chromatography-mass spectrometry (LC-MS/MS). RNase 4 endoribonuclease activity tolerates uridine base modifications such as pseudo-, N1-methyl-pseudo-, dihydro-, and 5-methoxy-uridine species (, m1 , D, and mo5U).

Reaction Conditions: NEBuffer r1.1, 37°C.

RNase I_f

NEB 3 RR 37° 70°

#M0243S 5,000 units
#M0243L 25,000 units

- *Eliminates RNA from DNA and protein preparations*
- *Degradation of single-stranded RNA to mono-, di- and trinucleotides*
- *Used in ribonuclease protection assays*

Description: Ribonuclease I_f (RNase I_f) is an RNA endonuclease which will cleave at all RNA dinucleotide bonds leaving a 5´ hydroxyl and 2´, 3´ cyclic monophosphate. It has a preference for single-stranded RNA over double-stranded RNA. RNase I_f is a recombinant protein fusion of RNase I (from *E. coli*) and maltose-binding protein. It has identical activity to RNase I.

Source: An *E. coli* strain containing a genetic fusion of the *E. coli* RNase I gene (2nd) and the gene coding for maltose binding protein (MBP).

Reaction Conditions: NEBuffer 3, 37°C. Heat inactivation: 70°C for 20 minutes.

Reagents Supplied:

• NEBuffer 3

Unit Definition: One unit is defined as the amount of enzyme required to fully digest 1 picomole of synthetic ssRNA 33-mer in a total reaction volume of 10 µl in 15 minutes in 1X NEBuffer 3 as visualized on a 20% acrylamide gel.

Concentration: 50,000 units/ml

Note: RNase I_f will not degrade DNA. It has a preference for single-stranded RNA over double-stranded RNA.

RNase H

NEB U RR 37° 65°

#M0297S 250 units
#M0297L 1,250 units

Description: Ribonuclease H (RNase H) is an endoribonuclease which specifically hydrolyzes the phosphodiester bonds of RNA which is hybridized to DNA. This enzyme does not digest single- or double-stranded DNA.

Source: An *E. coli* strain that carries the cloned RNase H gene (rnh) from *Escherichia coli*.

Reaction Conditions: RNase H Reaction Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied:

• RNase H Reaction Buffer

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of ribonucleotides from 20 picomoles of a fluorescently labelled 50 base pair RNA-DNA hybrid in a total reaction volume of 50 µl in 20 minutes at 37°C.

Concentration: 5,000 units/ml

Thermostable RNase H

#M0523S 250 units

- *Higher stringency RNA structure mapping and site-specific RNA cleavage*
- *Removal of poly(A) tails from mRNA hybridized to oligo(dT)*
- *Removal of mRNA during second strand cDNA synthesis*
- *Component of isothermal amplification methods*

Description: Thermostable RNase H specifically recognizes and cleaves the phosphodiester bonds of an RNA strand in an RNA-DNA hybrid while leaving the DNA strand intact. This thermostable nuclease exhibits the same enzymatic properties as *E. coli* RNase H, but is active at much higher temperatures.

Source: An *E. coli* strain carrying a codon optimized plasmid encoding RNase H from the extreme thermophile *Thermus thermophilus*.

Reaction Conditions: RNase H Reaction Buffer, ≥50°C.

Reagents Supplied:

• RNase H Reaction Buffer

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of ribonucleotides from 40 pmol of a fluorescently labeled 25 base pair RNA:DNA hybrid in a total reaction volume of 50 µl in 20 minutes at 50°C.

Concentration: 5,000 units/ml

RNase HII

#M0288S 250 units
#M0288L 1,250 units

- *Nicking of products generated with a polymerase that will incorporate ribonucleotides*
- *Generation of a double-stranded break at the site of an incorporated ribonucleotide when used with T7 Endo I*
- *Degradation of the RNA portion of Okazaki fragments or other RNA-DNA hybrids*

Description: Ribonuclease HII (RNase HII) is an endoribonuclease that preferentially nicks 5´ to a ribonucleotide within the context of a DNA duplex. The enzyme leaves 5´ phosphate and 3´ hydroxyl ends. RNase HII will also nick at multiple sites along the RNA portion of an Okazaki fragment.

Source: An *E. coli* strain containing a genetic fusion of the RNase HII gene (rnhB) from *E. coli* and the gene coding for maltose binding protein (MBP). Following affinity chromatography, RNase HII is cleaved from the fusion construct by Factor Xa and then purified away from both MBP and Factor Xa. RNase HII cleaved from MBP has four additional amino acids at its N-terminus (Ile-Ser-Glu-Phe).

Reaction Conditions: ThermoPol Reaction Buffer, 37°C.

Reagents Supplied:

• ThermoPol Reaction Buffer

Unit Definition: One unit is defined as the amount of enzyme required to yield a fluorescence signal consistent with the nicking of 100 picomol of synthetic double-stranded DNA substrate containing a single ribonucleotide near the quencher of a fluorophore/quencher pair in 30 minutes at 37°C in 1X ThermoPol Buffer.

Concentration: 5,000 units/ml

Note: Incubation with 0.1% SDS is sufficient to inactivate RNase HII.

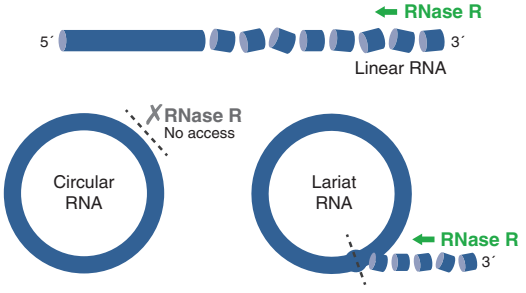
RNase R

#M0100S 400 units

- *Highly processive 3´ to 5´ exoribonuclease*
- *Digests nearly all linear RNAs with an accessible 3´ end, including rRNA and mRNA*
- *Enriches for circular RNAs and lariat RNAs from total RNA preps*
- *Requires magnesium for activity*
- *Inactivated with the addition of excess EDTA*

Description: Ribonuclease R (RNase R) is a processive 3' to 5' exoribonuclease. RNase R requires a 3' single-stranded RNA substrate that is approximately 10 nucleotides or longer, such as a poly (A) tail, for proper binding. RNase R is unique from other exoribonucleases for its ability to degrade highly structured RNAs without the need for an additional helicase. After RNase R digestion, circular RNAs and lariat RNAs are enriched, as these closed RNA structures are resistant to exoribonucleases.

Unit Definition: One unit is defined as the amount of enzyme required to convert 75 pmoles of 20-nucleotide single-stranded RNA sequence downstream of a 38-nucleotide DNA hairpin into acid soluble ribonucleotides in a total reaction volume of 20 µl in 15 minutes at 25°C.



RNase R digests linear RNA with an accessible 3´ end and can be used to enrich for circular and lariat RNA. *RNase R can be used to digest linear RNAs. Circular RNAs are closed RNA molecules that are resistant to RNase R digestion. Lariat RNAs have a looped structure with a single-stranded RNA region at the 3´ end. RNase R can digest the linear RNA at the 3´ end, but stops at the branch point of the lariat, preserving the looped RNA.*

ShortCut® RNase III

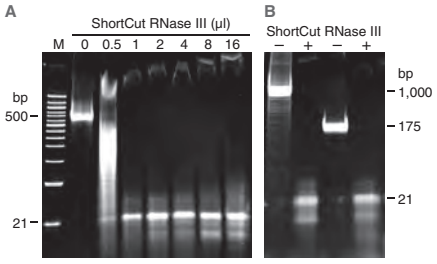
#M0245S 200 units
#M0245L 1,000 units

- *Generates siRNAs for RNA interference studies*
- *Removal of long dsRNAs*

Description: ShortCut RNase III converts long double-stranded RNA into a heterogeneous mix of short (18–25 bp) interfering RNAs (siRNA) suitable for RNA interference in mammalian cells.

Source: An *E. coli* strain containing a genetic fusion of the *E. coli* RNase III gene (rnc) and the gene coding for maltose binding protein (MBP).

Reaction Conditions: ShortCut Reaction Buffer, 37°C.



siRNA production by ShortCut RNase III. *A. Varying amounts of ShortCut RNase III were incubated with 2 µg of a 500 bp dsRNA for 20 minutes. B. dsRNA fragments (1 kb and 175 bp) were digested with ShortCut RNase III. Digests were analyzed by 20% TBE polyacrylamide gel electrophoresis.*

Reagents Supplied:

- ShortCut Reaction Buffer
- 10X EDTA
- $MnCl_2$
- Glycogen RNase-Free

Unit Definition: One unit is the amount of enzyme required to digest 1 µg of dsRNA to siRNA in 20 minutes at 37°C in a total reaction volume of 50 µl.

Concentration: 2,000 units/ml

Phosphorylation and Dephosphorylation

Quick CIP
#M0525S 1,000 units
#M0525L 5,000 units

Antarctic Phosphatase
#M0289S 1,000 units
#M0289L 5,000 units

Shrimp Alkaline Phosphatase (rSAP)
#M0371S 500 units
#M0371L 2,500 units

T4 Polynucleotide Kinase
#M0201S 500 units
#M0201L 2,500 units

NEB offers a selection of products for phosphorylation and dephosphorylation of DNA and RNA. Full product details can be found in the DNA Modifying Enzymes & Cloning Technologies chapter, or at www.neb.com.

FTO RNA Demethylase

#M0616S 20 µg

- *Demethylation of N6-methyladenosine (m6A) on RNA to form adenosine*

Description: FTO RNA Demethylase is a homolog of the Fe(II)/alpha-ketoglutarate (αKG)-dependent AlkB dioxygenases and is an N6-methyladenosine (m6A) demethylase of eukaryotic mRNA. *In vivo*, FTO demethylates internal m6A and cap N6, 2´-O-dimethyladenosine (m6AM) in mRNA and snRNA. FTO also mediates N1-methyladenosine (m1A) demethylation in tRNA. *In vitro*, FTO demethylates m6A, m6Am and m3U in ssRNA, as well as N6-methyl-deoxyadenosine (6mA) and 3-methylthymidine (3mT) on ssDNA.

Unit Definition: A 25 µl reaction in FTO Reaction Buffer containing 100 ng of a 1700-mer N6A methylated Cluc mRNA and 0.5 ug of FTO RNA Demethylase incubated for 1h at 37 °C results in >50% demethylation of the substrate mRNA as determined by LCMS.

XRN-1

NEB3 RR 37°

#M0338S 20 units
#M0338L 100 units

- *Removal of RNA containing 5´ monophosphate from an RNA mixture*

Description: XRN-1 is a highly processive 5´ to 3´ exoribonuclease, requiring a 5´ monophosphate. It also acts on 5´ monophosphate ssDNA with reduced efficiency.

Source: Purified from *E. coli* carrying a plasmid overexpressing the yeast XRN-1 gene (1).

Reaction Conditions: NEBuffer 3, 37°C.

Reagents Supplied:

• NEBuffer 3

Unit Definition: One unit is defined as the amount of enzyme that digests 1 µg of phosphorylated yeast RNA in 60 minutes at 37°C.

Concentration: 1,000 units/ml

Exonuclease T

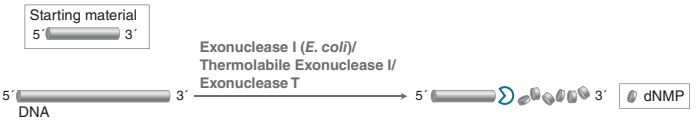
NEB4 RR 25° 65°

#M0265S 250 units
#M0265L 1,250 units

- *Generate blunt ends in DNA or RNA with 3´ overhangs*

Description: Exonuclease T (Exo T), also known as RNase T, is a single-stranded RNA or DNA specific nuclease that requires a free 3´ terminus and removes nucleotides in the 3´→ 5´ direction. Exo T can be used to generate blunt ends from RNA or DNA having 3´ extensions.

Source: Exonuclease T is overexpressed and purified as a C-terminal fusion to maltose-binding protein (MBP). MBP is removed from Exonuclease T by Factor Xa cleavage and Exonuclease T is then purified away from Factor Xa and MBP. Exonuclease T cleaved from MBP has an additional amino acid on the N-terminus and a Phe instead of a Met (i.e., Glu-Phe-Exo T instead of Met-Exo T).



Reaction Conditions: NEBuffer 4, 25°C.
Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to release 1 nmol of single dT nucleotides from 50 pmol of Fam-labeled polythymidine substrate in 30 minutes at 25°C.

Concentration: 5,000 units/ml

Note: Exonuclease T has a different activity on RNA vs. DNA. For RNA, 1 unit of Exonuclease T is required to completely digest 1.0 pmol of rA20 under standard reaction conditions as measured by gel electrophoresis.

Nucleoside Digestion Mix

NEBU 37°

#M0649S 50 reactions

- *Convenient one-step protocol*
- *Digests both DNA and RNA to single nucleosides*
- *Low-glycerol formulation significantly reduces glycerol-induced ion suppression during MS analysis*

Description: The Nucleoside Digestion Mix is a mixture of enzymes that provides a convenient one-step method to generate single nucleosides from DNA or RNA. Optimized for quantitative analysis by liquid chromatography-mass spectrometry (LC-MS), this reagent eliminates the need for sequential multi-step, time-consuming digestion protocols. The Nucleoside Digestion Mix digests ssDNA, dsDNA, DNA/RNA hybrids and RNA (except mRNA cap structures) containing epigenetically modified (m5C, hm5C, f5C, ca5C, m4C, m6A, etc.), unnatural, or damaged bases. Moreover, the low-glycerol formulation (< 1%) significantly reduces glycerol-induced ion suppression during mass spectrometry analysis.

Reaction Conditions: Nucleoside Digestion Mix Reaction Buffer, 37°C

Cloned at NEB | RR Recombinant Enzyme | PCR PCR Enzyme | Hot Start/WarmStart | SAM Requires SAM | 37° Incubation Temperature | 65° Heat Inactivation

DNase I (RNase-Free)

DNase I-XT

DNase I (RNase-free)
#M0303S 1,000 units
#M0303L 5,000 units

DNase I-XT
#M0570S 1,000 units
#M0570L 5,000 units

- *Removal of contaminating genomic DNA from RNA samples*
- *Degradation of DNA templates in transcription reactions*

GMP-grade reagent now available (DNase I (RNase-free) only).
See page 6 for details.

Description: DNase I is a DNA-specific endonuclease that degrades ds- and ss-DNA to release short oligos with 5´ phosphorylated and 3´-hydroxylated ends.

DNase I-XT is a salt tolerant enzyme that exhibits optimal activity between 50-100 mM salt and retains 65% and ~40% activity in 200 and 300 mM salt, respectively. Increased salt tolerance makes it ideal for DNA template removal from an *in vitro* transcription (IVT) reaction.

Both enzymes are RNase-free, allowing for complete removal of DNA from RNA preps, while maintaining RNA integrity.

Reaction Conditions: 1X DNase I Reaction Buffer, 37°C. DNase I can be heat inactivated at 75°C for 10 minutes, while DNase I-XT cannot.

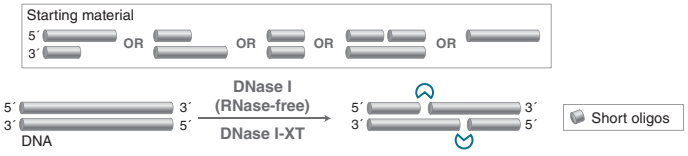
Reagents Supplied:

- DNase I Reaction Buffer (NEB #M0303)
- DNase I-XT Reaction Buffer (NEB #M0570)

Unit Definition: DNase I – One unit is defined as the amount of enzyme which will completely degrade 1 µg of pBR322 DNA in a total reaction volume of 50 µl in 10 minutes at 37°C. DNase I-XT – One unit is defined as the amount of enzyme required to release 210 pmol of FAM from a 35-mer FAM-BHQ1 labeled hairpin oligonucleotide in 1 minute at 30°C in a 50 µl reaction volume.

Concentration: 2,000 units/ml

Notes: DNase I-XT is supplied with an optimized reaction buffer. Use with supplied buffer, and not DNase I Reaction Buffer. Likewise, due to the sub-optimal salt concentration, do not use DNase I-XT Buffer with DNase I. When using DNase I, EDTA should be added to a final concentration of 5 mM to protect RNA from being degraded during enzyme inactivation.



NEW

Duplex DNase

#M7635S 150 units
#M7635L 750 units

- *DNA-specific endonuclease*
- *Specifically degrades dsDNA in the presence of ssDNA*
- *Cleaves DNA strand of DNA:RNA hybrid duplex*
- *Products are short oligos or ssRNA (if cleaving the DNA strand of DNA:RNA hybrid)*

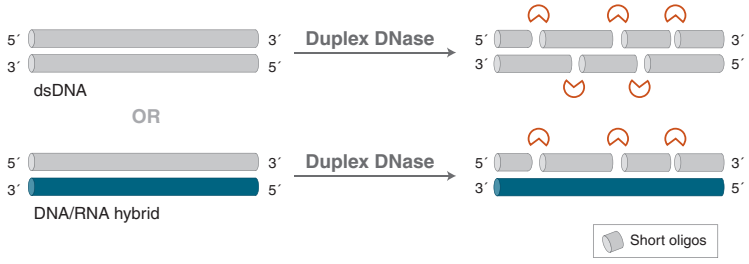
Description: Duplex DNase is an engineered double-strand-specific DNA endonuclease that preferentially degrades double-stranded DNA (dsDNA) over single-stranded DNA (ssDNA) or RNA. It will also cleave the DNA strand of a DNA/RNA hybrid duplex.

Source: A His-tagged engineered Duplex DNase expressed in *Pichia pastoris*.

Reaction Conditions: NEBuffer r1.1. Heat inactivation: 75°C for 10 minutes in the presence of 1 mM DTT. If the sample contains RNA, we recommend adding EDTA (10 mM final concentration) and DTT (1 mM final concentration), prior to heat inactivation. RNA may degrade at temperatures > 65°C in the presence of divalent metals such as Mg^{2+}..

Unit Definition: One unit is defined as the amount of enzyme required to release 50 pmol of FAM from a 35-mer FAM-BHQ1 labeled hairpin oligonucleotide in 1 minute at 30°C in a 50 µl reaction volume.

Concentration: 2,000 units/ml



RNase Inhibitor, Murine

RR

#M0314S 3,000 units
#M0314L 15,000 units

- *Inhibits common eukaryotic RNases*
- *Compatible with Taq Polymerase, AMV or M-MuLV Reverse Transcriptases*
- *cDNA synthesis & RT-PCR*
- *In vitro transcription/translation*

RNase Inhibitor, Murine has significantly **improved resistance to oxidation** compared to human & porcine RNase inhibitors.

GMP-grade reagent now available.
See page 6 for details.

Description: RNase Inhibitor, Murine is a 50 kDa recombinant protein of murine origin. It specifically inhibits RNases A, B and C by binding noncovalently in a 1:1 ratio with high affinity. It is not effective against RNase I, RNase T1, S1 Nuclease, RNase H or RNase from *Aspergillus*. No inhibition of polymerase activity is observed when used with *Taq* DNA Polymerase, AMV or M-MuLV Reverse Transcriptases, or Phage RNA Polymerases (SP6, T7, or T3).

Recombinant RNase Inhibitor, Murine does not contain the pair of cysteines identified in the human version that are very sensitive to oxidation and lead to inactivation of the inhibitor. As a result, RNase Inhibitor, Murine has significantly improved resistance to oxidation compared to the human/porcine RNase inhibitors, and is stable at low DTT concentrations (< 1 mM). This makes it ideal for RT-qPCR reactions.

Source: An E. coli strain that carries the Ribonuclease Inhibitor gene from mouse.

Unit Definition: One unit is defined as the amount of Murine RNase Inhibitor required to inhibit the activity of 5 ng of RNase A by 50%. Activity is measured by the inhibition of hydrolysis of cytidine 2', 3'-cyclic monophosphate by RNase A.

Concentration: 40,000 units/ml

RNase Inhibitor, Human Placenta

RR

#M0307S 2,000 units
#M0307L 10,000 units

- *Inhibits common eukaryotic RNases*
- *Compatible with Taq Polymerase, AMV or M-MuLV Reverse Transcriptases*
- *Active over a broad pH range (pH 5–8)*
- *cDNA synthesis reactions*
- *In vitro transcription/translation*

Description: RNase Inhibitor, Human Placenta is a recombinant human placental protein which specifically inhibits ribonucleases (RNases) A, B and C. It is not effective against RNase I, RNase T1, S1 Nuclease, RNase H or RNase from *Aspergillus*. In addition, no inhibition of polymerase activity is observed when RNase Inhibitor, Human Placenta is used with *Taq* DNA Polymerase, AMV or M-MuLV Reverse Transcriptases, or Phage RNA Polymerases (SP6, T7, or T3).

The 50 kDa protein inhibits RNases by binding noncovalently in a 1:1 ratio with an association constant greater than 10^{14}.

Source: An *E. coli* strain that carries the Ribonuclease Inhibitor gene from human placenta.

Unit Definition: One unit is defined as the amount of RNase Inhibitor, Human Placenta required to inhibit the activity of 5 ng of RNase A by 50%. Activity is measured by the inhibition of hydrolysis of cytidine 2, 3'-cyclic monophosphate by RNase A.

Concentration: 40,000 units/ml

Ribonucleoside Vanadyl Complex

#S1402S 10 ml

Description: Vanadium complexes are used in mRNA purifications as exogenous ribonuclease inhibitors. They are compatible with cell lysis techniques and with sucrose gradient fractionation of cytoplasmic components.

Ribonucleoside Vanadyl Complex is an equimolar mixture of all four ribonucleosides, complexed with oxovanadium IV by a modification of the procedures by Berger. Each lot of the complex is assayed for oxovanadium V content and inhibition of ribonuclease activity.

Source: This vanadyl complex is prepared from a modification of procedures by Lienhard using all four ribonucleosides (4).

Concentration: 200 mM

NEBNext Reagents for RNA Library Preparation

NEW

NEBNext UltraExpress RNA Library Prep Kit
#E3330S 24 reactions
#E3330L 96 reactions

NEBNext Ultra II Directional RNA Library Prep Kit for Illumina
#E7760S 24 reactions
#E7760L 96 reactions

NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads
#E7765S 24 reactions
#E7765L 96 reactions

NEBNext Ultra II RNA Library Prep Kit for Illumina
#E7770S 24 reactions
#E7770L 96 reactions

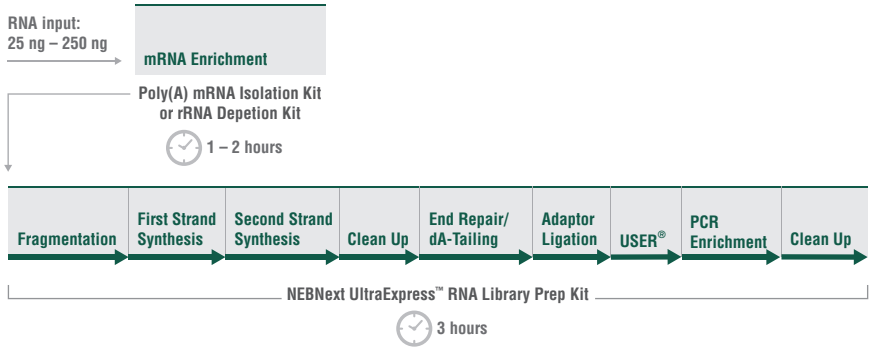
NEBNext Ultra II RNA Library Prep with Sample Purification Beads
#E7775S 24 reactions
#E7775L 96 reactions

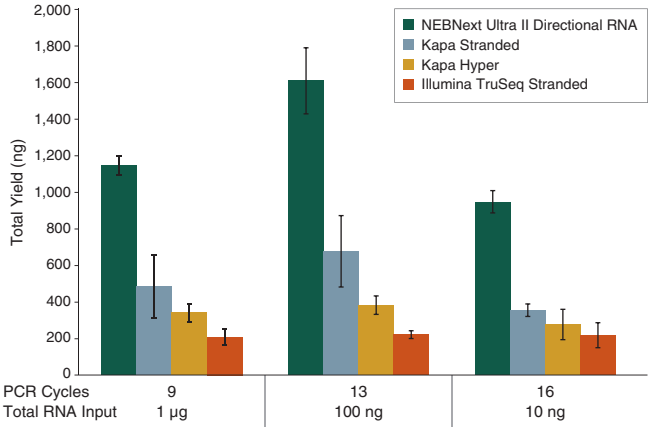
NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina
#E6420S 24 reactions
#E6420L 96 reactions

NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module
#E6421S 24 reactions
#E6421L 96 reactions

NEBNext Kits for RNA sample preparation for next generation sequencing keep pace with the use of ever-decreasing input amounts and sub-optimal sample quality, along with the need for superior performance, reliability, and automation compatibility. The fast and streamlined Ultra II Workflow is at the heart of our RNA library prep kits, including our NEBNext Single Cell/ Low Input Library Prep Kit for Illumina, and these are all available in flexible, user-friendly formats. The NEBNext UltraExpress RNA Library Prep Kit is the latest generation of NEBNext RNA library prep, and it has a fast, streamlined workflow. With a 3-hour library prep protocol, the kit enables creation of high-quality RNA directional libraries in a single day.

- *Get more of what you need, with the highest library yields*
- *Generate high quality libraries with limited amounts of RNA: 10 ng–1 µg Total RNA (polyA mRNA workflow); 10 ng–1 µg (rRNA depletion workflow)*
- *Minimize bias, with fewer PCR cycles required*
- *Maximize the flexibility to order reagents for your specific workflow needs*
- *Directional (strand-specific, using the "dUTP method") and non-directional workflow options available*
- *rRNA Depletion and poly(A) mRNA isolation reagents are available separately*
- *Save time with streamlined workflows, reduced hands-on time, and automation compatibility*
- *Enjoy the reliability of the gold standard SPRIselect size selection and clean-up beads, supplied in just the amounts you need*
- *Rely on robust performance, even with low quality RNA*





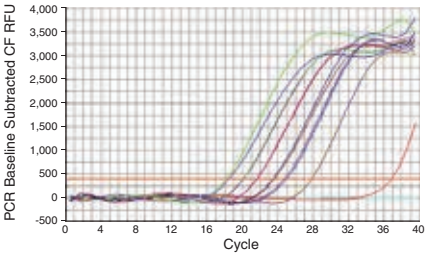
NEBNext Ultra II Directional RNA produces the highest yields, from a range of input amounts.
Poly(A)-containing mRNA was isolated from 10 ng, 100 ng and 1 µg of Universal Human Reference RNA (Agilent #740000) and libraries were made using the NEBNext Ultra II Directional RNA kit, Kapa™ Stranded mRNA-Seq kit, Kapa mRNA HyperPrep kit and Illumina® TruSeq® Stranded mRNA Kit. The input RNA amount and number of PCR cycles are indicated. Library yields from an average of three replicates are shown. Error bars indicate standard deviation. Library yields were assessed using the Agilent® Bioanalyzer®.

Magnetic mRNA Isolation Kit

#S1550S 25 isolations

- *Suitable for automated high-throughput applications*
- *Eliminates need for organic solvents*
- *No need to precipitate poly(A)+ transcripts in eluent*
- *Obtain intact poly(A)+ RNA in < 1 hour*
- *Negligible gDNA contamination*

Description: The New England Biolabs Magnetic mRNA Isolation Kit is designed to isolate intact poly(A)$^+$ RNA from cells and tissue without requiring phenol or other organic solvents. The technology is based on the coupling of Oligo d(T)$_{25}$ to 1 µm paramagnetic beads which are then used as the solid support for the direct binding of poly(A)$^+$ RNA. Thus, the procedure permits the manual processing of multiple samples and can be adapted for automated high-throughput applications. Additionally, magnetic separation technology permits elution of intact mRNA in small volumes eliminating the need for precipitating the poly(A)$^+$ transcripts in the eluent. Intact poly(A)$^+$ RNA which is fully representative of the mRNA population of the original sample can be obtained in less than one hour. Oligo d(T)$_{25}$ Magnetic Beads can be reused up to three times and the researcher has the option of eluting the isolated mRNA or using the bound dT DNA as a primer in a first-strand cDNA reaction.



Consistency and wide isolation range are demonstrated by poly(A)+ RNA isolation from duplicate samples of decreasing numbers of HEPG2 cells (5 x 10^5 to 1 x 10^3) by direct lysis/binding in microtiter plates followed by mRNA isolation with the magnetic method. *1/10th of isolated mRNA is converted to oligo (dT) primed?cDNA using ProtoScript M-MuLV First Strand cDNA Synthesis Kit (NEB #E6300) and qPCR done with validated primers for the peptidylpropyl isomerase, a low-abundance housekeeping gene.*

RNA REAGENTS

Oligo d(T)$_{25}$ Magnetic Beads

#S1419S 5 ml

Companion Product:

96-Well Microtiter Plate Magnetic Separation Rack
#S1511S 96 wells

- *Small-scale purification or immunoprecipitation of IgG species*
- *No centrifugation required*
- *Regenerate matrix without binding capacity loss*

Description: An affinity matrix for the small-scale isolation of mRNA from crude cell lysates and tissue. The isolation occurs through the hybridization of covalently coupled oligo d(T)$_{25}$ to the poly(A) region present in most eukaryotic mRNAs. Applications include direct mRNA isolation following lysis and second-round purification of previously isolated total RNA. The magnetic separation technology is scalable and permits elution of intact mRNA in small volumes eliminating the need for precipitation of the isolated mRNA. Beads can be reused up to three times, and the researcher has the option of eluting the isolated mRNA or using the bound d(T)$_{25}$ as a primer in a first-strand cDNA reaction.

Beads are supplied as a 5 mg/ml suspension in phosphate buffer (PBS) (pH 7.4), containing 0.05% Tween-20 and 0.05% NaN_3.

Concentration: 5 mg/ml

Support Matrix: 1 µm nonporous superparamagnetic microparticles.

Binding Capacity: 1 mg of Oligo d(T)$_{25}$ Beads will bind 10 µg of poly(A)$^+$ RNA.

Streptavidin Magnetic Beads

#S1420S 5 ml

Companion Products:

6-Tube Magnetic Separation Rack
#S1506S 6 tubes

96-Well Microtiter Plate Magnetic Separation Rack
#S1511S 96 wells

Description: Streptavidin Magnetic Beads are 1 µm superparamagnetic particles covalently coupled to a highly pure form of streptavidin. The beads can be used to capture of biotin labeled substrates including antigens, antibodies and nucleic acids. The strength of the biotin-streptavidin interaction coupled with low non-specific binding permits captured substrates to be useful as ligands in subsequent experiments including mRNA isolation and the capture of primary or secondary antibodies.

Beads are supplied as a 4 mg/ml suspension in phosphate buffer (PBS) (pH 7.4) containing 0.1% BSA, 0.05% Tween-20 and 0.05% NaN_3.

Concentration: 4 mg/ml

Support Matrix: 1 µM nonporous superparamagnetic microparticle

Binding Capacity: The beads will bind greater than 1000 pmol of free biotin per mg and greater than 500 pmol of single-stranded 25 bp biotinylated oligonucleotide per mg.

EpiMark® N6-Methyladenosine Enrichment Kit

#E1610S 20 reactions

- *Enrichment for m6A modified RNA in immunoprecipitation protocols*
- *Enriched RNA can be used directly for next gen sequencing or RT-qPCR*

Description: The EpiMark N6-Methyladenosine Enrichment Kit contains a rabbit monoclonal antibody specific for N6-Methyladenosine (m6A). The kit also contains two control RNAs, one with m6A modification (*Gaussia* luciferase) and one without (*Cypridina* luciferase) to monitor enrichment and depletion. The GLuc RNA control was transcribed in the presence of 20% m6ATP and 80% ATP.

This kit can be used to enrich m6A modified RNA in immunoprecipitation protocols for downstream analysis by next-generation RNA sequencing or RT-qPCR.

Modified RNA is isolated from a fragmented RNA sample by binding to the N6-Methyladenosine antibody attached to Protein G Magnetic Beads. After multiple wash and clean-up steps, the enriched RNA is eluted in nuclease-free water and is ready for further analysis.

Kit Includes:

- N6-Methyladenosine Antibody
- m6A Control RNA
- Unmodified Control RNA

N6-Methyladenosine Antibody is produced by Cell Signaling Technology, Inc. and sold by New England Biolabs, Inc.

Arandeni joined NEB in 2020 as a Production Technician in the Buffer Prep group. Deni is also part of the Running, Soccer, Plant, and Dog Owners Clubs.

Monarch Kits for Cleanup & Isolation

NEW
Monarch Spin RNA Isolation Kit (Mini)
#T2110S 50 preps

Monarch Spin RNA Cleanup Kit (10 µg)
#T2030S 10 preps
#T2030L 100 preps

Monarch Spin RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

Monarch Spin RNA Cleanup Kit (500 µg)
#T2050S 10 preps
#T2050L 100 preps

Companion Products:

Monarch Spin Columns S2A and Tubes
#T2047L 100 columns

Monarch Spin Columns S2C and Tubes
#T3017L 100 preps

Monarch Spin Collection Tubes
#T2118L 100 tubes

Monarch StabiLyse™ DNA/RNA Buffer
#T2111L 145 ml

- *Use with a wide variety of sample types*
- *Purify RNA of all sized, including miRNA & small RNA >20 nucleotides*
- *Includes DNase I, specialized columns, Proteinase K, and a stabilization reagent*
- *Efficiently remove contaminating genomic DNA*
- *Save money with value pricing for an all-in-one kit*

Description: The Monarch Spin RNA Isolation Kit (Mini) is a comprehensive solution for sample preservation, cell lysis, gDNA removal, and purification of total RNA from various biological samples, including cultured cells, mammalian tissues, microbes, plants, insects and blood. With just this single kit, users can purify up to 100 µg of high-quality total RNA from multiple sample types, from standard as well as tough-to-lyse samples, with included enzymes, reagents, and specialized gDNA removal columns. The kit uniquely enables binding capacities like RNA purification miniprep kits, combined with the low elution volumes of micro kits. Cleanup of enzymatic reactions and purification of RNA from TRIzol® -extracted samples is also possible using this kit. Purified RNA has metrics with $A_{260/280}$ and $A_{260/230}$ ratios typically > 2.0, high RNA integrity scores, and minimal residual gDNA. This kit captures RNA ranging in size from full-length rRNAs down to intact miRNAs. Purified RNA is suitable for downstream applications such as RT-qPCR, cDNA synthesis, RNA-seq, and RNA hybridization-based technologies.

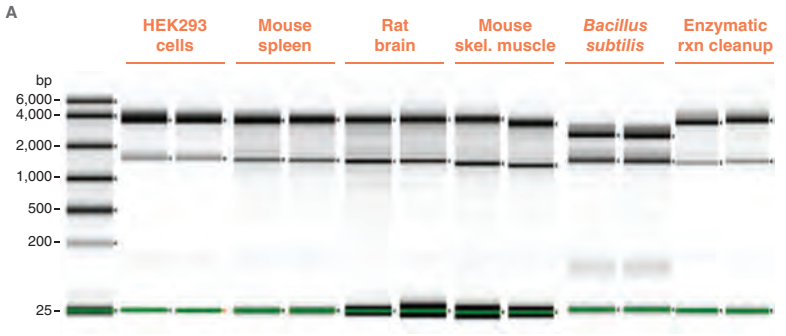
The Monarch Spin RNA Cleanup Kits provide a fast and simple silica spin column-based solution for RNA cleanup and concentration after any enzymatic reaction (including *in vitro* transcription, DNase I treatment, capping and labeling) and after other purification methods such as phenol/chloroform extraction. These kits can also be used to extract total RNA from cells, saliva and buccal/nasopharyngeal swabs. The Monarch Spin RNA Cleanup Kits are available in 3 different binding capacities (10 µg, 50 µg and 500 µg). Each kit contains unique columns, all designed to prevent buffer retention and ensure no carryover of contaminants, enabling low-volume elution of highly-pure RNA. Following the standard protocol, RNA ≥ 25 nt is purified with this kit; however, a modified protocol is available to enable the binding of RNA as small as 15 nt (including miRNAs).

The Monarch Spin RNA Isolation Kit (Mini) Includes:

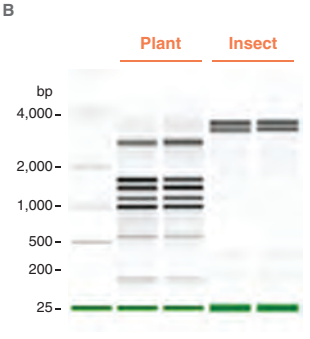
- gDNA Removal Columns
- RNA Purification Columns
- Collection Tubes
- Stabilization Reagent
- RNA Lysis Buffer
- DNase I & associated reaction buffers
- Proteinase K & associated reaction buffers
- RNA Priming Buffer
- RNA Wash Buffer
- Nuclease-free Water

The Monarch Spin RNA Cleanup Kits Include:

- Spin Columns (10, 50 or 500 µg)
- RNA Cleanup Binding Buffer
- RNA Cleanup Wash Buffer
- Collection Tubes
- Nuclease-free Water



RIN_e	10.0	10.0	8.9	9.2	8.8	9.6	9.4	9.6	10.0	10.0	10.0	10.0
$A_{260/280}$	2.08	2.08	2.09	2.10	2.10	2.10	2.15	2.09	2.15	2.13	2.07	2.08
$A_{260/230}$	2.36	2.31	2.21	2.27	2.27	2.08	2.13	2.15	2.39	2.35	2.08	2.09



$A_{260/280}$	2.11	2.11	2.15	2.16
$A_{260/230}$	2.38	2.34	2.04	1.95

Monarch Spin RNA Isolation Kit (Mini) is a versatile solution for high-quality RNA extraction from a wide variety of sample types. *RNA was extracted in duplicate using the Monarch Spin RNA Isolation Kit (Mini) from various samples representing a range of biological properties in the starting material, including fibrous, fatty and nuclease-rich mammalian tissues, tough-to-lyse samples such as Gram-positive bacteria, plants and insects, as well as enzymatic cleanup reactions. RNA quality was assessed using A_{260}/A_{280} and A_{260}/A_{230} ratios measured using a microvolume spectrophotometer (Lunatic®, Unchained Labs®). RNA integrity was measured using Agilent® automated electrophoresis systems according to sample types. For samples with known typical RNA profiles, Agilent RNA ScreenTape® was used on the TapeStation® 4200 (A). For samples with known atypical RNA profiles such as plant green tissue with plastidal content, and insects with ribosomal gene breaks, Agilent Bioanalyzer® 2100 used with NanoChip (B).*

Cloned at NEB | RR Recombinant Enzyme | PCR PCR Enzyme | Hot Start/WarmStart | SAM Requires SAM | 37° Incubation Temperature | 65 Heat Inactivation

RNA Markers & Ladders

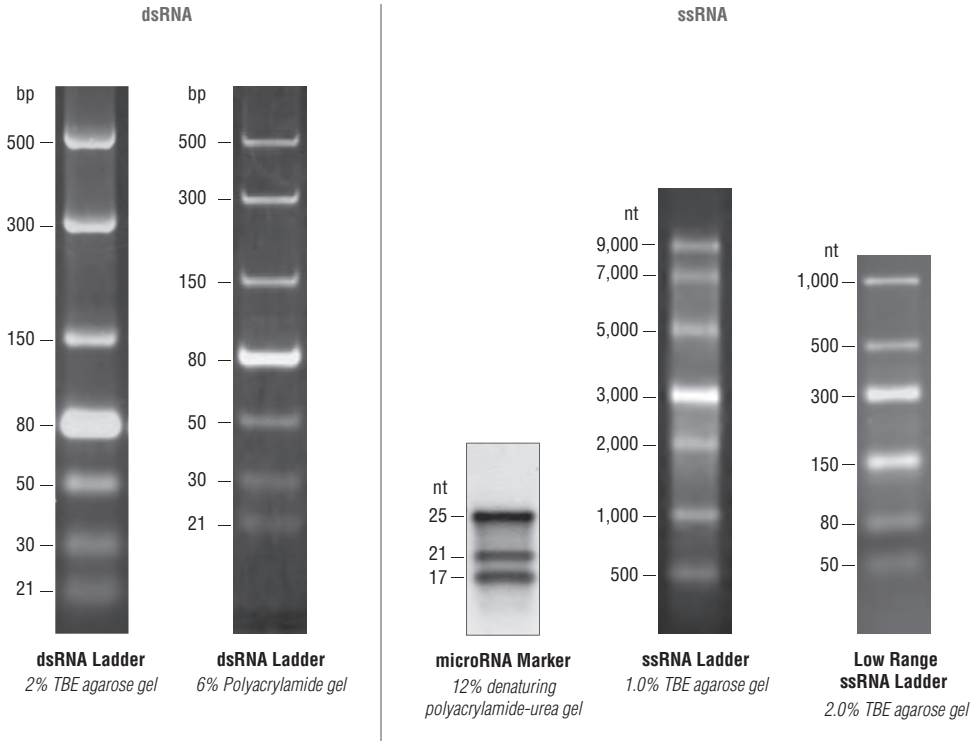
dsRNA Ladder
#N0363S 25 gel lanes

microRNA Marker
#N2102S 100 gel lanes

ssRNA Ladder
#N0362S 25 gel lanes

Low Range ssRNA Ladder
#N0364S 100 gel lanes

NEB offers several RNA Markers and Ladders with a size range from 17 to 9,000 bases. The Low Range ssRNA Ladder and the ssRNA Ladder are suitable for use as RNA size standards on denaturing or native gels. Both are supplied with RNA Loading Dye (2X) (NEB #B0363) and feature a higher intensity fragment to serve as a reference band. The microRNA Marker, provided in a ready-to-load denaturing solution, is ideally used as a size marker on denaturing polyacrylamide gels or northern blots and is best visualized stained with SYBR®-Gold. It is supplied with a 3´-biotinylated 21-mer oligonucleotide probe that can be labeled with g32-P-ATP and T4 PNK (NEB #M0201). The dsRNA Ladder is suitable for use as a size standard in dsRNA and RNAi analysis on both polyacrylamide and agarose gels.



dsRNA Ladder
2% TBE agarose gel

dsRNA Ladder
6% Polyacrylamide gel

microRNA Marker
12% denaturing polyacrylamide-urea gel

ssRNA Ladder
1.0% TBE agarose gel

Low Range ssRNA Ladder
2.0% TBE agarose gel

RNA Loading Dye, (2X)

#B0363S 4 ml

Description: The RNA Loading Dye, (2X) is a premixed loading dye for use with denaturing and non-denaturing PAGE/agarose gels.

RNA Loading Dye Composition: 1X RNA Loading Dye: 47.5% Formamide, 0.01% SDS, 0.01% Bromophenol Blue, 0.005% xylene cyanol and 0.5 mM EDTA.

Universal miRNA Cloning Linker

#S1315S 5 µg

Companion Product:

T4 RNA Ligase 2, truncated KQ
#M0373S 2,000 units
#M0373L 10,000 units

This 5-adenylated, 3´-blocked oligoribonucleotide can be used for cloning short RNAs according to the procedure of Bartel (1). RNA ligase recognizes the "activated" adenylated oligo and covalently links (ligates) its 5´ end to the 3´ OH of a second single-stranded sequence in the absence of ATP. In a mixture of nucleic acids use of the 5´ adenylated, 3´ blocked oligo with T4 RNA Ligase 2, truncated, T4 RNA Ligase 2, truncated K227Q or T4 RNA Ligase 1 (w/o ATP) results in ligation of the target oligo only.

The sequence of the adenylated DNA oligo is 5´-rAppCTGTAGGCACCATCAAT-NH_2 3´

(1) Lau et al. (2001) *Science*, 294, 858–856.

Listen to the NEB Podcast with Anne Madden.

LISTEN

A purple pigment isolated from naturally-occurring, purple-pigmented bacteria can kill a deadly fungus that affects anphibians
Credit: Fleur, Adobe Stock

Unveiling the Hidden Wonders of the Microbial World

Microbes are often misunderstood, perceived primarily as agents of disease and decay. Anne A. Madden, a dedicated microbiologist, is on a mission to change this perception. Recognizing that less than 1% of microbial species have been explored, she sees an untapped world of potential benefits waiting to be discovered. With a career spent uncovering 'wild' microbes that enhance human life — from novel antibiotics sourced from soil to patented brewing techniques using yeast found in wasps — she understands the profound impact these tiny organisms can have.

Inspired by the idea of creating a 'NASA for microbes,' Madden founded The Microbe Institute in 2020, a nonprofit organization devoted to fostering microbial discovery for a better tomorrow. Her vision is to make the microscopic world accessible, engaging and inspiring. *"I wanted to reshape the relationship humans have with microbes to help us facilitate future microbial science — those discoveries that ultimately advance human development, enhance conservation, and improve the health of our planet,"* she explained.

The Microbe Institute operates through a dynamic partnership model, collaborating with educators, scientists, companies and artists across various disciplines. Their innovative projects transcend traditional boundaries, combining research, education and art to highlight the beauty and utility of microbes. One such initiative is the Purple Microbe Project, a collaboration with Prof. Brooke Jude at Band College, began as a citizen science effort to map the biogeography of microbes that produce a vivid violet pigment and help amphibians combat deadly fungal infections.

The Purple Microbe Project evolved into a multifaceted endeavor. Students and participants worldwide engage in authentic research experiences, collecting data that aids scientists while learning valuable bioeconomy skills. The project also incorporates a participatory art element: using the purple dye derived from these microbes, participants create fabric squares that express themes of partnership and collaboration. This has grown into one of the largest participatory bio-art exhibits globally, uniting science and art in a tangible, impactful way.

Madden's approach emphasizes inclusivity and democratization of science. The Microbe Institute's resources and lesson plans have been integrated into university curricula and have reached international audiences through conferences, features in STEM education magazines, and media coverage — reaching audiences in the millions. Collaborations with Moroccan weavers have led to the development of environmentally sustainable dyeing practices, showcasing how microbial applications can have real-world benefits across cultures and industries.

At the heart of Madden's work is a deep-seated belief in the power of curiosity and wonder. *"I deeply believe that a better, more sustainable tomorrow exists,"* she said. *"By democratizing access to science, challenging historical convictions that exclude art and science disciplines from working together, and engaging the world in the process of discovery and awe, we can reach that future sooner."*

Madden's journey exemplifies how one individual's passion can bridge gaps between science, art and the public. Through The Microbe Institute, Madden unveils the microbial world's hidden wonders and inspires a new generation to look beyond misconceptions and see microbes as a source of hope and innovation. By fostering a global community engaged in microbial discovery, Madden is contributing to a more informed, connected and optimistic future.

Dr. Anne A. Madden
The Microbe Institute, Portland, ME, USA
2024 Passion in Science
Science Mentorship and Advocacy Award

Protein Expression & Purification

NEB offers an array of solutions for robust expression of your target protein.

At first glance, recombinant protein expression looks quite simple. Essentially, DNA encoding a target protein is cloned downstream of a promoter in an expression vector. This vector is then introduced into a host cell, and the cell's protein synthesis machinery produces the desired protein. In practice, however, protein expression can be very challenging, because so many factors may influence the process. For example, each protein folds in its own unique manner, a process that may be influenced by the choice of expression host. Similarly, some proteins require post-translational modifications or proper insertion into a biological membrane. Finally, some proteins may have an activity that is detrimental to the host. Thus, no single solution exists for successful production of all recombinant proteins. Instead, it is beneficial to have access to a wide range of expression tools, and a willingness to explore multiple approaches to better one's chances for success.

NEB offers an array of expression systems offering different advantages, enabling you to choose the strategy that best suits your protein expression and purification needs. Many share a compatible polylinker, enabling the gene of interest to be easily shuffled between systems. Additionally, a selection of competent cells is available for *in vitro* expression of difficult-to-express proteins.

Featured Products

Featured Tools & Resources

To learn more about NEB's portfolio of products for protein expression and purification, Visit **www.neb.com/Protein Expression** to learn more.

RM Recombinant Enzyme

Protein Expression Overview

Experts in protein expression and purification

Protein expression can be a very complex, multi-factorial process. Each protein requires a specific intracellular environment to correctly achieve its secondary and tertiary structures. Proteins may also require post-translational modifications or insertion into a cellular membrane for proper function. Other proteins, once expressed, may be toxic to the host. Thus, no single solution exists for the successful production of all recombinant proteins, and a broad range of expression tools is needed to ensure the successful expression of your target protein.

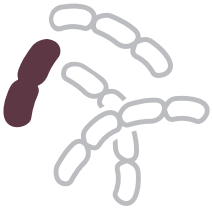
Our NEBExpress portfolio of products includes solutions for expression and purification, and is supported by access to scientists with over 40 years of experience in developing and using recombinant protein technologies in *E. coli*. We use these solutions in our own research and manufacturing processes and know that quality and performance are critical – all of our products are stringently tested so that you can be sure they will work optimally for your solution, just as we rely on them to work in ours.

Generate analytical amounts of protein with our cell-free expression systems

- NEBExpress Cell-free *E. coli* Protein Synthesis System, our novel *E. coli* cell-extract based transcription/translation system, is designed to synthesize proteins under the control of T7 RNA Polymerase at high yields for a wide variety of proteins
- The PURExpress® *In Vitro* Protein Synthesis Kit, our novel cell-free transcription/translation system, enables protein expression in approximately two hours and is ideal for high-throughput technologies

Generate and purify high yields of recombinant proteins

- The NEBExpress MBP Fusion and Purification System utilizes a pMAL vector and the *malE* gene for the expression of MBP-fusion proteins, which can be isolated by a two-step affinity purification
- The IMPACT™ Kit utilizes engineered protein splicing elements (inteins) to purify recombinant proteins in a single step
- For analysis, try our protein standards, which are available unstained, prestained, or with two colors for easy identification



Express a variety of proteins with our competent cells

- Our popular BL21 and BL21(DE3) Competent *E. coli* strains are available for routine expression
- Lemo21(DE3) Competent *E. coli* offers tunable T7 expression for difficult targets
- For expression of His-tagged proteins, we offer NiCo21(DE3) Competent *E. coli*
- SHuffle® strains are available for the expression of proteins with multiple disulfide bonds

Purify tagged proteins with our magnetic beads, columns and resins

- Nickel spin columns, magnetic beads and resin enable rapid purification of His-tagged proteins
- Amylose resins for purification of MBP-tagged proteins are available in a variety formats (standard, high flow and magnetic)
- Chitin resin and magnetic beads enable rapid purification of CBD-tagged proteins
- Remove affinity tags following your purification with TEV Protease

NEBExpress® Cell-free *E. coli* Protein Synthesis System

#E5360S	10 reactions
#E5360L	100 reactions

Companion Product:

NEBExpress GamS Nuclease Inhibitor
#P0774S 75 µg

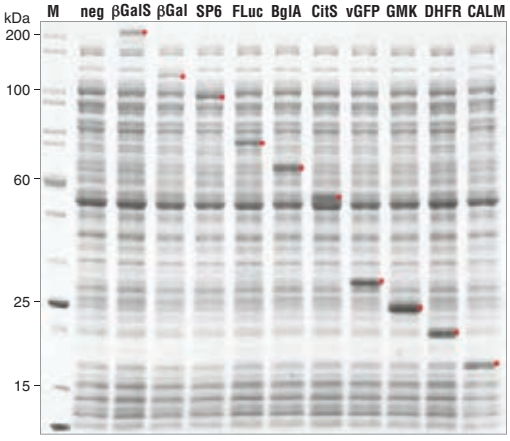
- *Synthesize high yields of protein (typically 0.5 mg/ml)*
- *Protein can be synthesized and visualized in approximately 2–4 hours*
- *Synthesize target proteins ranging from 17 to 230 kDa*
- *Templates can be plasmid DNA, linear DNA, or mRNA*
- *RNase contamination can be inhibited by the supplied RNase inhibitor, eliminating clean-up steps*
- *Flexible reaction conditions achieve maximum yield; protein synthesis can be sustained for 10 hours at 37°C or up to 24 hours at lower temperatures*
- *Reactions can be miniaturized or scaled up to yield milligram quantities of protein*

Description: The NEBExpress Cell-free *E. coli* Protein Synthesis System is a coupled transcription/translation system designed to synthesize proteins encoded by a DNA or mRNA template under the control of a T7 RNA Polymerase promoter. The system offers high expression levels, the ability to produce high molecular weight proteins, scalability, and is cost-effective for high-throughput expression applications. The speed and robustness of the system facilitates protein synthesis in applications such as protein engineering, mutagenesis studies and enzyme screening.

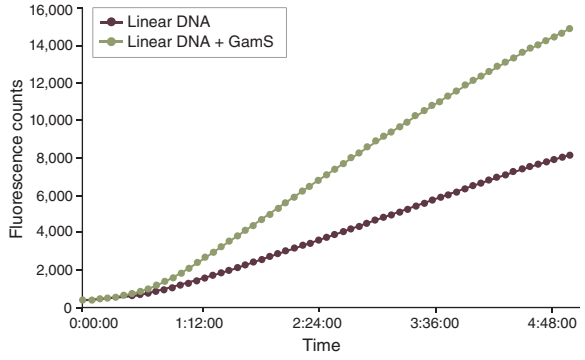
The NEBExpress Cell-free *E. coli* Protein Synthesis System contains all the components required for protein synthesis, except for the target template DNA. It is a combination of a highly active cell extract from a genetically engineered strain, a reaction buffer, and an optimized T7 RNA Polymerase, which together yield robust expression of a wide variety of protein targets ranging from 17 to 230 kDa.

Applications:

- High-throughput screening and liquid handling
- Mutation studies: effect of point mutations, deletions and insertions; rapidly identify active domains and functional residues
- Epitope mapping and protein folding
- Expression of toxic proteins



The NEBExpress Cell-free E. coli Protein Synthesis System can be used to express a wide range of proteins. *50 µl reactions containing 250 ng template DNA were incubated at 37°C for 3 hours. The red dot indicates the protein of interest. M = Unstained Protein Standard, Broad Range (NEB #P7717), "neg" = negative control, no DNA.*



NEBExpress GamS Nuclease Inhibitor enhances synthesis of linear DNA. *GamS inhibits Exonuclease V (RecBCD) activity and stabilizes linear DNA templates in E. coli based in vitro protein synthesis reactions. 50 µl reactions containing 100 ng linear template DNA, the components of the NEBExpress Cell-free E. coli Protein Synthesis System and 1.5 µg NEBExpress GamS Nuclease Inhibitor incubated for 5 hours at 37°C were monitored for activity as determined by fluorescence signal.*

Introduction to the NEBExpress Cell-free Protein Synthesis System.

PURExpress® *In Vitro* Protein Synthesis Kits

PURExpress *In Vitro* Protein Synthesis Kit
#E6800S 10 reactions
#E6800L 100 reactions

PURExpress Δ Ribosome Kit
#E3313S 10 reactions

PURExpress Δ (aa, tRNA) Kit
#E6840S 10 reactions

PURExpress Δ RF123 Kit
#E6850S 10 reactions

Companion Products:

PURExpress Disulfide Bond Enhancer
#E6820S 50 reactions

E. coli Ribosome
#P0763S 1 mg

- *Generation of analytical amounts of proteins for further characterization*
- *Confirmation of open reading frames*
- *Generation of truncated proteins to identify active domains and functional residues*
- *Introduction of modified, unnatural or labeled amino acids (NEB #E6840, #E6850)*
- *tRNA structure and function studies (NEB #E6840)*
- *Ribosome structure and function studies (NEB #E3313, #P0763)*
- *Release factor function studies/ribosome display (NEB #E6850)*
- *Epitope mapping*

PURExpress is based on the PURE system technology originally developed by Dr. Takuya Ueda at the University of Tokyo and commercialized as the PURESYSTEM™ by Biocomber (Tokyo, Japan).

PURESYSTEM™ is a trademark of Post Genome Institute.

Description: A rapid method for gene expression analysis, PURExpress is a novel cell-free transcription/translation system reconstituted from the purified components necessary for *E. coli* translation. The minimized nuclease activity and protease-free nature of the PURExpress platform preserves the integrity of DNA and RNA templates/complexes and results in proteins that are free of modification and degradation. Transcription and translation are carried out in a one-step reaction, and require the mixing of only two tubes. With results available in a few hours, PURExpress saves valuable laboratory time and is ideal for high-throughput technologies.

Advantages:

- Suitable for circular or linear DNA template
- Visualize synthesized protein directly on a Coomassie stained gel
- Protein expression in approximately 2 hours
- Transcription/translation components can be removed by affinity chromatography

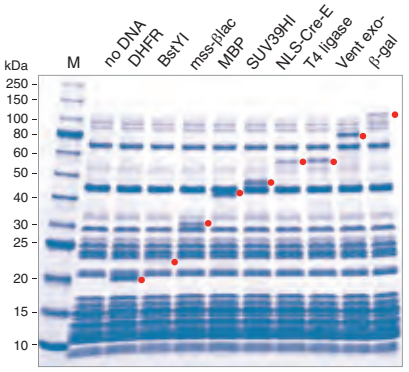
PURExpress Disulfide Bond Enhancer: This proprietary blend of proteins and buffer components is designed to correctly fold target proteins with multiple disulfide bonds produced in PURExpress reactions or NEBExpress *E. coli* S30 extracts. Added at the beginning of a reaction, the components assist with the oxidation of cysteine thiols and correcting mis-oxidized substrates, increasing yield of soluble and functionally active protein.

PURExpress Δ Ribosome Kit: The ribosomes are removed from this kit, allowing users to add their own modified ribosomes for studies on protein translation. Control ribosomes (sufficient for 2 reactions) are provided in a separate tube.

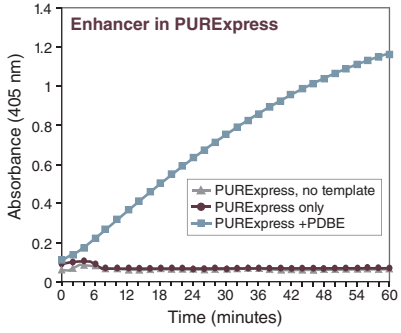
PURExpress Δ RF123 Kit: Release factors are involved in termination of protein translation by recognizing the stop codons in an mRNA sequence. In a ribosome display experiment using PURExpress, lack of release factors could stabilize the ternary complex of mRNA-ribosome-nascent protein. As a result, the cDNA recovery could be higher. In this kit, the three release factors are supplied separately, allowing users to perform a protein synthesis reaction/ribosome display experiment with/ without release factors of their choice.

PURExpress Δ (aa, tRNA) Kit: The tRNA and amino acids are supplied separately in this kit, allowing users to run a protein synthesis reaction by adding modified amino acids and tRNA mixtures to the reaction.

***E. coli* Ribosome:** The 70S *E. coli* Ribosome consists of a small subunit (30S) and a large subunit (50S). This preparation of ribosomes is highly active in NEB's PURExpress Protein Synthesis Kit (NEB #E6800), and can be used in ribosome structure and function studies, as a target for drug screening, and as starting material for isolation of native ribosomal RNAs (5S, 16S, 23S).



Protein expression using the PURExpress In Vitro Protein Synthesis Kit. *25 µl reactions containing 250 ng template DNA and 20 units RNase Inhibitor were incubated at 37°C for 2 hours. 2.5 µl of each reaction was analyzed by SDS-PAGE using a 10–20% Tris-glycine gel. The red dot indicates the protein of interest. Marker M is the Protein Standard.*



PURExpress Disulfide Bond Enhancer. *(PDBE) promotes proper folding of active vtPA. Reactions were set up according to PURExpress specifications with the vtPA template DNA. After a 2 hour incubation at 37°C, 5 µl of each reaction was used in an activity assay and cleavage of the chromogenic substrate was monitored for one hour.*

PURExpress Kit Components

PURExpress *In Vitro* Protein Synthesis Kit NEB #E6800	PURExpress Δ Ribosome Kit NEB #E3313	PURExpress Δ (aa, tRNA) Kit NEB #E6840	PURExpress Δ RF123 Kit NEB #E6850
• Solution A • Solution B • Control (DHFR) template	• Solution A • Factor Mix • Control (DHFR) template • Control Ribosomes	• Solution A (minus aa and tRNA) • Solution B • Control (DHFR) template • Amino Acid Mixture • *E. coli* tRNA	• Solution A • Solution B (minus RF1, RF2 and RF3) • Control (DHFR) template • RF1, RF2 and RF3

NEBExpress® MBP Fusion and Purification System

#E8201S 1 set

Companion Products:

TEV Protease
#P8112S 1,000 units

Amylose Resin
#E8021S 15 ml
#E8021L 100 ml

Anti-MBP Monoclonal Antibody
#E8032S 0.05 ml
#E8032L 0.25 ml

pMAL-c6T Vector
#N0378S 10 µg

- *Reliable E. coli expression: substantial yields (up to 100 mg/L)*
- *Fusion to MBP significantly enhances proper folding of target proteins*
- *Two-step purification: amylose elution followed by TEV Protease cleavage and Ni resin isolation results in a highly pure tag-free target protein*
- *Gentle elution with maltose: no detergents or harsh denaturants required*

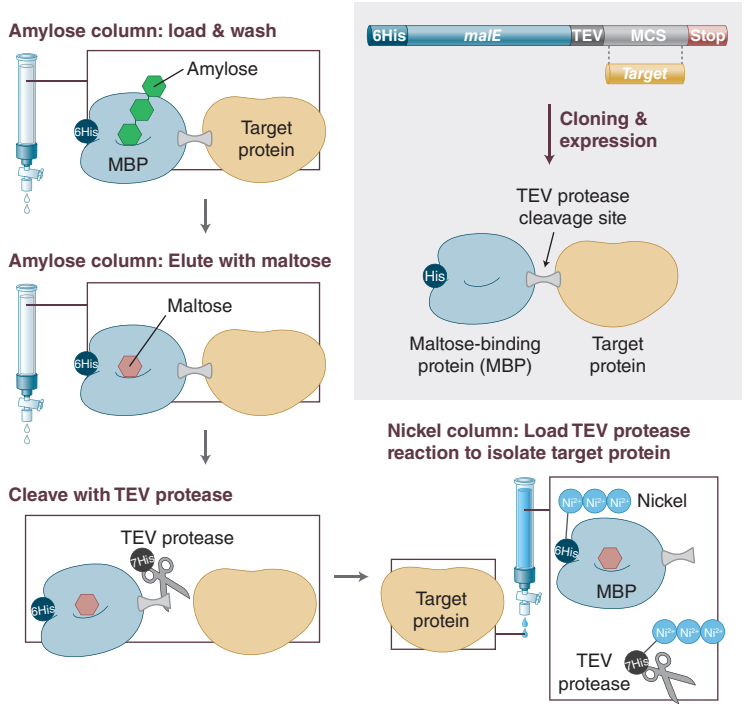
Description: In the NEBExpress MBP Fusion and Purification System, the pMAL-c6T vector provides a method for expressing and purifying a protein produced from a cloned gene or open reading frame. The cloned gene is inserted downstream from and in frame with the *malE* gene of *E. coli*, which encodes maltose-binding protein (MBP); this construct results in the expression of an MBP fusion protein. The pMAL-c6T vector expresses the *N*-terminal hexahistidine tagged *malE* gene (lacking its secretory signal sequence and engineered for tighter binding to amylose) followed by a multiple cloning site containing a TEV protease recognition sequence and stop codons in all three frames. The pMAL-c6T vector expresses the MBP fusion in the cytoplasm. The method uses the strong "tac" promoter and the *malE* translation initiation signals to yield high-level expression of the cloned sequences. The fusion protein is then purified by a one-step purification method using amylose resin and MBP's affinity for maltose.

Following amylose purification, the target protein can be cleaved from the MBP-tag using TEV Protease without adding any vector-derived residues to the protein. Both the MBP-tag and TEV Protease are polyhistidine-tagged for easy removal from the reaction. Loading the digest onto NEBExpress Ni Resin (NEB #S1428) sequesters both the MBP-tag and TEV Protease, thereby isolating the target protein in the column flow through. The target protein yield can be up to 100 mg/L, with typical yields in the range of 10–40 mg/L.

References: References for properties and applications of this product can be found at www.neb.com.

Kit Includes:

- pMAL-c6T Vector
- MBP6 Protein
- MBP6-TEV-Paramyosin ΔSal
- TEV Protease
- TEV Protease Reaction Buffer
- Anti-MBP Monoclonal Antibody
- *E. coli* ER2523 (NEB Express) (Glycerol Stock)
- Amylose Resin



Schematic illustration of the NEBExpress MBP Fusion and Purification System.

IMPACT™ Kit

#E6901S 1 set

Companion Products:

Anti-CBD Monoclonal Antibody
#E8034S 0.05 ml

pTWIN1 Vector
#N6951S 10 µg

pTXB1 Vector
#N6707S 10 µg

pTYB21 Vector
#N6709S 10 µg

Chitin Resin
#S6651S 20 ml
#S6651S 20 ml

Chitin Magnetic Beads
#E8036S 5 ml

- *Single-column purification without the use of proteases*
- *Produce target protein without vector-derived amino acids*
- *Fusion to either N- or C-terminus of target protein*
- *Ligation and labeling of recombinant proteins*
- *Isolation of proteins with or without N-terminal methionine*

Description: The IMPACT (Intein Mediated Purification with an Affinity Chitin-binding Tag) Kit utilizes engineered protein splicing elements (inteins) to purify recombinant proteins by a single column (Figure 1). This kit distinguishes itself from other protein fusion systems by its ability to separate a recombinant protein from the affinity tag without the use of a protease.

The IMPACT Kit allows fusion of a tag consisting of an intein and the chitin binding domain (CBD) from *Bacillus circulans*, to either the C-terminus (pTXB1) or the N-terminus (pTYB21) of a target protein (Figure 2). In the presence of thiols, such as DTT, the intein undergoes specific self-cleavage which releases the target protein. The pTXB1 vector can also be used to express a protein with a C-terminal thioester for use in Intein-mediated Protein Ligation (IPL). The IPL reaction, also referred to as expressed protein ligation, allows for ligation of a peptide or a protein with a N-terminal cysteine to a bacterially expressed protein with a C-terminal thioester through a native peptide bond for use in protein labeling and semisynthesis. For more information on the IMPACT System and IPL, visit www.neb.com.

pTXB1 is a *E. coli* expression vector that utilizes a mini-intein from the *Mycobacterium xenopi gyrA* gene [*Mxe* GyrA intein; 22 kDa]. This intein has been modified and combined with the CBD to create an affinity tag which can be bound to chitin beads (NEB #S6651). Release of the target protein is induced by thiol reagents such as DTT or 2-mercaptoethanesulfonic acid (for ligation).

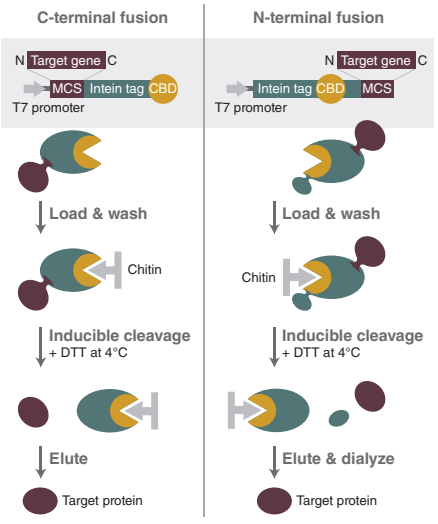
The pTYB21 vector allows for the fusion of the intein tag containing the *Saccharomyces cerevisiae* (*Sce*) *VMA*1 intein and CBD to the N-terminus of the target protein.

pTWIN1 Vector is available separately and enables isolation of proteins with an N-terminal cysteine and/or a C-terminal thioester. The polylinker is designed for the in-frame fusion of a target gene between the modified *Ssp* DnaB and *Mxe* GyrA inteins. The presence of the CBD facilitates purification.

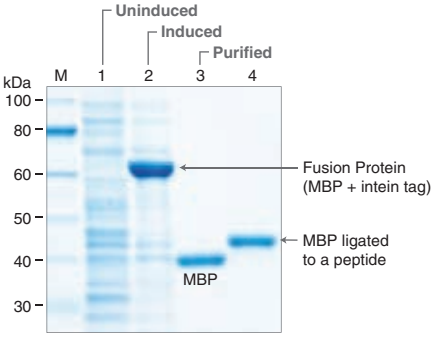
References: References for properties and applications of this product can be found at www.neb.com.

Kit Includes:

- *E. coli* ER2566
- Blue Protein Loading Dye
- pTXB1 Vector
- pMXB10 Control Plasmid
- pTYB21 Vector
- Anti-CBD Monoclonal Antibody
- DTT
- Chitin Resin



Schematic of the IMPACT System.



Purification of Maltose Binding Protein (MBP) in a single affinity purification step. *Lane 1: uninduced cell extract. Lane 2: induced cell extract showing expressed fusion protein. Lane 3: MBP fraction eluted after inducing cleavage overnight at 4°C. Lane 4: MBP ligated to a peptide containing an N-terminal cysteine. Marker M is the protein ladder.*

Guide to IMPACT vectors and applications

Vectors	Site of Target Protein Fusion	Intein Tag (kDa)	Recommended Cloning Sites[a]	Preferred Residues at Cleavage Site[b]	Method of Cleavage[c,d]	Applications
pTXB1	C-terminus	Mxe GyrA intein (28)	NdeI-SapI/SpeI	Y, F, O, N, T, K, A, H, M Unfavorable residues: S, P, D, G	DTT (or MESNA) pH 8.0-8.5, 4°C	Purification; C-terminal thioester for ligation and modification
pTYB21	N-terminus	Sce VMA1 intein (56)	SapI/BsmI/ NdeI-PstI	A, Q, M, G, L, N, W, F, Y Unfavorable residues: P, S, C, T, R	DTT pH 8.0-8.5, 25°C	Purification
pTWIN1	C-terminus (Intein 2)	Mxe GyrA intein (28)	NdeI-SapI/SpeI	M, Y, F, LEM Unfavorable residues: S, P, E, D	DTT (or MESNA) pH 8.0-8.5, 4°C	Purification; C-terminal thioester for ligation and modification

[a] NEBuilder HiFi DNA Assembly Cloning Kit (NEB #E5520) can be used to generate construct without the use of restriction enzymes.
[b] Actual cleavage efficiency is dependent on the adjacent residues as well as the folding of the fusion protein.
[c] Dithiothreitol (DTT) is used only for protein purification. 2-mercaptoethanesulfonic acid (MESNA) is used for isolation of proteins possessing a C-terminal thioester for ligation, labeling and cyclization.
[d] Cysteine can be used in the place of DTT.

K. lactis Protein Expression Kit

#E1000S 1 set

Companion Products:

SacII
#R0157S 2,000 units
#R0157L 10,000 units

Yeast Carbon Base Medium Powder
#B9017S 12 g

K. lactis GG799 Competent Cells
#C1001S 5 reactions

BstXI
#R0113S 1,000 units
#R0113L 5,000 units

Enterokinase, light chain
#P8070S 480 units
#P8070L 2,560 units

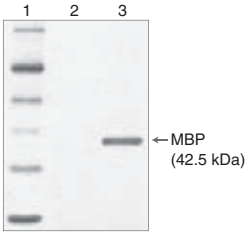
- *Clone and express genes toxic to E. coli*
- *Simultaneous expression of multiple genes*
- *No expensive antibiotics or methanol required*
- *Easy-to-use protocols for those inexperienced with yeast systems*
- *Attractive commercial sublicensing*

A restriction map for pKLAC2 can be found in the technical reference section or at **www.neb.com.**

Description: The *K. lactis* Protein Expression Kit provides an easy method for expressing a gene of interest in the yeast *Kluyveromyces lactis*. The gene is cloned into the integrative pKLAC-series of vectors, and may be expressed intracellularly or secreted. The *K. lactis* system offers several advantages over other yeast and bacterial expression systems. Abundant overexpression of protein is achieved through high culture densities as well as the ability to integrate multiple copies of the vector. The pKLAC-series of vectors use a strong *LAC4* promoter, which has been modified to lack expression in *E. coli*, making this system useful for expressing toxic genes. For the selection of transformants, no expensive antibiotics are required. In addition, no methanol is required in growth media. Finally, the *K. lactis* system can express post-translationally modified proteins, making it a useful alternative to bacterial expression systems.

pKLAC2 is a general purpose expression vector. Using this vector, proteins may be produced intracellularly or may be fused to the *K. lactis* α-mating factor sequence for secreted expression. Vector pKLAC2 contains an MCS that is compatible with other expression systems available from NEB.

GG799 competent cells are provided in the *K. lactis* Protein Expression Kit. GG799 cells are characterized by very high cell density growth and efficient expression of foreign proteins. GG799 cells have no genetic modifications.

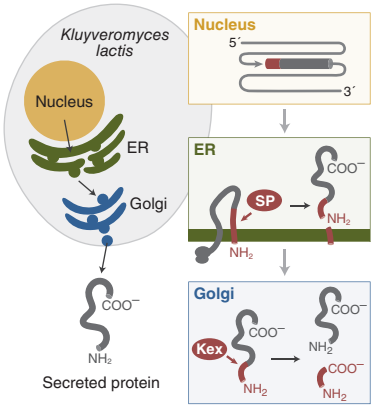


Protein Expression in K. lactis. *SDS-polyacrylamide gel electrophoresis separation of secreted recombinant maltose-binding protein (MBP) detected directly in peptone rich growth medium by Coomassie staining. Lane 1: Protein Molecular Weight Markers. Lane 2: spent culture medium (15 µl) from wild-type K. lactis cells. Lane 3: spent culture medium (15 µl) from K. lactis cells harboring an integrated expression cassette containing the E. coli malE gene.*

References: References for properties and applications of these products can be found at www.neb.com.

Kit Includes:

- SacII
- pKLAC1-*malE* Control Plasmid
- pKLAC2 Vector
- rCutSmart
- Yeast Carbon Base Medium Powder (12 g)
- Acetamide solution (sterile) (10 ml)
- Integration Primer 2
- Integration Primer 1
- Integration Primer 3
- *K. lactis* GG799 Competent Cells
- NEB Yeast Transformation Reagent (5 ml)



Secreted protein processing. *In the nucleus, an integrated expression vector encoding a fusion between the α-MF domain (blue) and a desired protein (black) is expressed. A signal peptide in the α-MF domain directs entry of the fusion protein into the endoplasmic reticulum (ER) and is removed by signal peptidase (SP). The fusion protein is transported to the Golgi apparatus where the Kex protease removes the α-MF domain. The protein of interest is then secreted from the cell.*

Meet two of our Corporate and Business Development Managers: Teni (left) and Irene (right). They both joined NEB in 2024. Irene's interests involve RNA and protein expression-related applications, while Teni is focused on amplification and next generation sequencing.

Competent Cells for Protein Expression

NEB offers a wide selection of competent cell strains ideal for expression of a variety of proteins. Proteins with multiple disulfide bonds are correctly oxidized to significantly higher yields with SHuffle® strains. Tunable T7 expression is achieved with Lemo21(DE3), an ideal strain for difficult targets including membrane proteins. NiCo21(DE3) is designed for the expression and purification of His-tagged proteins. NEBExpress and T7 Express are offered with varying levels of control. Only NEB offers exceptional control of T7 expression by the *lysY* gene, which is ideal for proteins that are difficult to express or toxic to the cell. Each strain is provided with a protocol for optimal expression.

Strain	NEB #	Characteristics	Size
NEBExpress Competent *E. coli* (High Efficiency)*	C2523H C2523I	• Versatile non-T7 expression strain • Protease deficient	20 x 0.05 ml 6 x 0.2 ml
NEBExpress I^q Competent *E. coli* (High Efficiency)	C3037I	• Control of IPTG induced expression from Plac , Ptac and Ptrc • Protease deficient	6 x 0.2 ml
T7 Express Competent *E. coli* (High Efficiency)	C2566H C2566I	• Most popular T7 expression strain • Protease deficient	20 x 0.05 ml 6 x 0.2 ml
T7 Express *lysY* Competent *E. coli* (High Efficiency)	C3010I	• T7 expression • Protease deficient • Better reduction of basal expression	6 x 0.2 ml
T7 Express *lysY*/I^q Competent *E. coli* (High Efficiency)	C3013I	• T7 expression • Protease deficient • Highest level of expression control	6 x 0.2 ml
SHuffle Express Competent *E. coli*	C3028J	• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • Protease deficient/B strain	12 x 0.05 ml
SHuffle T7 Express Competent *E. coli*	C3029J	• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression • Protease deficient/B strain	12 x 0.05 ml
SHuffle T7 Express *lysY* Competent *E. coli*	C3030J	• T7 expression • Protease deficient/B strain • Tightly controlled expression of toxic proteins • Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm	12 x 0.05 ml
SHuffle T7 Competent *E. coli*	C3026J	• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression/K12 strain	12 x 0.05 ml
BL21 Competent *E. coli*	C2530H	• Routine expression for non-T7 Vectors • Protease deficient	20 x 0.05 ml
BL21(DE3) Competent *E. coli*	C2527H C2527I	• Routine T7 Expression • Protease deficient	20 x 0.05 ml 6 x 0.2 ml
Lemo21(DE3) Competent *E. coli*	C2528J	• Tunable T7 Expression for difficult targets • Protease deficient	12 x 0.05 ml
NiCo21(DE3) Competent *E. coli*	C2529H	• Expression and purification of His-tagged proteins • Protease deficient	20 x 0.05 ml

Note: Store Competent Cells at –80°C. Once thawed, do not refreeze. Storage at –20°C will result in a significant decrease in transformation efficiency. Cells lose efficiency whenever they are warmed above –80°C, even if they do not thaw.

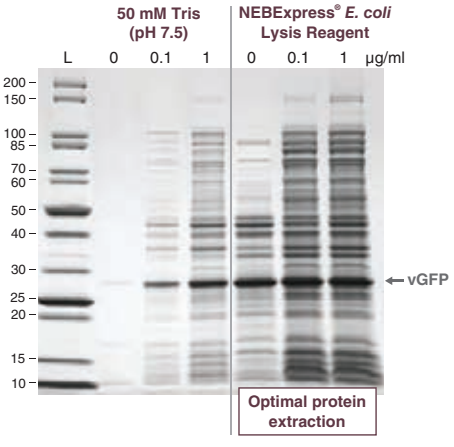
* NEBExpress is the recommended strain for the NEBExpress MBP Fusion and Purification System.

NEBExpress® T4 Lysozyme

#P8115S 200 µg
#P8115L 1,000 µg

- *200-fold more active than chicken egg white lysozyme*
- *Lysis reactions are scalable and compatible with high throughput workflows*
- *Lysis efficiency increases 2-fold when used in combination with NEBExpress E. coli Lysis Reagent*
- *Fast and non-mechanical bacterial lysis; the lysate is ready to use and compatible with affinity resins.*
- *Recombinant, animal-free and REACH compliant*

NEBExpress T4 Lysozyme is a recombinant murein hydrolase that breaks down the bacterial cell wall by hydrolyzing the β-1,4 linkage between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan of prokaryotic cells (gram-negative and some gram-positive bacteria). It can be used to extract soluble proteins, membrane proteins, DNA, RNA or metabolites.



Optimal protein extraction with NEBExpress T4 Lysozyme in the presence of NEBExpress E. coli Lysis Reagent. *T7Express E. coli expressing vGFP was lysed with NEBExpress T4 Lysozyme (T4L) in 50 mM Tris-HCl pH 7.5 or in NEBExpress E. coli Lysis Reagent (NEB #P8116S): 4 UOD600 of cell pellet were resuspended in 200 µl of Tris buffer or NEBExpress E. coli Lysis Reagent and lysed for 5 minutes at room temperature in the presence of NEBExpress T4 Lysozyme at 0, 0.1 or 1 ug per 1 ml of cell suspension. The soluble proteins were harvested by centrifugation and analyzed on SDS-PAGE.*

NEBExpress® *E. coli* Lysis Reagent

25°

#P8116S 100 ml
#P8116L 500 ml

- *Scalable lysis reactions from small to large bacterial cell pellets and compatible with high-throughput workflows*
- *Compatible with analyses such as SDS-PAGE, Western blots, activity assay, immunoprecipitation, and downstream purification*

NEBExpress® *E. coli* Lysis Reagent is a chemical lysis solution composed of a proprietary mix of non-ionic and zwitterionic detergents and Tris-based buffer. It allows disruption of *E. coli* cells without denaturing soluble proteins. It is ideal for extracting proteins, especially thermosensitive proteins vulnerable to mechanical lysis procedures, and can disrupt most Gram-negative bacterial cells. Provided as a ready-to-use liquid that is stable at room temperature.

Purification Beads, Columns and Resin Selection Chart

Isolation of pure substrates or proteins for downstream experiments is a common, yet time consuming, task. New England Biolabs offers a variety of resins and magnetic beads that are easy-to-use, highly specific, and available in several different formats for rapid isolation and purification of proteins, nucleic acids and immunoglobulins. NEB's magnetic beads are ideally suited for applications involving high-throughput proteomic screening, small-scale protein isolation, immunomagnetic isolations or cell separation experiments. With magnetic beads, affinity purification of tagged proteins, antigens, antibodies and nucleic acids can be done conveniently and quickly. Immobilized substrates remain biologically active and can be eluted in small volumes or serve as ligands in subsequent pull-down or target interaction experiments involving DNA or proteins. NEB's resins enable simple, one-step purification strategies for tagged proteins, resulting in proteins of high yield and purity. For the full list of products available for protein expression and purification, visit **www.neb.com/ ProteinExpression**.

Product	Protein Purification	Large-scale Purifications	Use in Automated Chromatography	High-throughput	Biotinylated Substrate Binding	Protein Pull-down	Nucleic Acid Pull-down	mRNA Purification/ Pull-down	Immuno-precipitation
NEBExpress Ni-NTA Magnetic Beads (NEB #S1423)	● His-tag			●		●			
NEBExpress Ni Spin Columns (NEB #S1427)	● His-tag			●		●			
NEBExpress® Ni Resin (NEB #S1428)	● His-tag	●	●			●			
Amylose Resin (NEB #E8021)	● MBP	●				●			
Amylose Resin High Flow (NEB #E8022)	● MBP	●	●			●			
Amylose Magnetic Beads (NEB #E8035)	● MBP			●		●			
Chitin Resin (NEB #S6651)	● Intein-CBD tag	●				●			
Chitin Magnetic Beads (NEB #E8036)	● Intein-CBD tag			●		●			
Oligo d(T)$_{25}$ Magnetic Beads (NEB #S1419)				●			● Poly(A)-tailed	● Poly(A)-tailed	
Streptavidin Magnetic Beads (NEB #S1420)				●	●	● Biotinylated bait	● Biotinylated bait		
Hydrophilic Streptavidin Magnetic Beads (NEB #S1421)				●	●	● Biotinylated bait	● Biotinylated bait		
Protein A Magnetic Beads (NEB #S1425)				●					●
Protein G Magnetic Beads (NEB #S1430)				●					●
Magnetic mRNA Isolation Kit (NEB #S1550)				●				● Poly(A)-tailed	

Polyhistidine-tagged Protein Purification

NEBExpress Ni-NTA Magnetic Beads
#S1423S 1 ml
#S1423L 5 ml

NEBExpress® Ni Resin
#S1428S 25 ml

NEBExpress Ni Spin Columns
#S1427S 10 Each
#S1427L 25 Each

TEV Protease
#P8112S 1,000 units

NEBExpress Ni-NTA Magnetic Beads: An affinity matrix for the small-scale isolation and purification of polyhistidine-tagged (His-tagged) fusion proteins in manual or automated formats. High specific binding yields purities of > 95% in a single-purification step. Matrix tolerates a wide range of conditions, including the presence of denaturants and detergents. Compatible with commercially available detergent-based cell lysis reagents. Elution can be achieved by protonation, ligand exchange (with imidazole) or extraction of the metal ion by a strong chelator (e.g., EDTA).

- Support Matrix: Spherical, agarose based superparamagnetic microparticles ranging in size from 20-100 µm.
- Binding Capacity: Varies with target, typically ≥ 7.5 mg His-tagged fusion protein/ml bed volume.

NEBExpress Ni Resin: NEBExpress Ni Resin is an affinity matrix for the isolation and purification of polyhistidine-tagged (His-tagged) fusion proteins. It is intended for use in gravity or pressure flow columns and batch purifications, and high specific binding yields purities of > 95% in a single-purification step. NEBExpress Ni Resin is comprised of a highly uniform and chemical-tolerant resin that is pre-charged with nickel ions on the matrix surface. It is resistant to a wide range of chemicals, including NaOH, EDTA, and commonly used reducing agents such as TCEP, DTT, and β-mercaptoethanol. Can be used under native or denaturing conditions.

- Support Matrix: Spherical, agarose based microparticles ranging in size from 10-100 µm.
- Binding Capacity: 1 ml of NEBExpress Ni Resin will bind ≥ 10 mg of His-tagged fusion protein.

NEBExpress Ni Spin Columns: NEBExpress Ni Spin columns are pre-packed with agarose-based microparticles ranging in size from 10-100 µm for the small-scale isolation and purification of polyhistidine-tagged (His-tagged) fusion proteins. Purification can be performed under native or denaturing conditions, including conditions in which EDTA or reducing reagents are required, yielding highly pure target protein in a single purification step. This enables screening of expression conditions and streamlines the functional and structural characterization of the target protein.

- Support Matrix: Spherical, agarose based microparticles ranging in size from 10-100 µm.
- Binding Capacity: Varies with target, ≥ 1 mg His-tagged fusion protein per column.

TEV Protease: TEV Protease, also known as Tobacco Etch Virus (TEV) Protease, is a highly specific cysteine protease that recognizes the amino-acid sequence Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser) and cleaves between the Gln and Gly/Ser residues. It is often used for the removal of affinity purification tags such as maltose-binding protein (MBP) or polyhistidine from fusion proteins. TEV Protease has a 7XHis-tag for easy removal from a reaction using nickel affinity resins and has been engineered to improve thermal stability and decrease autolysis.

Maltose Binding Protein (MBP) Purification

Amylose Resin
#E8021S 15 ml
#E8021L 100 ml

Amylose Resin High Flow
#E8022S 15 ml
#E8022L 100 ml

Amylose Magnetic Beads
#E8035S 25 mg

Anti-MBP Monoclonal Antibody
#E8032S 0.05 ml
#E8032L 0.25 ml

Amylose Resin and Amylose Resin High Flow: Affinity matrix used for isolation of proteins fused to maltose-binding protein. It is a composite amylose/agarose bead. Amylose Resin High Flow is a more rigid bead, suitable for use in automated chromatography systems.

Binding Capacity: Amylose Resin and Amylose Resin High Flow: > 4 mg MBP5-paramyosin ΔSal fusion protein/ml of bed volume.

Amylose Magnetic Beads: Affinity matrix for the small-scale isolation and purification of MBP-fusion proteins. Amylose is covalently coupled to a paramagnetic particle through a linkage that is stable and leak resistant over a wide pH range.

Support Matrix: 10 µM superparamagnetic particles.

Binding Capacity: 1 mg of Amylose Magnetic Beads will bind ≥ 10 µg of MBP-fusion protein.

Anti-MBP Monoclonal Antibody: Anti-MBP Monoclonal Antibody is a murine anti-maltose-binding protein antibody, isotype IgG2a. It is purified from tissue culture supernatant by protein A affinity chromatography.

Chitin Binding Domain (CBD) Purification

Chitin Resin
#S6651S 20 ml
#S6651L 100 ml

Chitin Magnetic Beads
#E8036S 5 ml

Anti-CBD Monoclonal Antibody
#E8034S 0.05 ml

Companion Product:

IMPACT Kit
#E6901S 1 set

Chitin Resin: An affinity matrix for the isolation of target proteins fused on an intein-chitin binding domain (CBD). Strong specific binding enables purification of highly pure protein from crude lysates in one step. Removal of CBD-tag during elution typically yields highly pure, native protein without the use of a protease.

Support Matrix: Approximately 50-70 µm microparticles

Binding Capacity: 2.0 mg maltose-binding protein/ml bed volume released from the resin after cleavage of the fusion protein expressed from pMYB5.

Chitin Magnetic Beads: An affinity matrix for the small-scale isolation of target proteins fused to a chitin binding domain (CBD). Chitin beads have been preparedwith encapsulated magnetite, thereby permitting the magnetic isolation of CBD-fusion proteins from cell culture supernatants. Removal of CBD-tag during elution typically yields highly pure, native protein.

Support Matrix: Approximately 50-70 µm paramagnetic microparticles

Binding Capacity: 2 mg chitin binding domain protein / ml bed volume released

Anti-CBD Monoclonal Antibody: Anti-CBD Monoclonal Antibody is a murine anti-chitin binding domain (CBD) antibody, isotype IgG1. It has high purity and specificity for chitin binding domain tag, and is verified for use in both Western blotting and ELISA.

Magenetic Bead Purification Products

Oligo $d(T)_{25}$ Magnetic Beads
#S1419S 5 ml

Magnetic mRNA Isolation Kit
#S1550S 25 isolations

Streptavidin Magnetic Beads
#S1420S 5 ml

Hydrophilic Streptavidin Magnetic Beads
#S1421S 5 ml

Protein A Magnetic Beads
#S1425S 1 ml

Protein G Magnetic Beads
#S1430S 1 ml

Companion Product:

96-Well Microtiter Plate Magnetic Separation Rack
#S1511S 96 wells

- *Small-scale purification or immunoprecipitation of IgG species*
- *No centrifugation required*
- *Regenerate matrix without binding capacity loss*

Oligo $d(T)_{25}$ Magnetic Beads: These beads enable small-scale isolations of mRNA from a variety of samples, including *in vitro* transcribed mRNA, total RNA, crude cell lysates and tissue. The selectivity for mRNA results from the annealing of bead-linked oligo $d(T)_{25}$ to the poly(A) region present in most eukaryotic mRNAs.

- Support Matrix: 1 µm non-porous superparamagnetic microparticles
- Binding Capacity: ≥ 5 µg rA30 per mg of beads

Magnetic mRNA Isolation Kit: The Magnetic mRNA Isolation Kit is designed to isolate intact poly(A)$^+$ RNA from cells and tissue without requiring phenol or other organic solvents. The technology is based on the coupling of Oligo $d(T)_{25}$ to 1 µm paramagnetic beads, which is then used as the solid support for the direct binding of poly(A)$^+$ RNA.

Streptavidin Magnetic Beads: The beads provide fast magnetic response times and reaction kinetics, and they have high binding capacity and sensitivity while retaining their physical integrity. They can be used to capture biotin-labeled substrates including DNA, RNA, peptides, antigens, antibodies and other proteins of interest in manual or automated workflows. These beads typically exhibit lower non-specific binding of proteins.

- Support Matrix: 1 µm non-porous superparamagnetic microparticles
- Binding Capacity: ≥ 30 µg biotinylated antibody per mg of beads or > 500 pmol of single-stranded 25 bp biotinylated oligonucleotide per mg of beads

Hydrophilic Streptavidin Magnetic Beads: The beads provide rapid magnetic response times and reaction kinetics, and they have high binding capacity and sensitivity while retaining their physical integrity. They can be used to capture biotin-labeled substrates including DNA, RNA, peptides, antigens, antibodies and other proteins of interest in manual or automated workflows. These beads typically exhibit lower non-specific binding of nucleic acids.

- Support Matrix: 2 µM non-porous superparamagnetic microparticles
- Binding Capacity: > 400 pmol of single-stranded 25 bp biotinylated oligonucleotide per mg of beads

Protein A and Protein G Magnetic Beads: The beads allow for isolation of most mammalian immunoglobulins (IgGs) and are amenable to immunoprecipitation. Predominant Fc-binding allows optimal IgG orientation upon binding to the outer surface of the Protein A and Protein G Magnetic Beads, enabling Fab regions to efficiently bind antigen. These beads can be used to immunoprecipitate target proteins from crude cell lysates using a selected primary antibody. In addition, specific antibodies can be chemically cross-linked to the Protein A- or Protein G-coated surface to create a reusable immunoprecipitation bead, thereby avoiding the co-elution of antibody with the target antigen.

- Support Matrix: 2 µm non-porous superparamagnetic microparticles
- Binding Capacity: > 280 µg of Human IgG per ml of beads

Magnetic Separation Racks

Product	Application	Magnets	Capacity	Convenience
6-Tube Magnetic Separation Rack (NEB #S1506)	Designed for small-scale separations using magnetic particles	Neodymium rare earth permanent magnets	6 tubes (1.5 ml)	Use with magnetic particle-based affinity purification for rapid, small-scale purifications
50 ml Magnetic Separation Rack (NEB #S1507)	Designed for small-scale separations using magnetic particles	Neodymium rare earth permanent magnets	4 tubes (50 ml)	Use with magnetic particle-based affinity purification for rapid, streamlined purifications
12-Tube Magnetic Separation Rack (NEB #S1509)	Designed for small-scale separations using magnetic particles	Neodymium rare earth permanent magnets	12 tubes (1.5 ml)	Use with magnetic particle-based affinity purification for rapid, small-scale purifications
96-Well Microtiter Plate Magnetic Separation Rack (NEB #S1511)	Designed for use with commercially available high-flanged 100 µl to 300 µl flat-bottom 96-well microplates	24 side-pull magnetic pins attract magnetic beads from solution to the side walls of four adjacent wells	96-well	The orientation of the magnetic field ensures complete removal of the magnetic beads from solution during pipetting steps, thereby minimizing sample loss
NEBNext Magnetic Separation Rack (NEB #S1515)	Designed for rapid and effective small-scale separations of magnetic particles	Anodized aluminum rack with Neodymium Iron Boron (NdFeB) rare earth magnets	24 tubes (0.2 ml)	Next generation sequencing library preparation workflows include magnetic bead-based purification and size-selection steps. It is important for library yield and quality that bead separation be highly efficient and fast, and this is enabled by the powerful fixed magnet cores in this rack.

View bees flying in a pollinator garden in 3D.

EXPLORE 3D

Wildflowers
Credit: S.H.exclusiv, Adobe Stock

Encouraging Sustainable Labs and Green Spaces for Well-being

Research laboratories are not only energy-intensive environments — consuming 3 – 10 times more energy than standard office spaces — but these spaces can also contribute to high stress levels among researchers. Recognizing these dual issues, Jim Chadwick, a Ph.D. student at The Pirbright Institute in the UK, embarked on two initiatives. Through a campaign promoting sustainable lab practices and the creation of a community allotment and wildflower garden to enhance well-being, he tackles both environmental impact and mental health from the ground up.

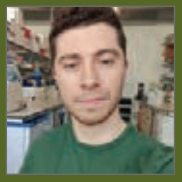
Chadwick's first initiative was an energy literacy study called the "Switch-off Study". It addressed the undersupply of information and discussion about energy use by employing simple yet effective strategies focused on raising energy awareness and promoting sustainable behaviors. Using a nudge-based approach, he developed a system of color-coded stickers placed on lab equipment. These stickers, designed like traffic lights, indicated whether equipment should be turned off when not in use (green), turned off at the end of the day (orange), or left on continuously (red) due to critical functions like refrigeration. This visual cue system encouraged researchers to consider their energy consumption habits and make conscious decisions about equipment use.

To measure the impact, Chadwick collaborated with the institute's engineering department to monitor energy usage at the circuit level — a challenging task since energy monitoring typically occurs at the building or floor scale. Over time, the study revealed a significant reduction in energy consumption, averaging savings of 30 kilowatt-hours per day across the participating labs. This grassroots effort reduced the institute's carbon footprint and sparked broader conversations among lab members, shifting the culture to one that prioritizes sustainability. It was so successful that it contributed to a white paper, *Transforming to Zero: Changing Behaviors to Decarbonize Laboratories,* which outlines practical strategies for achieving net-zero emissions in research spaces.

Parallel to his energy initiative, Chadwick's Community Allotments project focuses on improving mental health among researchers, particularly Ph.D. students. Stress, anxiety and burnout are common in high-pressure research environments. His project involved revitalizing 2,000 sq. meters of neglected space at the research institute, transforming it into a community allotment and pollinator-friendly wildflower garden. Plans for the garden include community events, social gatherings, and even friendly gardening competitions to strengthen social bonds and create a supportive environment.

Chadwick's motivation stems from a deep commitment to combating climate change and improving mental health within the research community. Recognizing that small, consistent actions contribute to significant change, he asked himself, *"Why shouldn't it be me who makes a difference?"* He took initiative where others might have hesitated.

Ultimately, Jim Chadwick's initiatives highlight the profound impact that individual passion and leadership can have on larger systemic issues. By addressing sustainability and mental health in tandem, he offers a model for how the scientific community can evolve to meet the challenges of the future. His work serves as an inspiration, demonstrating that grassroots projects can lead to meaningful change when driven by dedication and a collaborative spirit.



Jim Chadwick
University of Oxford, Oxford, UK
2024 Passion in Science
Environmental Stewardship Award

Competent Cells

NEB® has a competent cell strain for your needs.

Choose the right cells for your cloning and protein expression applications from NEB's portfolio of high efficiency competent cell strains.

For cloning experiments, choose from a variety of formats, including chemical and electrocompetent. These *E. coli* strains are T1 phage resistant and are Endonuclease I-deficient for high-quality plasmid preparations. Additionally, all competent cells from NEB are free of animal products.

NEB also offers a wide variety of competent cell strains ideal for many protein expression applications. These strains address the needs of difficult protein expression control, toxic protein expression and cytoplasmic disulfide bond formation. NEBExpress®, T7 Express and SHuffle® strains are available with varying levels of control. I^q strains feature added control from increased supply of Lac repressor (*lacI*^{q}). Only NEB offers the exceptional control of expression from the *lysY* gene that reduces basal expression from T7 strains without inhibiting IPTG-induced expression. Our Lemo21(DE3) strain features tunable T7 expression for difficult targets. Each strain is provided with a detailed protocol for optimal expression.

NO DRY ICE CHARGES
with Competent Cells from NEB

Featured Products

Featured Tools & Resources

Visit **www.neb.com** to find additional online tools, including our Competitor Cross-reference Tool for comparing NEB strains to other commercially available strains.

Find tips for successful transformation.

Competent Cell Strain Properties

There are many properties to consider when choosing a strain for your experiments. Requirements such as plasmid preparation, blue/white screening, proper disulfide bond formation and fast colony growth necessitate specific strain choices. The following selection chart highlights the characteristics of NEB's strains to help select the optimal strain for a particular experiment.

CAUTION: Chemically Competent *E. coli* contain DMSO, a hazardous material. Review the MSDS before handling.

Cloning Strain Properties

Strain Properties	Features	Transformation Efficiency (cfu/µg)		Available Formats [7]	Outgrowth Medium & Control Plasmid Included?	Strain Background	Library Construction
		Chemical	Electrocompetent				
dam⁻/dcm⁻	• Dam/Dcm methyltransferase free plasmid growth	1-3 x 10^6	N/A	50, 200	●	K12	
NEB Turbo (High Efficiency)	• Fastest growth – colonies visible after 6.5 hours • Plasmid preparation after 4 hours	1-3 x 10^9	N/A	50, 200	●	K12	●
NEB 5-alpha (High Efficiency)	• Versatile cloning strain • DH5α™ derivative	1-3 x 10^9 [8]	N/A	50, 200, 96, 384, Strips	●	K12	●
NEB 5-alpha F´ I^q (High Efficiency)	• Toxic gene cloning • F´ strain with extremely high transformation efficiency	1-3 x 10^9	N/A	50, 200	●	K12	●
NEB 10-beta (High Efficiency)	• Large plasmid and BAC cloning • DH10B™ derivative	1-3 x 10^9 [9]	> 2 x 10^{10}	50, 200, 96	●	K12	●
NEB Stable (High Efficiency)	• Cloning unstable inserts • Isolating and propagating retroviral/ lentiviral clones	1-3 x 10^9	N/A	50, 200	●	K12	●
NEB 5-alpha (Subcloning Efficiency)	• Ideal for subcloning efficiency transformations, such as plasmid transformation or routine subcloning	> 1 x 10^6	N/A	400		K12	

Protein Expression Strain Properties

Strain Properties	Features	Chemical Transformation Efficiency (cfu/µg)	Available Formats [7]	Outgrowth Medium & Control Plasmid Included?	Strain Background	Library Construction
NEBExpress	• Versatile non-T7 expression strain • Protease deficient	0.6-1 x 10^9	50, 200	●	B	●
BL21(DE3)	• Routine T7 expression	1-5 x 10^7	50, 200	●	B	
Lemo21(DE3)	• Tunable T7 expression for difficult targets	1-3 x 10^7	50	● [1]	B	
NiCo21(DE3)	• Improved purity of target proteins isolated by IMAC	1-5 x 10^7	50	●	B	
BL21	• Routine non-T7 expression	1-5 x 10^7	50	●	B	
T7 Express	• Most popular T7 expression strain • Protease deficient	0.6-1 x 10^9	50, 200	●	B	●
T7 Express *lysY*	• T7 expression • Protease deficient • Better reduction of basal expression	0.6-1 x 10^9	200		B	●
T7 Express *lysY/I^q*	• T7 expression • Protease deficient • Highest level of expression control	0.6-1 x 10^9	200		B	●
SHuffle T7	• T7 expression/K12 strain • Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm	1 x 10^6	50		K12	
SHuffle Express	• Protease deficient/B strain • Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm	1 x 10^7	50		B	
SHuffle T7 Express	• T7 expression • Protease deficient/B strain • Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm	1 x 10^7	50		B	
SHuffle T7 Express *lysY*	• T7 expression • Protease deficient/B strain • Tightly controlled expression of toxic proteins • Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm	1 x 10^7	50		B	
NEBExpress I^q	• Control of IPTG induced expression from P_{lac}, P_{tac}, P_{trc} and $T5_{lac}$ • Protease deficient	0.6-1 x 10^9	200		B	●

	lacI^{q}	F´	*endA*$^{-}$ (2)	*recA*$^{-}$	Blue/White Screening	Drug Resistance (5)	Methylation Phenotype
			•			cam, str, nit	*Dam*$^{-}$, *Dcm*$^{-}$, M. EcoKI^{+}
	•	•	•		•	nit	*Dam*$^{+}$, *Dcm*$^{+}$, M. EcoKI^{-}
			•	•	•	none	*Dam*$^{+}$, *Dcm*$^{+}$, M. EcoKI^{+}
	•	•	•	•	•	tet	*Dam*$^{+}$, *Dcm*$^{+}$, M. EcoKI^{+}
			•	•	•	str	*Dam*$^{+}$, *Dcm*$^{+}$, M. EcoKI^{-}
	•	•	•	•	•	tet, str	*Dam*$^{+}$, *Dcm*$^{+}$, M. EcoKI^{-}
			•	•	•	none	*Dam*$^{+}$, *Dcm*$^{+}$, M. EcoKI^{+}

(1) Rhamnose solution is provided instead of SOC; control plasmid is included.
(2) Important for high-quality plasmid preparation.
(3) Lacks Lon and OmpT protease activity.
(4) Constitutively expresses a chromosomal copy of the disulfide bond isomerase DsbC.
(5) nit = nitrofurantoin, tet = tetracycline, cam = chloramphenicol, str = streptomycin, spec = spectinomycin
(6) Resistance to low levels of streptomycin may be observed.
(7) 50 = 50 µl tubes
200 = 200 µl tubes
96 = 96 well plate
384 = 384 well plate
strips = 96 tube strips (50 µl/tube)
400 = 400 µl tubes
(8) 1-5 x 10^{8} for R-format.
(9) 1-3 x 10^{8} for P-format.

	lacI^{q}	F´	*endA*$^{-}$	*lysY*	Protease Deficient (3)	T7 RNA Polymerase	Cytoplasmic Disulfide Bond Formation (4)	Drug Resistance (5)	Methylation Phenotype
			•		•			nit	*Dam*$^{+}$, *Dcm*$^{-}$, M. EcoKI^{-}
					•	•		none	*Dam*$^{+}$, *Dcm*$^{-}$, M. EcoKI^{-}
				•	•	•		cam	*Dam*$^{+}$, *Dcm*$^{-}$, M. EcoKI^{-}
					•	•		none	*Dam*$^{+}$, *Dcm*$^{-}$, M. EcoKI^{-}
					•			none	*Dam*$^{+}$, *Dcm*$^{-}$, M. EcoKI^{-}
			•		•	•		nit	*Dam*$^{+}$, *Dcm*$^{-}$, M. EcoKI^{-}
			•	•	•	•		cam, nit	*Dam*$^{+}$, *Dcm*$^{-}$, M. EcoKI^{-}
	•		•	•	•	•		cam, nit	*Dam*$^{+}$, *Dcm*$^{-}$, M. EcoKI^{-}
	•	•				•	•	str, spec, nit	*Dam*$^{+}$, *Dcm*$^{+}$, M. EcoKI^{+}
	•		•		•		•	spec(6), nit	*Dam*$^{+}$, *Dcm*$^{-}$, M. EcoKI^{-}
	•		•		•	•	•	spec(6), nit	*Dam*$^{+}$, *Dcm*$^{-}$, M. EcoKI^{-}
	•		•	•	•	•	•	cam, spec(6), nit	*Dam*$^{+}$, *Dcm*$^{-}$, M. EcoKI^{-}
	•		•		•			cam, nit	*Dam*$^{+}$, *Dcm*$^{-}$, M. EcoKI^{-}

Competitor Cross Reference

Using another competent cell strain? Try our **Competitor Cross Reference Tool** to find out which NEB strain is compatible.

NEBcloner®

For help with choosing the right competent cell strain, try **NEBcloner at NEBcloner.neb.com**.

Learn how to perform a transformation.

NEB Cloning Competent *E. coli* Sampler

#C1010S 8 x 0.05 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Outgrowth medium and control plasmid included*
- *Value pricing*
- *Free of animal products*

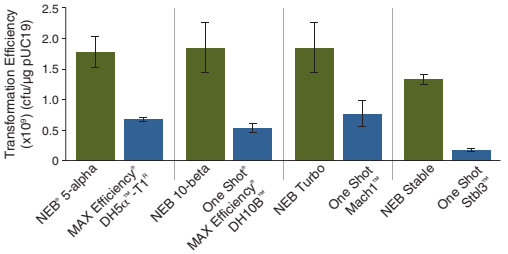
Description: A sample pack of four cloning strains of *E. coli* suitable for high efficiency transformation.

Please refer to the individual datacards for each reagent's recommended use and storage conditions.

Transformation Efficiency: 1-3 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 *(fhuA2)*

Sensitivity: Nit, Spec



Benefit from high transformation efficiencies. *Transformation efficiencies were compared using manufacturers' recommended protocols. Values shown are the average of triplicate experiments.*

Kit Includes:

- NEB 5-alpha Competent *E. coli* (High Efficiency)
- NEB 10-beta Competent *E. coli* (High Efficiency)
- NEB Stable Competent *E. coli* (High Efficiency)
- NEB Turbo Competent *E. coli* (High Efficiency)
- SOC Outgrowth Medium
- NEB 10-beta/Stable Outgrowth Medium
- pUC19 Vector

NEB Turbo Competent *E. coli* (High Efficiency)

#C2984H 20 x 0.05 ml
#C2984I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Tight expression control ($lacI^q$)*
- *Colonies visible after 6.5 hours*
- *Isolate DNA after 4 hours growth*
- *5 minute transformation protocol with Amp^R plasmids*
- *Free of animal products*

Description: *E. coli* cells featuring fast colony growth (6.5 hours) and tight expression control.

Genotype: F' *$proA^+B^+$ $lacI^q$ Δ(lacZ)M15 / fhuA2 Δ(lac-proAB) glnV galK16 galE15 R(zgb-210::Tn10)*Tet^S *endA1 thi-1 Δ(hsdS-mcrB)5*

Features:

- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Suitable for blue/white screening

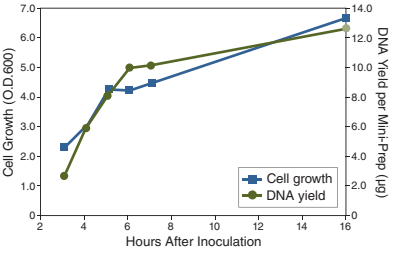
Transformation Efficiency:
1 - 3 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 *(fhuA2),* Nit

Sensitivity: Amp, Cam, Kan, Spec, Str, Tet

Reagents Supplied:

- pUC19 Vector
- SOC Outgrowth Medium



Miniprep DNA can be prepared from a single overnight colony after inoculation and only 3 hours growth. *DNA yield doubles after an additional hour of growth.*

NEB Turbo Transformation: *With NEB Turbo, colonies are visible after only 8 hours. Ligation products were transformed into 50 µl of NEB Turbo Competent E. coli and plated on LB/Amp. Plates were incubated for 8 hours, 10 hours and 12 hours at 37°C. NEB Turbo features fast colony growth and blue/white selection to simplify cloning experiments.*

What is the difference between chemical transformation and electroporation?

NEB 10-beta Competent *E. coli*

NEB 10-beta Competent *E. coli* (High Efficiency)
#C3019H 20 x 0.05 ml
#C3019I 6 x 0.2 ml
#C3019P 1 96-well plate

NEB 10-beta Electrocompetent *E. coli*
#C3020K 6 x 0.1 ml

Companion Product:

NEB 10-beta/Stable Outgrowth Medium
#B9035S 100 ml

- *Clone large plasmids and BACs*
- *DH10B derivative*
- *Free of animal products*

Description: A DH10B derivative suitable for a wide range of applications, including large plasmid and BAC cloning.

Genotype: *Δ(ara-leu) 7697 araD139 fhuA ΔlacX74 galK16 galE15 e14- Φ80dlacZΔM15 recA1 relA1 endA1 nupG rpsL* (Str^R) *rph spoT1 Δ(mrr-hsdRMS-mcrBC)*

Features:

- Efficient transformation of methylated DNA derived from eukaryotic sources or unmethylated DNA derived from PCR, cDNA and many other sources
- Suitable for blue/white screening without IPTG
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Reduced recombination of cloned DNA (*recA1*)

Transformation Efficiency: High Efficiency: 1–3 x 10^9 cfu/µg pUC19 DNA (NEB #C3019H, #C3019I); 1–3 x 10^8 cfu/µg pUC19 DNA (NEB #C3019P)

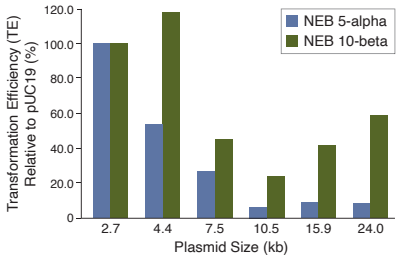
Electrocompetent: > 2 x 10^{10} cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 (*fhuA*), Str

Sensitivity: Amp, Cam, Kan, Nit, Spec, Tet

Reagents Supplied:

- NEB 10-beta/Stable Outgrowth Medium
- pUC19 Vector



Effect of Plasmid Size on Transformation Efficiency. *NEB 10-beta chemically competent cells are more efficiently transformed with large plasmids than NEB 5-alpha cells. The difference in TE between the two cell lines increases with the size of the plasmid being transformed.*

NEB 5-alpha Competent *E. coli*

NEB 5-alpha Competent *E. coli* (High Efficiency)
#C2987H 20 x 0.05 ml
#C2987I 6 x 0.2 ml
#C2987P 1 96-well plate
#C2987R 1 384-well plate
#C2987U 96 x 0.05 ml

NEB 5-alpha Competent *E. coli* (Subcloning Efficiency)
#C2988J 6 x 0.4 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *DH5α derivative*
- *Free of animal products*

Description: A DH5α derivative and versatile *E. coli* cloning strain.

Genotype: *fhuA2Δ(argF-lacZ)U169 phoA glnV44 Φ80Δ(lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*

Features:

- Efficient transformation of unmethylated DNA derived from PCR, cDNA and many other sources (*hsdR*)
- Suitable for blue/white screening
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Reduced recombination of cloned DNA (*recA1*)

Transformation Efficiency: High Efficiency: 1–3 x 10^9 cfu/µg pUC19 DNA (NEB #C2987H, #C2987I, #C2987P, #C2987U); 1–5 x 10^8 cfu/µg pUC19 DNA (NEB #C2987R)

Subcloning Efficiency: > 1 x 10^6 cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 (*fhuA2*)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:

- SOC Outgrowth Medium
- pUC19 Vector

* NEB 5-alpha Competent *E. coli* (Subcloning Efficiency) is not supplied with SOC Outgrowth Medium or pUC19 Control DNA.

NEB 5-alpha F'I^q Competent *E. coli* (High Efficiency)

#C2992H 20 x 0.05 ml
#C2992I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Tight expression control (lacI^q)*
- *F´ Strain with extremely high TE*
- *DH5α derivative*
- *Free of animal products*

Description: An F´ *E. coli* strain with extremely high transformation efficiency suitable for toxic gene cloning.

Genotype: F´ *proA$^+$B$^+$ lacI^q Δ(lacZ)M15 zzf::Tn10* (Tet^R) */ fhuA2Δ(argF-lacZ)U169 phoA glnV44 Φ80Δ(lacZ) M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*

Features:

- Efficient transformation of unmethylated DNA derived from PCR, cDNA and many other sources (*hsdR*)
- Suitable for blue/white screening
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Reduced recombination of cloned DNA (*recA1*)
- Suitable for propagation of M13 clones

Transformation Efficiency:
1-3 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 *(fhuA2),* Tet

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str

Reagents Supplied:

- SOC Outgrowth Medium
- pUC19 Vector

NEB Stable Competent *E. coli* (High Efficiency)

#C3040H 20 x 0.05 ml
#C3040I 6 x 0.2 ml

Companion Product:

NEB 10-beta/Stable Outgrowth Medium
#B9035S 100 ml

- *T1 phage resistance (fhuA)*
- *Free of animal products*
- *Carries endA mutation (isolated plasmids are free of EndoI)*
- *Ideal for cloning unstable inserts, as well as isolating and propagating retroviral/ lentiviral clones*
- *Compatible with DNA assembly reactions and ligation reactions*

Description: Chemically competent *E. coli* cells suitable for high efficiency transformation and isolation of plasmid clones containing repeat elements.

Genotype: F' *proA*$^{+}$*B*$^{+}$ *lacI*^{q} *Δ(lacZ)M15 zzf::Tn10* (Tet^{R})*/Δ(ara-leu) 7697 araD139 fhuA ΔlacX74 galK16 galE15 e14- Φ80dlacZΔM15 recA1 relA1 endA1 nupG rpsL* (Str^{R}) *rph spoT1 Δ(mrr-hsdRMS-mcrBC)*

Features:

- Activity of nonspecific endonuclease I (*endA1*) abolished for highest quality plasmid preparations
- Reduced recombination of cloned DNA (*recA1*)

Transformation Efficiency:
1-3 x 10^{9} cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 *(fhuA),* Str, Tet

Sensitivity: Amp, Cam, Kan, Nit, Spec

Reagents Supplied:

- NEB 10-beta/Stable Outgrowth Medium
- pUC19 Vector

dam^{-}*/dcm*$^{-}$ Competent *E. coli*

#C2925H 20 x 0.05 ml
#C2925I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Isolate plasmids free of Dam and Dcm methylation*
- *Free of animal products*

Description: Methyltransferase deficient *E. coli* cells suitable for growth of plasmids free of *Dam* and *Dcm* methylation.

Genotype: *ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 R(zgb210::Tn10)* Tet^{S} *endA1 rspL136* (Str^{R}) *dam13::Tn9* (Cam^{R}) *xylA-5 mtl-1 thi-1 mcrB1 hsdR2*

Features:

- Allows for propagation of plasmids free of *Dam* and *Dcm* methylation
- Activity of nonspecific endonuclease I (*endA1*) abolished for highest quality plasmid preparations

Transformation Efficiency:
1-3 x 10^{6} cfu/µg pUC19 DNA

Resistance: Phage T1 resistant (*fhuA31*), Cam, Nit, Str

Sensitivity: Amp, Kan, Spec, Tet

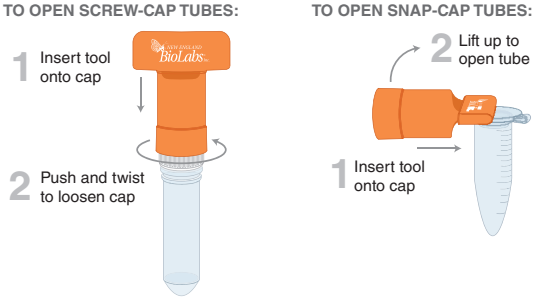
Reagents Supplied:

- pUC19 Vector
- SOC Outgrowth Medium

NEB Tube Opener

#C1008S 2 Each

Description: Use to open a variety of microcentrifuge tubes. Can be used for snap-on caps or screw-on caps.



BL21 Competent *E. coli*

#C2530H 20 x 0.05 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Ideal for P_{lac}, P_{tac}, P_{trc}, ParaBAD expression vectors*
- *Resistance to phage T1 (fhuA2)*
- *Protease deficient B strain*
- *Free of animal products*

Description: Widely used non-T7 expression *E. coli* strain. Suitable for transformation and protein expression. This strain does not express the T7 RNA Polymerase.

Genotype: *fhuA2 [lon] ompT gal [dcm] ΔhsdS*

Features:

- Deficient in proteases Lon and OmpT

Transformation Efficiency:
1–5 x 10^7 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 (*fhuA2*)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:

- pUC19 Vector
- SOC Outgrowth Medium

BL21(DE3) Competent *E. coli*

#C2527H 20 x 0.05 ml
#C2527I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Routine T7 expression*
- *Resistance to phage T1 (fhuA2)*
- *Protease deficient B strain*
- *Free of animal products*

Description: Widely used T7 expression *E. coli* strain.

Genotype: *fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5*

Features:

- Deficient in proteases Lon and OmpT

Transformation Efficiency:
1–5 x 10^7 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 (*fhu*A2)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:

- SOC Outgrowth Medium
- pUC19 Vector

Lemo21(DE3) Competent *E. coli*

#C2528J 12 x 0.05 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Expression of difficult targets*
- *Membrane protein expression*
- *Ideal for periplasmic expression*
- *Expression of toxic proteins*
- *Proteins with solubility issues*

Description: Lemo21(DE3) Competent *E. coli* is a tunable T7 expression strain designed for the expression of challenging proteins. A derivative of BL21(DE3), Lemo21(DE3) offers the host features of this popular expression strain, with the added benefit of being able to control expression levels by varying the level of T7 lysozyme (*lysY*), the natural inhibitor of T7 RNA Polymerase. The fine control of expression makes Lemo21(DE3) ideal for membrane proteins, toxic proteins, secreted proteins and proteins prone to insoluble expression.

Genotype: *fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS/* pLemo(Cam^R) *λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5*
pLemo = pACYC184-*PrhaBAD-lysY*

Features:

- Enhanced BL21(DE3) derivative
- Fine control of expression
- Greatest range of expression of any T7 strain (0-2,000 µM rhamnose)
- Potential elimination of inclusion body formation

Transformation Efficiency:
1–3 x 10^7 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 (fhuA2), Cam

Sensitivity: Amp, Kan, Nit, Spec, Str, Tet

Reagents Supplied:

- pUC19 Vector
- L-rhamnose solution

NiCo21(DE3) Competent *E. coli*

#C2529H 20 x 0.05 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Superior alternative to BL21(DE3) for routine protein expression*
- *Improved purity of target proteins isolated by IMAC*
- *Free of animal products*

Description: Poly-histidine tagged recombinant proteins that are isolated by immobilized metal affinity chromatography (IMAC) are often contaminated with significant amounts of endogenous *E. coli* metal binding proteins. The protein expression strain NiCo21(DE3) has been engineered to minimize *E. coli* protein contamination of IMAC fractions: GlmS is mutated to eliminate binding to IMAC resins and three other proteins (SlyD, ArnA and Can) are tagged to enable rapid removal by chitin affinity chromatography.

Genotype: *can::CBD fhuA2 [lon] ompT gal (λ DE3) [dcm] arnA::CBD slyD::CBD glmS6Ala ΔhsdS λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5*

Features:

- Identical growth characteristics as BL21(DE3)
- Deficient in proteases Lon and OmpT

Transformation Efficiency:
1–5 x 10^7 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 (*fhuA2*)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:

- pUC19 Vector
- SOC Outgrowth Medium

NEBExpress® Competent *E. coli* (High Efficiency)

#C2523H 20 x 0.05 ml
#C2523I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Enhanced BL21 derivative ideal for P_{lac}, P_{tac}, P_{trc} expression vectors*
- *Fast growth from colonies*
- *Free of animal products*
- *Protease deficient*

Description: A versatile non-T7 expression *E. coli* strain. NEBExpress is the recommended host strain for the NEBExpress MBP Protein Fusion and Purification System (NEB #E8201).

Genotype: *fhuA2 [lon] ompT gal sulA11 R(mcr-73::miniTn10--Tet^S)2 [dcm] R(zgb-210::Tn10--Tet^S) endA1 Δ(mcrC-mrr)114::IS10*

Features:

- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA

Transformation Efficiency:
0.6-1 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 *(fhuA2),* Nit

Sensitivity: Amp, Cam, Kan, Spec, Str, Tet

Reagents Supplied:

- SOC Outgrowth Medium
- pUC19 Vector

NEBExpress® I^q Competent *E. coli* (High Efficiency)

#C3037I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Enhanced BL21 derivative ideal for P_{lac}, P_{tac}, P_{trc}, P_{T5} expression vectors*
- *Better control of IPTG induced expression with non-T7 plasmids*
- *Fast growth from colonies*
- *$lacI^q$ reduces basal expression*
- *Protease deficient*
- *Free of animal products*

Description: *E. coli* cells featuring control of IPTG induced expression with non-T7 plasmids.

Genotype: MiniF *$lacI^q$* (Cam^R) / *fhuA2 [lon] ompT gal sulA11 R(mcr-73::miniTn10--Tet^S)2 [dcm] R(zgb-210::Tn10--Tet^S) endA1 Δ(mcrC-mrr)114::IS10*

Features:

- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA
- Ideal for controlled protein expression from pUC19 and pUC19 derivatives

Transformation Efficiency:
0.6-1 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 *(fhuA2),* Cam, Nit

Sensitivity: Amp, Kan, Spec, Str, Tet

T7 Express Competent *E. coli* (High Efficiency)

#C2566H 20 x 0.05 ml
#C2566I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Enhanced BL21 derivative*
- *Popular T7 expression strain*
- *Fast growth from colonies*
- *Free of animal products*

Description: Enhanced BL21 *E. coli* derivative for T7 expression.

Genotype: *fhuA2 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10--*Tet^S*) endA1 Δ(mcrC-mrr)114::IS10*

Features:

- T7 RNA Polymerase in the *lac* operon - no λ prophage
- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA

Transformation Efficiency:
0.6-1 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 *(fhuA2),* Nit

Sensitivity: Amp, Cam, Kan, Spec, Str, Tet

Reagents Supplied:

- pUC19 Vector
- SOC Outgrowth Medium

T7 Express *lysY* Competent *E. coli* (High Efficiency)

#C3010I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Enhanced BL21 derivative*
- *T7 Lysozyme for expression control*
- *Clone toxic genes*
- *Fast growth from colonies*
- *Free of animal products*

Description: Enhanced BL21 *E. coli* derivative for T7 expression with enhanced reduction of basal expression.

Genotype: MiniF *lysY* (Cam^R) */ fhuA2 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10--*Tet^S*) endA1 Δ(mcrC-mrr)114::IS10*

Features:

- T7 RNA Polymerase in the lac operon - no λ prophage
- Control of T7 RNA Polymerase by T7 lysozyme allows potentially toxic genes to be expressed
- LysY is a variant of T7 lysozyme lacking amidase activity, thus cells are less susceptible to lysis during induction
- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA
- No Cam requirement

Transformation Efficiency:
0.6-1 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 *(fhuA2),* Cam, Nit

Sensitivity: Amp, Kan, Spec, Str, Tet

T7 Express *lysY/I^q* Competent *E. coli* (High Efficiency)

#C3013I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Enhanced BL21 derivative*
- *Tight control of expression (lacI^q)*
- *Highest level of expression control*
- *Clone toxic genes*
- *Fast growth from colonies*
- *Free of animal products*

Description: Enhanced BL21 *E. coli* derivative with highest level of T7 expression control.

Genotype: MiniF *lysY lacI^q*(Cam^R) */ fhuA2 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10--*Tet^S*) endA1 Δ(mcrC-mrr) 114::IS10*

Features:

- T7 RNA Polymerase in the *lac* operon - no λ prophage
- Tight control of expression by *lacI^q* allows potentially toxic genes to be cloned
- Control of T7 RNA Polymerase by T7 lysozyme allows toxic genes to be expressed
- LysY is a variant of T7 lysozyme lacking amidase activity, thus cells are less susceptible to lysis during induction
- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA
- No Cam requirement

Transformation Efficiency:
0.6-1 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 *(fhuA2),* Cam, Nit

Sensitivity: Amp, Kan, Spec, Str, Tet

Disulfide Bonds

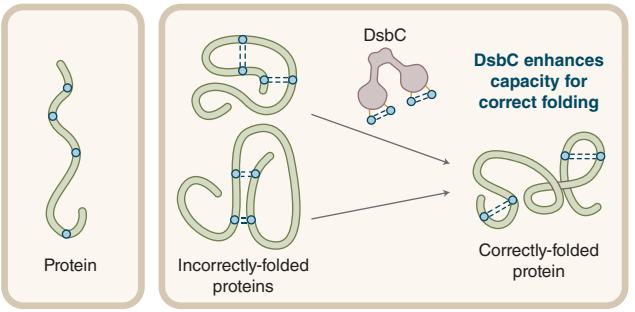
Features of SHuffle® Strains:

- Engineered *E. coli* K12 or B strains promote disulfide bond formation in the cytoplasm
- Constitutively expresses a chromosomal copy of the disulfide bond isomerase DsbC
- DsbC promotes the correction of mis-oxidized proteins into their correct form
- The cytoplasmic DsbC is a chaperone that can also assist in the folding of proteins that do not require disulfide bonds
- Alternative expression strain for proteins that do not fold in wild-type *E. coli*, independent of redox state

SHuffle strains from NEB are engineered *E. coli* strains capable of expressing proteins with increasing disulfide bond complexity in the cytoplasm. SHuffle strains express the disulfide bond isomerase DsbC within the cytoplasm. DsbC isomerizes mis-oxidized substrates into their correctly folded state, greatly enhancing the fidelity of disulfide bond formation. Cytoplasmic expression also results in significantly higher protein yields of disulfide bonded proteins when compared to periplasmic expression. SHuffle strains are sensitive to kan, amp, tet and in most cases, cam, which makes them able to express proteins from a wide variety of expression vectors, offering greater versatility in experimental design.

References:

References for properties and applications for these products can be found at www.neb.com.



Disulfide bond formation in the cytoplasm of wild type E. coli is not favorable, while SHuffle is capable of correctly folding proteins with multiple disulfide bonds in the cytoplasm.

SHuffle® Express Competent *E. coli*

#C3028J 12 x 0.05 ml

- *Folds disulfide bonded proteins in the cytoplasm*
- *Protease deficient B strain*
- *Enhanced BL21 derivative*
- *Free of animal products*

Description: *E. coli* cells with enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm.

Genotype: *fhuA2 [lon] ompT ahpC gal* λatt::*pNEB3-r1-cDsbC* (Spec^R, *lacI*^{q}) *ΔtrxB sulA11* R(*mcr-73::miniTn10--*Tet^S)*2 [dcm] R(zgb-210::Tn10 --*Tet^S) *endA1 Δgor Δ(mcrC-mrr)114::IS10*

Transformation Efficiency:

1 x 10^7 cfu/µg pUC19 DNA

Resistance:

Resistance to phage T1 (*fhuA2*), Nit, Spec and Str*.
*Note that resistance to low levels of streptomycin may be observed.

Sensitivity: Amp, Cam, Kan, Tet

SHuffle® T7 Express Competent *E. coli*

#C3029J 12 x 0.05 ml

- *Folds disulfide bonded proteins in the cytoplasm*
- *T7 expression*
- *Protease deficient B strain*
- *Enhanced BL21 derivative*
- *Free of animal products*

Description: T7 Expression *E. coli* strain with enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm.

Genotype: *fhuA2 lacZ::T7 gene1 [lon] ompT ahpC gal λatt::pNEB3-r1-cDsbC* (Spec^R, *lacI*^{q}) *ΔtrxB sulA11 R(mcr-73::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10 --*Tet^S*) endA1 Δgor Δ(mcrC-mrr)114::IS10*

Transformation Efficiency:

1 x 10^7 cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 *(fhuA2)*, Nit, Spec and Str*.

*Note that resistance to low levels of streptomycin may be observed.

Sensitivity: Amp, Cam, Kan, Tet

What is a disulfide bond?

SHuffle® T7 Competent *E. coli*

#C3026J 12 x 0.05 ml

- *Folds disulfide bonded proteins in the cytoplasm*
- *T7 expression*
- *K12 strain*
- *Free of animal products*

Description: T7 Expression *E. coli* strain with enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm.

Genotype: F´ *lac, pro, lacI*^{q} */ Δ(ara-leu)7697 araD139 fhuA2 lacZ::T7 gene1 Δ(phoA)PvuII phoR ahpC* galE (or U) galK λatt::pNEB3-r1-cDsbC* (Spec^{R}, *lacI*^{q}) *ΔtrxB rpsL150*(Str^{R}) *Δgor Δ(malF)3*

Transformation Efficiency:
1 x 10^{6} cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 *(fhuA2)*, Nit, Str, Spec

Sensitivity: Amp, Cam, Kan, Tet

SHuffle® T7 Express *lysY* Competent *E. coli*

#C3030J 12 x 0.05 ml

- *Express toxic proteins (lysY)*
- *Folds disulfide bonded proteins in the cytoplasm*
- *T7 expression*
- *Protease deficient B strain*
- *Free of animal products*
- *Enhanced BL21 derivative*

Description: *E. coli* strain with tight T7 Expression control and enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm.

Genotype: MiniF *lysY* (Cam^{R}) */ fhuA2 lacZ::T7 gene1 [lon] ompT ahpC gal λatt::pNEB3-r1-cDsbC* (Spec^{R}, *lacI*^{q}) *ΔtrxB sulA11 R(mcr-73::miniTn10--*Tet^{S}*)2 [dcm] R(zgb-210::Tn10 --*Tet^{S}*) endA1 Δgor Δ(mcrC-mrr)114::IS10*

Transformation Efficiency:
1 x 10^{7} cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 (*fhuA2*), Cam, Nit, Spec and Str*.

*Note that resistance to low levels of streptomycin may be observed.

Sensitivity: Amp, Kan, Tet

How do I express my protein in SHuffle cells?

Currently there are two SHuffle cell lines available from NEB; SHuffle (NEB #C3026) based on *E. coli* K12, and SHuffle Express (NEB #C3028, #C3029, #C3030) based on *E. coli* B.

We recommend testing both B and K12 expression strains, as we do see variability in expression depending on the protein of interest (see table). If T7 expression is not necessary, then we recommend comparing NEB #C3026 and #C3028. If T7 expression is necessary, test NEB #C3026 and #C3029. If T7-driven expression of a protein is toxic, switch to a non-leaky *lysY* version (NEB #C3030). Once the strain is chosen expression conditions should be optimized. This can include temperature as well as auto expression (1).

Reference:
(1) Ke, N. and Berkmen, M. (2014) *Current Protocols Molecular Biology* 16.1B.21.

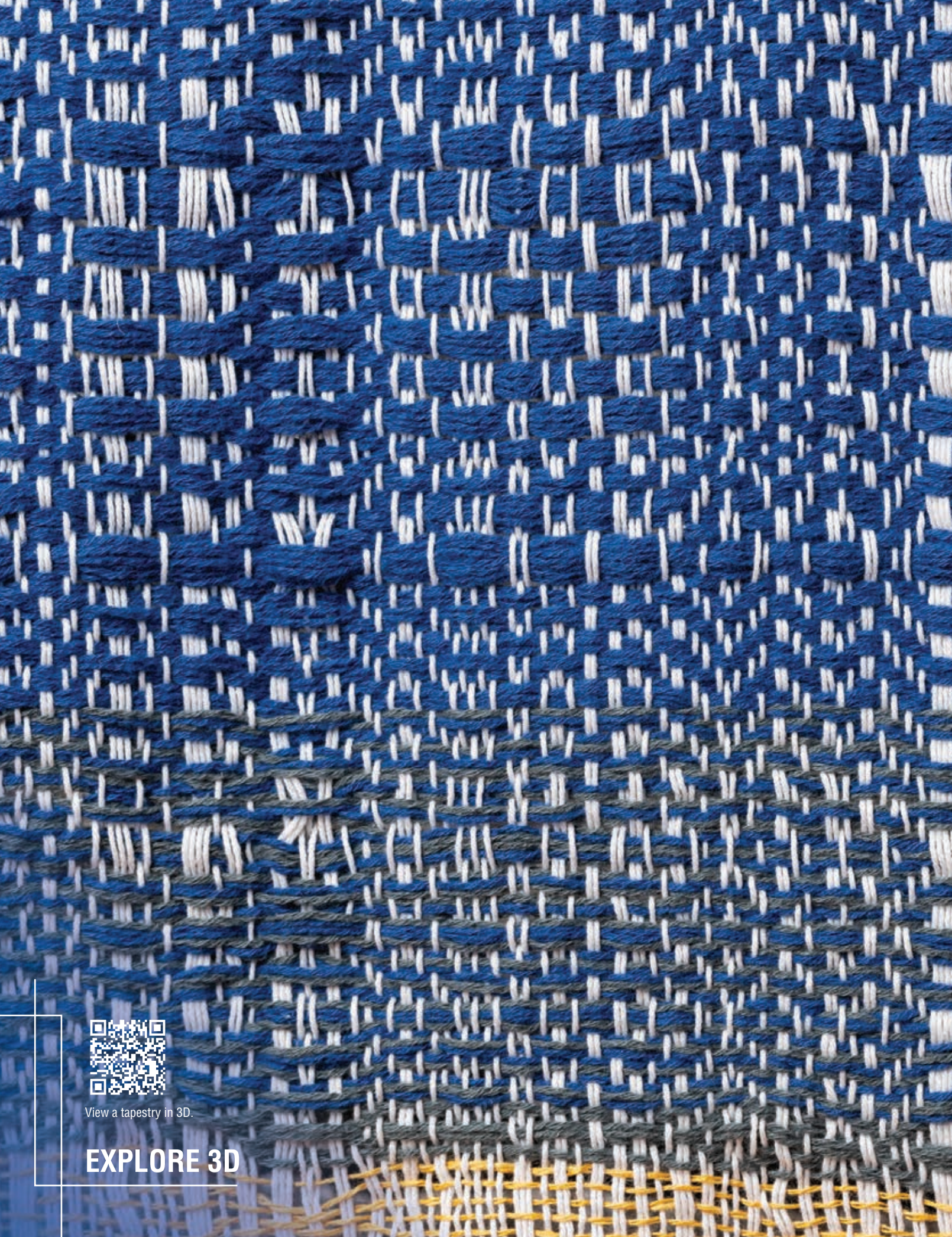
View our online tutorial for tips on setting up reactions with SHuffle.

Percentage of relative solubility of various proteins using SHuffle (K12 and B strains):

Protein	Relative % Soluble		# Cysteines
	K12	B	
Gluc	65	100	10
Urokinase	60	100	24
vtPA	5	100	12
BSA	100	0	35
Polymerase	100	0	0
Nuclease	100	10	4

Results are determined based on protein levels detected by SDS-PAGE (not shown)

Each year, NEB recognizes employees who execute novel ideas that help make NEB a better and more efficient place to work. In 2024, Sean, Rev, Vladimir and Jackson received an Innovation Award for their work in enzyme discovery and screening.



View a tapestry in 3D.

EXPLORE 3D

Woven mitochondrial DNA sequence
Credit: Ji Hyun Kong

The Threads of Ancestry in Mitochondrial Weaving

In a compelling fusion of science and art, Ji Hyun (Sally) Kong has created 'Mitos' — a project that transforms her own mitochondrial DNA (mtDNA) sequence into intricate handwoven textiles, offering a visual and tactile representation of her genetic heritage. By bridging molecular biology and traditional weaving, she presents a deeply personal yet universally relatable exploration of identity, heritage, and the threads that connect generations.

The idea for Mitos emerged as Kong learned weaving around the same time she was exploring her own mitochondrial DNA. Unlike nuclear DNA, mtDNA is inherited exclusively from the mother, passing down an unbroken genetic line that traces back through countless generations of maternal ancestors. Intrigued by this matrilineal inheritance, she felt compelled to channel this biological wonder into her art. *"Upon discovering that the root word of mitochondria, mitos, means thread in Greek, I felt that my mitochondrial DNA sequence was meant to be woven,"* she explained.

The project involved three main stages: sequencing, translation and physicalization. Kong isolated, amplified and sequenced the hypervariable region of her mitochondrial DNA. She then wrote a Python™ script, utilizing the versatile programming language for data analysis, to map the nucleotide sequence into weaving instructions. Using Houdini™, a 3D visual effects software known for its advanced modeling capabilities, she visualized the resulting patterns. Finally, she handwove the patterns using a Schacht® four-shaft floor loom, exploring different weaving techniques such as basket weave and twill.

By mapping the nucleotides — adenine (A), thymine (T), guanine (G), and cytosine (C) — to specific weaving instructions and patterns, Kong transformed her personal genetic code into tangible art. *"I hope these handwoven pieces evoke a sense of embrace by a long lineage of mothers who brought us into existence,"* she said.

Kong's work exemplifies the fusion of technology and creativity, linking molecular biology with textile design in a way that resonates with a wider audience. She envisions a future where this concept could be shared more broadly. *"I would love to continue this project by introducing molecular cloning to weavers and organizing workshops where we extract and amplify genes that are meaningful to us and see how we could manifest this information using different weaving techniques,"* she said. She dreams of mothers weaving blankets from their own DNA sequences for their children, creating heirlooms that embody both physical and emotional heritage.

Mitos stands out for its originality and unification of computer programming, molecular biology and weaving. The project is personal in nature but highlights the shared human experience encoded in our mitochondrial DNA. Kong's innovative approach not only honors the integrity of scientific data but also imbues it with personal meaning and aesthetic beauty.

By weaving her mitochondrial DNA, Kong offers a unique lens on the convergence of science and art, making the microscopic threads of life visible and tangible. Her work invites us to consider the threads that connect us to our ancestors and each other, reminding us of the intricate tapestry that is human existence.

Ji Hyun (Sally) Kong
Genspace, New York, NY, USA
2024 Passion in Science Arts and Creativity Award

Glycobiology & Protein Analysis Tools

Trust NEB's expertise in enzymology when you need reagents for glycan and protein analysis.

Glycan Analysis Tools

Proteomics, the systematic study of proteins in biological systems, has expanded the knowledge of protein expression, modification, interaction and function. However, in eukaryotic cells, the majority of proteins are post-translationally modified (1). A common post-translational modification, essential for cell viability, is the attachment of glycans. Glycosylation defines the adhesive properties of glycoconjugates, and it is largely through glycan–protein interactions that cell–cell and cell–pathogen (including viruses) contacts occur, a fact that accentuates the importance of glycobiology.

Glycan analysis relies on effective enzymatic and analytical techniques for the correlation of glycan structure with function. Glycan molecules modulate many other processes important for cell and tissue differentiation, metabolic and gene regulation, protein activity, protein clearance, transport and more (2-9).

Protein Analysis Tools

Not only are proteins a major structural component of living systems, they can also be effector molecules whose states determine downstream activities. Therefore, studying the protein complement within a cell can reveal the mechanisms behind many of the cell's responses to its environment. Given the vast number of applications for protein analysis, several tools and methods for its study exist; determining the correct method for your application is paramount to success.

Phage display technology is an *in vitro* screening technique for identifying ligands for proteins and other macromolecules. At the crux of phage display technology is the ability to express peptide or protein sequences as fusions to the coat proteins of a bacteriophage. Libraries of phage-displayed peptides or proteins are thereby physically linked to their encoding nucleic acid, allowing selection of binding partners for myriad target types by iterative rounds of in vitro panning and amplification, followed by DNA sequencing.

All NEB products pass stringent quality control assays to ensure the highest level of functionality and purity.

Featured Products

273 Rapid™ PNGase F

286 TEV Protease

286 Thermolabile Proteinase K

Featured Tools & Resources

Protein Analysis Tools & Glycomics Overview

PNGase F Overview & Selection Chart

Visit **www.neb.com/glycosidase** to view our online tutorial on *N*- and *O*-linked glycosylation.

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(2) Varki A. (1993) Glycobiology, 3, 97–130. PMID: 8490246.
(3) Zhao Y.Y. et al. (2008) Cancer Sci. 99, 1304–1310. PMID: 18492092.
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(5) Skropeta D. (2009) Bioorg. Med. Chem. 17, 2645–2653. PMID: 19285412.
(6) Neu U. et al. (2011) Curr. Opin. Struct. Biol. 21, 610–618. PMID: 21917445.
(7) Cerliani J.P. et al. (2011) J. Clin. Immunol. 31, 10–21. PMID: 21184154.
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(9) Arnold J.N. (2006) Immunol. Lett. 106, 103–110. PMID: 16814399.

Rec Recombinant Enzyme

Glycosidases

- *Enabling Novel Technologies*
- *Unique Specifications*
- *Exceptional Value*
- *High Purity*

NEB offers a selection of endoglycosidases and exoglycosidases for glycobiology research. Many of these reagents are recombinant, and all undergo several quality control assays, enabling us to provide products with lower unit cost, high purity, and reduced lot-to-lot variation.

All of our glycosidases are tested for contaminants. Since *p*-nitrophenyl-glycosides are not hydrolyzed by some exoglycosidases, we use only fluorescently-labeled oligosaccharides to screen for contaminating glycosidases.

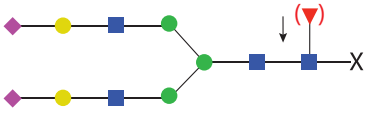
NEB's glycosidases are provided with 10X buffer to ensure optimal activity. Using more than one glycosidase simultaneously is a common timesaving procedure. Selecting the best buffer to provide reaction conditions that optimize enzyme activity is an important consideration.

Reaction Buffer Compositions:
Visit **www.neb.com/glycosidase** for details.

Endo F2

NEBU 37° 65°

#P0772S 480 units



- *Removal of complex biantennary N-linked glycans from glycoproteins and glycopeptides*
- *Useful for determining N-glycosylation sites*

Description: Endo F2 is a highly specific recombinant endoglycosidase which cleaves within the chitobiose core of asparagine-linked complex biantennary and high mannose oligosaccharides from glycoproteins and glycopeptides. Endo F2 cleaves biantennary glycans at a rate approximately 20 times greater than high mannose glycans. The activity of Endo F2 is identical on biantennary structures with and without core fucosylation. However, Endo F2 is not active on hybrid or tri- and tetra-antennary oligosaccharides. Endo F2 is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol free for optimal performance in HPLC and MS intensive methods.

Source: Cloned from *Elizabethkingia miricola* (formerly *Flavobacterium meningosepticum*) and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 4, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
• GlycoBuffer 4

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the carbohydrate from 10 µg Porcine Fibrinogen in 1 hour at 37°C in a total reaction volume of 10 µl.

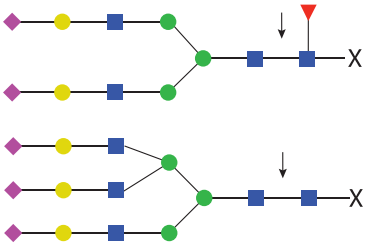
Molecular Weight: 39 kDa.

Concentration: 8,000 units/ml

Endo F3

NEBU 37° 65°

#P0771S 240 units



- *Removal of complex biantennary and triantennary N-linked glycans from glycoproteins and glycopeptides*
- *Useful for determining N-glycosylation sites*

Description: Endo F3 is a highly specific recombinant endoglycosidase which cleaves within the chitobiose core of asparagine-linked fucosylated-biantennary and triantennary complex oligosaccharides from glycoproteins. Endo F3 is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol free for optimal performance in HPLC and MS intensive methods.

Source: Cloned from *Elizabethkingia miricola* (formerly *Flavobacterium meningosepticum*) and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 4, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
• GlycoBuffer 4

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the carbohydrate from 10 µg Porcine Fibrinogen in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 38 kDa.

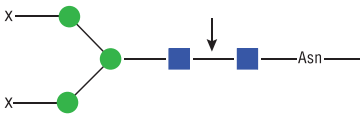
Concentration: 8,000 units/ml

Find an overview of glycobiology.

NEBU Unique NEBuffer · Cloned at NEB · RR Recombinant Enzyme · BSA Requires BSA · 65° Heat Inactivation

Endo D

#P0742S	1,500 units
#P0742L	7,500 units



- *Removal of paucimonnose N-linked glycans from glycoproteins and glycopeptides*
- *Useful for determining N-glycosylation sites*

Description: Endo D, also known as Endoglycosidase D, is a recombinant glycosidase, which cleaves within the chitobiose core of paucimannose *N*-linked glycans, with or without extensions in the antennae.

Endo D is tagged with a chitin binding domain (CBD) for easy removal from a reaction, and is supplied glycerol-free for optimal performance in HPLC and MS intensive methods.

Source: A truncated Endo D gene cloned from *Streptococcus pneumoniae* and expressed in *E. coli* as a fusion to chitin binding domain

Reaction Conditions: GlycoBuffer 2, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- DTT
- GlycoBuffer 2

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of glycosidase-trimmed (trimannosyl core) Fetuin in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 140 kDa.

Concentration: 50,000 units/ml

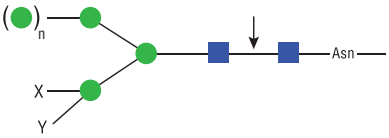
Endo H

Endo H

#P0702S	10,000 units
#P0702L	50,000 units

Endo H_f

#P0703S	100,000 units
#P0703L	500,000 units



Endo H and Endo H_f cleave only high mannose structures (n = 2–150, x = (Man)1–2, y = H) and hybrid structures (n = 2, x and/or y = AcNeu-Gal-GlcNAc).

- *Removal of high mannose N-glycans from glycoproteins*

Description: Endoglycosidase H is a recombinant glycosidase which cleaves within the chitobiose core of high mannose and some hybrid oligosaccharides from *N*-linked glycoproteins.

Endo H_f is a recombinant protein fusion of Endoglycosidase H and maltose binding protein. It has identical activity to Endo H.

Source: Endo H and Endo H_f have been cloned from *Streptomyces plicatus* and overexpressed in *E. coli*.

Reaction Conditions: Denature glycoprotein in 1X Glycoprotein Denaturing Buffer at 100°C for 10 minutes. Incubate in 1X GlycoBuffer 3 at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- Glycoprotein Denaturing Buffer
- GlycoBuffer 3

Molecular Weight:

- Endo H: 29 kDa
- Endo H_f: 70 kDa

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: Endo H concentration: 500,000 units/ml, Endo H_f concentration: 1,000,000 units/ml

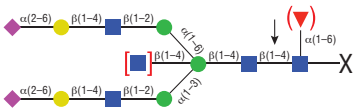
Note: Enzymatic activity is not affected by SDS. To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

Mike joined NEB in 2016 as the Director of GMP-grade Operations and is currently the Global Operations Director in the International Business Team. He is part of NEB's Rock Climbing and Mountain Biking Clubs.

Gal Glc Man GalNAc GlcNAc Fuc NeuAc **R** = any sugar

Endo S

#P0741S 6,000 units
#P0741L 30,000 units



- *Removal of N-glycans from native IgG*
- *Useful for determining N-glycosylation sites*

Description: Endo S is an endoglycosidase with a uniquely high specificity for removing *N*-linked glycans from the chitobiose core of the heavy chain of native IgG. Endo S is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol-free for optimal performance in HPLC- and MS-intensive methods.

Source: Endo S is cloned from *Streptococcus pyogenes* and overexpressed as a fusion to the chitin binding domain in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Heat inactivation: 55°C for 10 minutes.

Reagents Supplied:

• GlycoBuffer 1

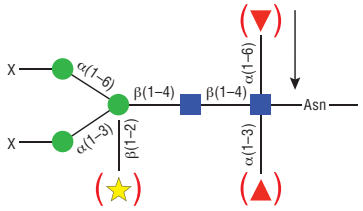
Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 5 µg of native mouse monoclonal IgG in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 136 kDa.

Concentration: 200,000 units/ml

PNGase A

#P0707S 150 units
#P0707L 750 units



- *Removal of N-linked glycans from glycoproteins*

Description: PNGase A is a recombinant amidase, which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and short complex oligosaccharides such as those found in plant and insect cells from *N*-linked glycoproteins and glycopeptides. PNGase A differs from PNGase F in that it cleaves *N*-linked glycans with or without α(1,3)-linked core fucose residues.

Source: Cloned from *Oryza sativa* (rice) and expressed in *Pichia pastoris*.

Reaction Conditions: GlycoBuffer 3, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

• GlycoBuffer 3
• Glycoprotein Denaturing Buffer
• NP-40

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 1 µg of denatured recombinant Avidin produced in Maize in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 63 kDa.

Concentration: 5,000 units/ml

Note: PNGase A is active on both glycoproteins and glycopeptides. PNGase A cannot cleave larger *N*-glycans such as those from Fetuin, Fibrinogen, IgG, Lactoferrin and Transferrin. PNGase A is able to cleave high mannose *N*-glycan structures from Man 3 up to Man 9.

Jenna joined NEB in 2022 as a Customer Support Representative. She has participated in the summer cornhole tournaments for the last two years.

NEBU Unique NEBuffer Cloned at NEB RR Recombinant Enzyme BSA Requires BSA 65° Heat Inactivation

PNGase F & PNGase F, Recombinant

PNGase F
#P0704S 15,000 units
#P0704L 75,000 units

PNGase F (Glycerol-free)
#P0705S 15,000 units
#P0705L 75,000 units

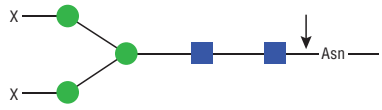
PNGase F, Recombinant
#P0708S 15,000 units
#P0708L 75,000 units

PNGase F (Glycerol-free), Recombinant
#P0709S 15,000 units
#P0709L 75,000 units

Companion Products:

RNase B
#P7817S 250 µg

Endoglycosidase Reaction Buffer Pack
#B0701S 4 ml



PNGase F hydrolyzes nearly all types of N-glycan chains from glycopeptides/proteins [x = H or oligosaccharide].

- *Removal of N-linked glycans from glycoproteins*

Description: Peptide-N-Glycosidase F, also known as PNGase F, is an amidase which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid and complex oligosaccharides from N-linked glycoproteins. A glycerol-free version of PNGase F is also offered for HPLC methods.

Source: NEB #P0704 and #P0705 are purified from *Elizabethkingia miricola* (formerly *Flavobacterium meningosepticum*).

NEB #P0708 and #P0709 are purified from *Elizabethkingia miricola* (formerly *Flavobacterium meningosepticum*) and expressed in *E. coli*.

Reaction Conditions: Denature glycoprotein in 1X Glycoprotein Denaturing Buffer at 100°C for 10 minutes. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:

- Glycoprotein Denaturing Buffer
- GlycoBuffer 2
- 10% NP-40

Molecular Weight: 36 kDa.

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 500,000 units/ml

Note: Since PNGase F activity is inhibited by SDS, it is essential to have NP-40 present in the reaction mixture.

To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

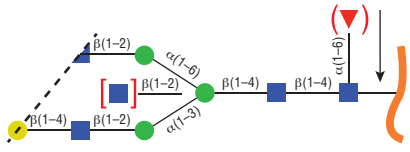
Rapid™ PNGase F & Rapid PNGase F (non-reducing format)

Rapid PNGase F
#P0710S 50 reactions

Rapid PNGase F (non-reducing format)
#P0711S 50 reactions

Companion Product:

Rapid PNGase F Antibody Standard
#P6043S 250 µg



- *Complete deglycosylation of antibodies and fusion proteins in minutes*
- *Release of all N-glycans rapidly and without bias*
- *Optimal activity is ensured for 12 months, if stored properly*
- *Purified to > 95% homogeneity, as determined by SDS-PAGE*

Description: Rapid PNGase F is an improved reagent that allows the complete and rapid deglycosylation of antibodies and fusion proteins in minutes. All *N*-glycans are released rapidly and without bias, and are ready to be prepared for downstream chromatography or mass spectrometry analysis. Rapid PNGase F creates an optimized workflow which reduces processing time without compromising sensitivity or reproducibility.

Developed for proteomic applications, Rapid PNGase F (non-reducing format) enables complete and rapid deglycosylation while preserving disulfide bonds. This facilitates high-throughput proteomics applications and methods for antibody characterization by mass spectrometry such as intact mass analysis. Rapid PNGase F (non-reducing format) combines the advantages of Rapid PNGase F (fast processing time), with non-reducing conditions, preserving quaternary structure.

Heat inactivation: 75°C for 10 minutes.

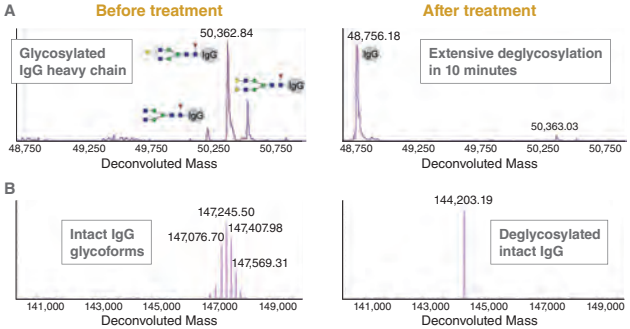
Reagents Supplied (NEB #P0710):

- Rapid PNGase F
- Rapid PNGase F Reaction Buffer

Reagents Supplied (NEB #P0711):

- Rapid PNGase F (non-reducing format)
- Rapid PNGase F (non-reducing format) Buffer

Specificity: Rapid PNGase F cleaves all complex, hybrid and high-mannose type glycans from antibodies and related proteins. Core α1-3 fucosylation (found in immunoglobulins expressed in plant or insect cells) is resistant to both PNGase F and Rapid PNGase F.



ESI-TOF analysis of an antibody before and after treatment with (A) Rapid PNGase F and (B) Rapid PNGase F (non-reducing format).

Gal Glc Man GalNAc GlcNAc Fuc NeuAc **R** = any sugar

Remove-iT® PNGase F

NEB U | 37° | 75°

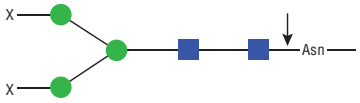
#P0706S 6,750 units
#P0706L 33,750 units

Companion Products:

Chitin Magnetic Beads
#E8036S 5 ml

6-Tube Magnetic Separation Rack
#S1506S 6 tubes

12-Tube Magnetic Separation Rack
#S1509S 12 tubes



Description: Remove-iT PNGase F is an amidase which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from *N*-linked glycoproteins. Remove-iT PNGase F is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol-free for optimal performance in HPLC- and MS-intensive methods.

Source: Remove-iT PNGase F is purified from *Elizabethkingia miricola* (formerly *Flavobacterium meningosepticum*).

Reaction Conditions: GlycoBuffer 2, 37°C. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 2
- DTT

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 5 µg of DTT denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 41 kDa.

Concentration: 225,000 units/ml

Note: Remove-iT PNGase F yields a higher activity concentration of 500,000 U/ml in reactions containing 1X GlycoProtein Denaturing Buffer; however, it is essential to have the non-ionic detergent NP-40 in the reaction mixture to minimize SDS inhibition of Remove-iT PNGase F. It is not known why this non-ionic detergent counteracts the SDS inhibition. Removal of Remove-iT PNGase F from the deglycosylation reaction can be scaled up linearly with larger volumes of chitin magnetic beads.

O-Glycosidase

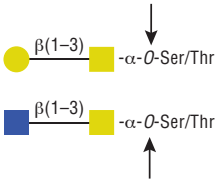
NEB U | RR | 37° | 65°

#P0733S 2,000,000 units
#P0733L 10,000,000 units

Companion Products:

O-Glycosidase & α2-3,6,8 Neuraminidase Bundle
#E0540S 1 set

α2-3,6,8 Neuraminidase
#P0720S 2,000 units
#P0720L 10,000 units



- *Removal of Core 1 and Core 3 O-linked disaccharide glycans from glycoproteins*

Description: *O*-Glycosidase, also known as Endo-a-*N*-Acetylgalactosaminidase, catalyzes the removal of Core 1 and Core 3 *O*-linked disaccharides from glycoproteins.

Source: Cloned from *Enterococcus faecalis* and expressed in *E. coli* (1).

Reaction Conditions: GlycoBuffer 2, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 2
- Glycoprotein Denaturing Buffer
- NP-40

Unit Definition: One unit is defined as the amount of enzyme required to remove 0.68 nmol of *O*-linked disaccharide from 5 mg of neuraminidase-digested, non-denatured fetuin (2) in 1 hour at 37°C in a total reaction volume of 100 µl (1 unit of both *O*-Glycosidase and PNGase F will remove equivalent molar amounts of *O*-linked disaccharides and *N*-linked oligosaccharides, respectively).

Molecular Weight: 147 kDa.

Concentration: 40,000,000 units/ml

Boletopsis grisea Lectin (BGL)

RR | 25° | No

#P0867S 1 ml

- *Binds to N-glycans having at least one terminal outer arm GlcNAc as well as O-glycans containing Gal-β1,3-GalNAc-α- within their structure.*
- *Enables enrichment of GlcNAc-capped N-glycans or mucin type O-glycopeptides from complex samples in glycomics and glycoproteomics analytical workflows.*

Description: *Boletopsis grisea* Lectin (BGL) is a recombinant 15 kDa lectin from the *Boletopsis grisea* mushroom that has been expressed in *E. coli*. BGL has two separately functioning ligand binding sites. Site 1 binds to O-glycans bearing the Tn antigen (GalNAc-α-Ser/Thr) or Thomsen-Friedenreich antigen (TF-antigen; Gal-β1,3-GalNAc-α-) and Site 2 binds N-glycans with terminal GlcNAc residues.

Molecular Weight: 15 kDa.

Concentration: 1 mg/ml

This is an **Enzyme for Innovation (EFI)**.
To learn more, visit
www.neb.com/EnzymesforInnovation.

NEB U Unique NEBuffer | Cloned at NEB | RR Recombinant Enzyme | BSA Requires BSA | 65° Heat Inactivation

NEW

N-Glycopeptide Binding Protein

#P0872S 1 mg

- *Enrich for N-glycopeptides in complex proteomics samples with this engineered and improved lectin without requiring the use of HILIC columns or cartridges.*
- *Significantly improved N-glycopeptide spectra match and glycoprotein identification and discovery.*
- *Broad specificity to high mannose, complex, and hybrid N-glycans, as well as fucosylated and non-fucosylated, sialylated and non-sialylated, with bi-, tri- and tetra-antennary structures.*

Description: N-Glycopeptide Binding Protein, also known as Fbs1-GYR lectin, is engineered to selectively bind and enrich a wide variety of N-glycopeptides in a complex proteomic sample to assist N-glycoprotein identification and discovery, without requiring the use of traditional lectin affinity chromatography (LAC) techniques, HILIC columns or cartridges. This engineered lectin, originating from human Fbs1 protein, exhibits improved specificity towards high mannose, complex, and hybrid N-glycans, as well as towards fucosylated and non-fucosylated, sialylated and non-sialylated, with bi-, tri- and tetra-antennary structures. N-Glycopeptide Binding Protein includes a SNAP-tag for immobilizing to create versatile workflows.

Source: An *E. coli* strain that carries human Fbs1 mutant gene.

Molecular Weight: 45 kDa.

Concentration: 1 mg/ml

Protein Deglycosylation Mix II

#P6044S 20 reactions

- *Fast reaction setup*
- *Enzyme mixture ensures effective deglycosylation of N-and O-linked glycans*
- *Can be used under native and reducing conditions*
- *Enzymatic deglycosylation leaves intact core structures suitable for mass spectrometry analysis*

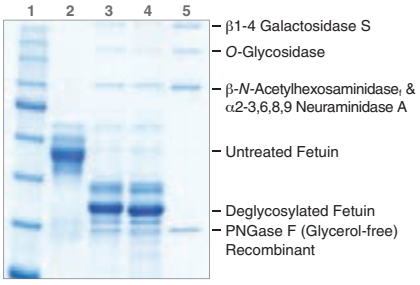
Description: The Protein Deglycosylation Mix II contains all of the enzymes, reagents and controls necessary to remove almost all *N*-linked and simple *O*-linked glycans, as well as some complex *O*-linked glycans. This mix contains enzymes sufficient for 20 reactions or the cleavage of as much as 2 mg of glycoprotein.

Deglycosylation Enzyme Mix II:

- PNGase F (Glycerol-free), Recombinant: 10,000 units/vial
- *O*-Glycosidase: 80,000 units/vial
- α2-3,6,8,9 Neuraminidase A: 400 units/vial
- β1-4 Galactosidase S: 960 units/vial
- β-*N*-Acetylhexosaminidase$_f$: 300 units/vial

Reagents Supplied:

- Deglycosylation Mix Buffer 1
- Deglycosylation Mix Buffer 2
- Fetuin



Enzymatic Deglycosylation of Bovine Fetuin under both native (10X Deglycosylation Mix Buffer 1) and reducing (10X Deglycosylation Mix Buffer 2) conditions. *20 µg reactions were loaded onto a 10-20% Tris-glycine SDS-PAGE gel. Lane 1: Color Prestained Protein Standard, Broad Range (11-245 kDa), Lane 2: 20 µg untreated Fetuin control, Lane 3: 20 µg Fetuin deglycosylated under native conditions with Deglycosylation Mix Buffer 1, Lane 4: 20 µg Fetuin deglycosylated under reducing conditions with Deglycosylation Mix Buffer 2, Lane 5: 5 µl Protein Deglycosylation Mix II.*

Fetuin

#P6042S 500 µg

Description: Fetuin is a glycoprotein containing sialylated *N*-linked and *O*-linked glycans that can be used as a positive control for endoglycosidase enzymes.

Source: Fetal Calf Serum

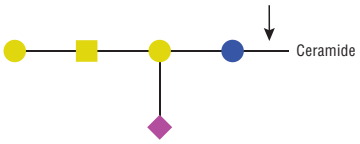
Molecular Weight: 48 kDa.

Concentration: 10 mg/ml

Note: 500 µg is enough for approximately 20 reactions. Due to heterogeneous glycosylation; Fetuin runs as a doublet on an SDS-PAGE gel.

Endoglycoceramidase I (EGCase I)

#P0773S 150 milliunits



Description: Endoglycoceramidase I (ECGase I) catalyzes the hydrolysis of the β-glycosidic linkage between oligosaccharides and ceramides in various glycosphingolipids. One unit of *R. triatomea* EGCase I is defined as the amount of enzyme required to hydrolyze 1 µmol of ganglioside GM1a per minute at 37°C.

Source: Cloned from *Rhodococcus triatomea* and expressed in *E. coli*.

Reaction Conditions: EGCase I Reaction Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied:

- EGCase I Reaction Buffer

Unit Definition: One unit of *R. triatomea* EGCase I was defined as the amount of enzyme required to hydrolyze 1 µmol of ganglioside GM1a per minute at 37°C.

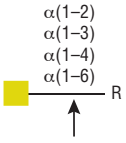
Molecular Weight: 50 kDa.

Concentration: 6 units/ml

This is an **Enzyme for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

α-*N*-Acetylgalactosaminidase

#P0734S 3,000 units



Description: α-*N*-Acetylgalactosaminidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α-linked d-*N*-Acetylgalactosamine residues from oligosaccharides and *N*-glycans attached to proteins.

Source: Cloned from *Chryseobacterium meningosepticum* and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 100 µg/ml Recombinant Albumin (Low-glycerol). Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1
- Recombinant Albumin (Low-glycerol)

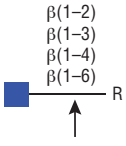
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-D-*N*-acetylgalactosamine from 1 nmol (GalNAcα1-3)(Fucα1-2)Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 47 kDa.

Concentration: 20,000 units/ml

β-*N*-Acetylglucosaminidase S

#P0744S 100 units
#P0744L 500 units



- *Removal of bisecting β-GlcNAc residues*

Description: β-*N*-Acetylglucosaminidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of terminal, non-reducing β-*N*-Acetylglucosamine residues from oligosaccharides.

Source: Cloned from *Streptococcus pneumoniae* and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C.

Reagents Supplied:

- GlycoBuffer 1

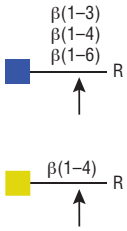
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, non-reducing β-*N*-Acetylglucosamine from 1 nmol GlcNAcβ1-4GlcNAcβ1-4GlcNAc-7-amino-4-methylcoumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 125 kDa.

Concentration: 4,000 units/ml

β-*N*-Acetylhexosaminidase$_f$

#P0721S 500 units



Description: β-*N*-Acetylhexosaminidase$_f$ is a recombinant protein fusion of β-*N*-Acetylhexosaminidase and maltose binding protein with identical activity to β-*N*-Acetylhexosaminidase. It catalyzes the hydrolysis of terminal β-*N*-Acetylgalactosamine and glucosamine residues from oligosaccharides.

Source: Cloned from *Streptomyces plicatus* and overexpressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1

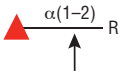
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal β-D-*N*-Acetylgalactosamine from 1 nmol of GalNAcβ1-4Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 100 kDa.

Concentration: 5,000 units/ml

α1-2 Fucosidase

#P0724S 1,000 units



■ *Active only on linear substrates*

Description: α1-2 Fucosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of linear α1-2 linked fucose residues from oligosaccharides. In this case, a linear substrate is defined as having no branching on the adjacent residue.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 100 µg/ml Recombinant Albumin (Low-glycerol). Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1
- Recombinant Albumin (Low-glycerol)

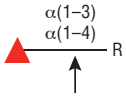
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the α-L-fucose from 1 nmol of Fucα1-2Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 70 kDa.

Concentration: 20,000 units/ml

α1-3,4 Fucosidase

#P0769S 200 units



Description: α1-3,4 Fucosidase, (also known as AMF) is a broad specificity exoglycosidase that catalyzes the hydrolysis of α1-3 and α1-4 linked fucose residues from oligosaccharides and glycoproteins.

Source: Cloned from the sweet almond tree (*Prunus dulcis*) and expressed in *Pichia pastoris*.

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 100 µg/ml Recombinant Albumin (Low-glycerol). Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1
- Recombinant Albumin (Low-glycerol)

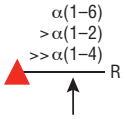
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the α-fucose from 1 nmol of Galβ1-4GlcNAcβ1-3(Fucα1-3)Galβ1- 4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl

Molecular Weight: 56 kDa.

Concentration: 4,000 units/ml

α1-2,4,6 Fucosidase O

#P0749S 80 units
#P0749L 400 units



Description: α1-2,4,6 Fucosidase O is a broad specificity exoglycosidase that catalyzes the hydrolysis of terminal α1-2, α1-4 and α1-6 linked fucose residues from oligosaccharides. α1-2,4,6 Fucosidase O cleaves α1-6 fucose residues more efficiently than other linkages.

Source: Cloned from *Omnitrophica* bacterium and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1

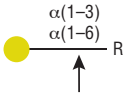
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the fucose from 1 nmol of G0F from human IgG [GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc(Fucα1-6)-AMAC], in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 49 kDa.

Concentration: 2,000 units/ml

α1-3,6 Galactosidase

#P0731S 100 units



Description: a1-3, 6 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of a1-3, 6 linked d-galactopyranosyl residues from oligosaccharides.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 100 µg/ml Recombinant Albumin (Low-glycerol). Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1
- Recombinant Albumin (Low-glycerol)

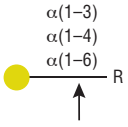
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, α-D-galactose from 1 nmol Galα1-3Galβ1-4Gal-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 70 kDa.

Concentration: 4,000 units/ml

α1-3,4,6 Galactosidase

#P0747S 200 units
#P0747L 1,000 units



Description: a1-3,4,6 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-3, α1-4 and α1-6 linked d-galactopyranosyl residues from oligosaccharides.

Source: Cloned from green coffee bean and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 100 µg/ml Recombinant Albumin (Low-glycerol). Heat inactivation: 65°C.

Reagents Supplied:

- GlycoBuffer 1
- Recombinant Albumin (Low-glycerol)

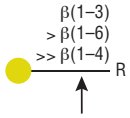
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, α-D-galactose from 1 nmol Galα1-3Galβ1-4Gal- 7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 39.7 kDa.

Concentration: 8,000 units/ml

β1-3 Galactosidase

#P0726S 500 units



Description: β1-3 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-3 and, at a much lower rate, β1-6 linked d-galactopyranosyl residues from oligosaccharides. The approximate kinetic data show > 100-fold preference for β1-3 over β1-6 linkages and > 500-fold preference from β1-3 over β1-4 linkages.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 100 µg/µl Recombinant Albumin (Low-glycerol). Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1
- Recombinant Albumin (Low-glycerol)

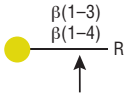
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal β-D-galactose from 1 nmol of Galβ1-3GlcNAcβ1-3Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 66 kDa.

Concentration: 10,000 units/ml

β1-3,4 Galactosidase

#P0746S 400 units



Description: β1-3,4 Galactosidase, cloned from bovine testes and also known as BTG, is a highly specific exoglycosidase that catalyzes the hydrolysis of terminal β1-3 and β1-4 linked galactose residues from oligosaccharides.

Source: Cloned from bovine testes and expressed in *Pichia pastoris*.

Reaction Conditions: GlycoBuffer 4, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 4

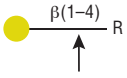
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, β-D-galactose from 1 nmol Galβ1-4GlcNAcβ1-3Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 71 kDa.

Concentration: 8,000 units/ml

β1-4 Galactosidase S

#P0745S 400 units
#P0745L 2,000 units



Description: β1-4 Galactosidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-4 linked galactose residues from oligosaccharides.

Source: Cloned from *Streptococcus pneumoniae* and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, β-D-galactose from 1 nmol Galβ1-4GlcNAcβ1- 3Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 231 kDa.

Concentration: 8,000 units/ml

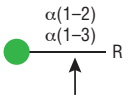
Team members of NEB's subsidiary office in China.

Gal Glc Man GalNAc GlcNAc Fuc NeuAc **R** = any sugar

α1-2,3 Mannosidase

NEBU RR 37° 65° rAlbumin

#P0729S 640 units



Description: α1-2,3 Mannosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-2 and α1-3 linked d-mannopyranosyl residues from oligosaccharides.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli.*

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 100 µg/ml Recombinant Albumin (Low-glycerol). Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1
- Recombinant Albumin (Low-glycerol)

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the non-reducing terminal α-D-mannose from 1 nmol Manα1-3Manβ1-4GlcNAc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

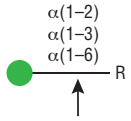
Molecular Weight: 90 kDa.

Concentration: 32,000 units/ml

α1-2,3,6 Mannosidase

NEBU RR 37° 95°

#P0768S 80 units



Description: α1-2,3,6 Mannosidase, cloned from Jack Bean, and also known as JBM, is a broad specificity exoglycosidase that catalyzes the hydrolysis of terminal α1-2, α1-3 and α1-6 linked mannose residues from oligosaccharides. α1-2,3,6 Mannosidase has a slight preference for α1-2 mannose residues over α1-3 and α1-6 mannose residues.

Source: Cloned from *Canavalia ensiformis* (Jack Bean) and expressed in *Pichia pastoris.*

Reaction Conditions: GlycoBuffer 4, 37°C. Supplement with 1X Zinc. Heat inactivation: 95°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 4
- Zinc

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal mannose from 1 nmol of Man(α1,3)-Man(β1,4)-GlcNAc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

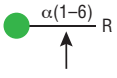
Molecular Weight: 110 kDa.

Concentration: 2,000 units/ml

α1-6 Mannosidase

NEBU RR 37° 65° rAlbumin

#P0727S 800 units



Description: α1-6 Mannosidase is a highly specific exoglycosidase that removes unbranched α1-6 linked d-mannopyranosyl residues from oligosaccharides. When used in conjunction with α1-2,3 Mannosidase, the α1-6 Mannosidase will cleave α1-6 Mannose residues from branched carbohydrate substrates.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli.*

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 100 µg/ml Recombinant Albumin (Low-glycerol). Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1
- Recombinant Albumin (Low-glycerol)

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-D-mannose from 1 nmol of Manα1-6Manα1-6Man-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

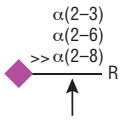
Molecular Weight: 51 kDa.

Concentration: 40,000 units/ml

Note: *p*-nitrophenyl-α-D-mannopyranoside is NOT a substrate for this enzyme.

α2-3,6,8 Neuraminidase

#P0720S 2,000 units
#P0720L 10,000 units



- *Active from pH 4.5 to 8.5*

Description: Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). This Neuraminidase catalyzes the hydrolysis of α2-3, α2-6 and α2-8 linked *N*-acetylneuraminic acid residues from glycoproteins and oligosaccharides.

Source: Cloned from *Clostridium perfringens* and overexpressed in *E. coli.*

Reaction Conditions: GlycoBuffer 1, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1

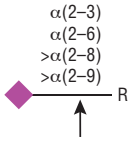
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 5 minutes at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 43 kDa.

Concentration: 50,000 units/ml

α2-3,6,8,9 Neuraminidase A

#P0722S 800 units
#P0722L 4,000 units



- *Removes branched sialic acid residues that are linked to an internal residue*

Description: Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). α2-3,6,8,9 Neuraminidase A catalyzes the hydrolysis of all linear and branched non-reducing terminal sialic acid residues from glycoproteins and oligosaccharides. The enzyme releases α2-3 and α2-6 linkages at a slightly higher rate than α2-8 and α2-9 linkages.

Source: Cloned from *Arthrobacter ureafaciens* and expressed in *E. coli.*

Reaction Conditions: GlycoBuffer 1, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1

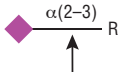
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 100 kDa.

Concentration: 20,000 units/ml

α2-3 Neuraminidase S

#P0743S 400 units
#P0743L 2,000 units



Description: Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). α2-3 Neuraminidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of α2-3 linked *N*-acetylneuraminic acid residues from glycoproteins and oligosaccharides.

Source: Cloned from *Streptococcus pneumoniae* and expressed in *E. coli.*

Reaction Conditions: GlycoBuffer 1, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1

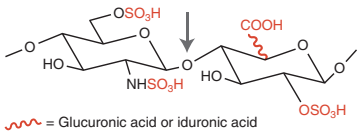
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 74 kDa.

Concentration: 8,000 units/ml

Bacteroides Heparinase I

#P0735S 240 units
#P0735L 600 units



= Glucuronic acid or iduronic acid
All structural determinants for enzyme specificity are displayed in red.

- *Degradation of heparin and heparan sulfate glycosaminoglycans*

Description: *Bacteroides* Heparinase I cloned from *Bacteroides eggerthii*, also called Heparin Lyase I, is active on heparin and the highly sulfated domains of heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.

Source: Cloned from *Bacteroides Eggerthii* and expressed in *E. coli*.

Reaction Conditions: Bacteroides Heparinase Reaction Buffer, 30°C. Heat inactivation: 100°C for 1 minute.

Reagents Supplied:

- Bacteroides Heparinase Reaction Buffer

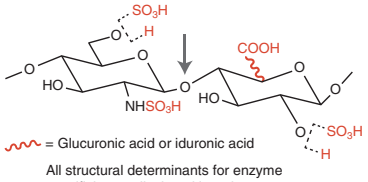
Unit Definition: One unit is defined as the amount of enzyme that will liberate 1.0 µmol unsaturated oligosaccharides from porcine mucosal heparin per minute at 30°C and pH 7.0 in a total reaction volume of 100 µl.

Molecular Weight: 42 kDa.

Concentration: 12,000 units/ml

Bacteroides Heparinase II

#P0736S 80 units
#P0736L 200 units



= Glucuronic acid or iduronic acid
All structural determinants for enzyme specificity are displayed in red.

- *Degradation of heparin and heparan sulfate glycosaminoglycans*

Description: *Bacteroides* Heparinase II cloned from *Bacteroides eggerthii*, also called Heparin Lyase II, is active on heparin and heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.

Source: Cloned from *Bacteroides eggerthii* and expressed in *E. coli*.

Reaction Conditions: Bacteroides Heparinase Reaction Buffer, 30°C. Heat inactivation: 100°C for 1 minute.

Reagents Supplied:

- Bacteroides Heparinase Reaction Buffer

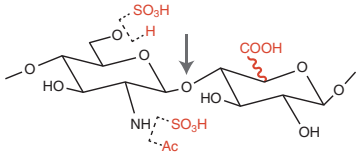
Unit Definition: One unit is defined as the amount of enzyme that will liberate 1.0 µmol unsaturated oligosaccharides from porcine mucosal heparin per minute at 30°C and pH 7.0 in a total reaction volume of 100 µl.

Molecular Weight: 86 kDa.

Concentration: 4,000 units/ml

Bacteroides Heparinase III

#P0737S 14 units
#P0737L 35 units



= Glucuronic acid or iduronic acid
All structural determinants for enzyme specificity are displayed in red.

- *Degradation of heparan sulfate glycosaminoglycans*

Description: *Bacteroides* Heparinase III cloned from *Bacteroides eggerthii*, also called Heparin Lyase III, is active on heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.

Source: Cloned from *Bacteroides eggerthii* and expressed in *E. coli*.

Reaction Conditions: Bacteroides Heparinase Reaction Buffer, 30°C. Heat inactivation: 100°C for 1 minute.

Reagents Supplied:

- Bacteroides Heparinase Reaction Buffer

Unit Definition: One unit is defined as the amount of enzyme that will liberate 1.0 µmol unsaturated oligosaccharides from heparan sulfate per minute at 30°C and pH 7.0 in a total reaction volume of 100 µl.

Molecular Weight: 75 kDa.

Concentration: 700 units/ml

Proteases

Proteins found in nature vary greatly in size from 5 kDa to greater than 400 kDa. While it is possible to study intact proteins and the modifications present on these proteins by mass spectrometry, the most common proteomic approaches utilize digestion with site-specific proteases to generate smaller fragments, called peptides, as a first step in the analysis. Peptides are easier to characterize and can be separated using reverse phase supports, such as C18, combined with high performance liquid chromatography (HPLC). HPLC coupled with tandem Mass Spectrometry is used to obtain fragmentation data of individual peptides.

IdeZ Protease (IgG-specific)

#P0770S 4,000 units

human IgG1, IgG3, IgG4: CPAPELLG▼GPSVF
human IgG2: CPAPPVA▼GPSVF
murine IgG2a: CPAPNLLG▼GPSVF
murine IgG3: CPPGNILG▼GPSVF

- *Complete fragmentation of antibodies and immunoglobulin fusion proteins in 30 minutes under native conditions*

Description: IdeZ Protease (IgG-specific) is a recombinant antibody specific protease that recognizes all human, sheep, monkey, and rabbit IgG subclasses, specifically cleaving at a single recognition site below the hinge region, yielding a homogenous pool of F(ab´)2 and Fc fragments. IdeZ Protease more effectively cleaves murine IgG2a than IdeS.

Source: Cloned from *Streptococcus equi* subspecies *zooepidemicus* and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 2, 37°C. Heat inactivation: 65°C for 10 minutes.

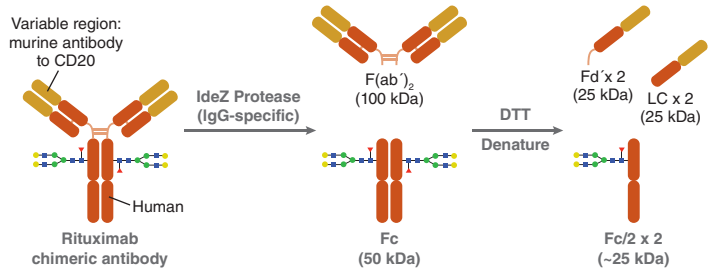
Reagents Supplied:

- GlycoBuffer 2

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of 1 µg of human IgG, in 15 minutes at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 35.6 kDa.

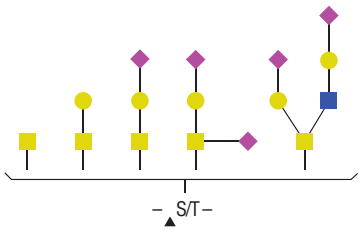
Concentration: 80,000 units/ml



Digestion of IgG with IdeZ Protease (IgG-specific), followed by denaturation.

O-Glycoprotease (IMPa)

#P0761S 200 reactions



- *Efficiently cleaves glycoproteins with or without sialic acid; no neuraminidase treatment necessary*
- *200 reactions is sufficient for the cleavage of up to 2 mg of glycoprotein*

Description: O-Glycoprotease is a highly specific protease that cleaves the peptide bonds of a glycoprotein or glycopeptide immediately *N*-terminal to a serine or threonine residue containing a mucin-type *O*-linked glycan with or without sialylation.

Source: Cloned from *Pseudomonas aeruginosa* and expressed in *E. coli*.

Reaction Conditions: 20 mM Tris-HCl, pH 8.0, 37°C. Heat inactivation: 95°C for 10 minutes.

Unit Definition: One unit of O-Glycoprotease (IMPa) will cleave > 90% of 2 pmol FAM-labelled O-glycopeptide in a total reaction volume of 20 µl in 2 hours at 37°C in 20mM Tris-HCl, pH 8.0.

Molecular Weight: 97 kDa.

Concentration: 1,000 units/ml

Gal Glc Man GalNAc GlcNAc Fuc NeuAc **R** = any sugar

α-Lytic Protease

37° 95°

#P8113S 20 µg
#P8113L 100 µg

XX-T/A/S/V▼XX

- *Analyze complex proteomes*
- *Suitable for both in-gel and solution digests*
- *Optimal activity and stability for up to 24 months*
- *Ideal for digestion of proteins for proteomic analysis by mass spectrometry*

Description: α-Lytic Protease (aLP) cleaves after Threonine (T), Alanine (A), Serine (S) and Valine (V) residues. Its specificity makes it an orthogonal and alternative protease to others commonly used in proteomics applications, including trypsin and chymotrypsin. Peptides generated by aLP are of similar average length to those of Trypsin.

Source: Purified from *Lysobacter enzymogenes*

Molecular Weight: 19,860 daltons.

Concentration: 0.4 mg/ml

Heat Inactivation: 95°C

Note: α-Lytic Protease is stable for at least 2 years at –20°C. No loss of activity is observed after 10 freeze-thaw cycles. To avoid autolysis, α-Lytic Protease should be stored long term in 10 mM Sodium Acetate pH 5.0.

Endoproteinase LysC

37°

#P8109S 20 µg

XX-Lys▼XXX

- *Ideal for proteomic analysis by mass spectrometry*
- *Free of contaminating proteases*
- *Best suited for peptide identification*

Description: LysC is a serine endoproteinase, isolated from *Lysobacter enzymogenes*, that cleaves peptide bonds C-terminal to lysine residues. LysC is a sequencing grade enzyme and is suitable for proteomics and glycobiology applications.

Source: Isolated from *Lysobacter enzymogenes*

Molecular Weight: 30,000 daltons.

Reconstitution: Endoproteinase LysC should be reconstituted in 200 µl high-purity water to make a 100 ng/µl solution in 10 mM Tris-HCl, pH 8.0. Rapid autolysis is a function of enzyme concentration.

Note: Storage Conditions: Supplied in dry format from a Tris-HCl buffer. The solution can be stored at 4°C for several days or in single-use aliquots at –20°C for several months. Use only freshly reconstituted protease for best results.

Team members of NEB's subsidiary office in the UK.

Endoproteinase GluC

#P8100S 50 µg

XX-Glu▼XX

- *Ideal for proteomic analysis by mass spectrometry*
- *Protein & peptide identification*
- *Free of contaminating proteases. Produced from a protease-deficient Bacillus subtilis strain*

Description: Endoproteinase GluC (*Staphylococcus aureus* Protease V8) is a serine proteinase which selectively cleaves peptide bonds C-terminal to glutamic acid residues. Endoproteinase GluC also cleaves at aspartic acid residues at a rate 100–300 times slower than at glutamic acid residues.

Source: *Staphylococcus aureus* Protease V8 gene cloned and expressed with histidine-tag in *Bacillus subtilis.*

Reaction Conditions: GluC Reaction Buffer, 37°C.

Reagents Supplied:

• GluC Reaction Buffer

Molecular Weight: 29,849 daltons.

Reconstitution: Endoproteinase GluC should be reconstituted by the addition of 50-500 µl of high purity water. Finger flick the volume of water in the tube to fully resuspend the enzyme. Rapid autolysis is a function of enzyme concentration; any sample reconstituted in a small volume should be used immediately. To get the most use out of the enzymes, resuspend in 500 ml H_2O and aliquot 50 ml each in 10 tubes. Freeze the tubes that are not being used immediately at -20°C for up to two weeks or less. Storage at - 80°C will prolong enzyme stability approximately 2-4 additional weeks.

Endoproteinase AspN

#P8104S 50 µg

XX▼Asp-XXX

- *Ideal for proteomic analysis by mass spectrometry*
- *Free of contaminating proteases*
- *Best suited for peptide identification*

Description: Endoproteinase AspN (flavastacin) is a zinc metalloendopeptidase which selectively cleaves peptide bonds N-terminal to aspartic acid residues.

Source: Purified from *Flavobacterium menigosepticum.*

Reaction Conditions: Endoproteinase AspN Reaction Buffer, 37°C.

Reagents Supplied:

• Endoproteinase AspN Reaction Buffer

Molecular Weight: 40,089 daltons.

Reconstitution:
Endoproteinase AspN should be reconstituted by the addition of 50-500 µl of high purity water. Rapid autolysis is a function of enzyme concentration.

Note: Storage Conditions: Supplied in dry format. Can be stored frozen in solution at -20°C for up to 2 weeks. A decrease in activity will occur if stored in solution. Use only freshly reconstituted protease for best results.

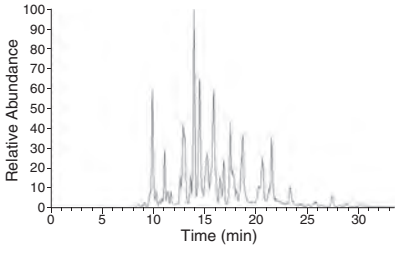
Trypsin-digested BSA MS Standard (CAM-modified)

#P8108S 500 pmol

Description: A complex mixture of peptides produced by Trypsin digestion of Bovine Serum Albumin (BSA) that was reduced and alkylated with Iodoacetimide (CAM modified). This peptide mixture can be used to test a Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) or Electrospray Ionization (ESI) mass spectrometer (TOF, Q-TOF or Ion Trap).

Source: BSA (GENBANK P02769) was digested using Trypsin (TPCK-treated).

Reconstitution: Suggested volume to resuspend: 500 µl. Avoid repeated freeze/thaw cycles once in solution.



One hundred fmol of resuspended peptide mix may be analyzed by reverse phase liquid chromatography with on-line MS/MS analysis, for example with a Proxeon EASY-nLC and by Orbitrap Mass Spectrometer. *Both analytical methods reveal a range of peptides in the standard. At least sixty percent sequence coverage is seen after database search, with greater than 15 unique peptides being identified.*

Proteinase K, Molecular Biology Grade

#P8107S 2 ml

- *Isolation of plasmid and genomic DNA*
- *Isolation of RNA*
- *Inactivation of RNases, DNases and enzymes in reactions*

Description: Proteinase K is a subtilisin-related serine protease that will hydrolyze a variety of peptide bonds.

Source: *Engyodontium album (Tritirachium album)*

Unit Definition: One unit will digest urea-denatured hemoglobin at 37°C (pH 7.5) per minute to produce equal absorbance as 1.0 µmol of L-tyrosine using Folin & Ciocalteu's phenol reagent (6).

Molecular Weight: 28 kDa.

Concentration: 800 units/ml

Note: Active in a wide range of buffers, including all NEB-specific restriction endonuclease buffers. It is highly active between pH 7.5 and 12 and temperatures 20-60°C. Proteinase K is also active in chelating agents such as EDTA and activity is stimulated in up to 2% SDS or 4M urea.

Thermolabile Proteinase K

#P8111S 30 units

- *Heat inactivated following incubation at 55°C for 10 minutes*
- *Isolation of plasmid and genomic DNA*
- *Inactivation of RNases, DNases and enzymes in reactions*
- *Removal of enzymes from DNA to improve cloning efficiency*
- *PCR purification*

Description: Thermolabile Proteinase K is an engineered, subtilisin-related serine protease that will hydrolyze a variety of peptide bonds.

Source: Cloned from *Engyodontium album* (formerly *Tritirachium album*), mutagenized to increase thermolability of the enzyme and expressed in *Pichia pastoris*.

Unit Definition: One unit is defined as the amount of enzyme required to release 1.0 µmol of 4-nitroaniline per minute from N-Succinyl-Ala-Ala-Pro-Phe-p-nitroanilide at 25°C, in a total reaction volume of 105 µl.

Molecular Weight: 29 kDa.

Concentration: 120 units/ml

Note: Active in a wide range of buffers. It is highly active between pH 7.0 and 9.5 and temperatures 20-40°C. It is active in chelating agents such as EDTA up to 10 mM.

TEV Protease

#P8112S 1,000 units

E-N-L-Y-F-Q▼(S/G/A/M/C/H)

- *Removal of affinity purification tags such as MBP or poly-histidine from fusion proteins*
- *Contains a His-tag for easy removal from a reaction using NEBExpress Ni Resin (NEB #S1428), NEBExpress NiSpin Columns (NEB #S1427) or NEBExpress Ni-NTA Magnetic Beads (NEB #S1423)*

Description: TEV Protease, also known as Tobacco Etch Virus (TEV) Protease, is a highly specific cysteine protease that recognizes the amino-acid sequence Glu-Asn-Leu-Tyr-Phe-Gln-(Ser/Gly/Met/Ala/Cys/His) and cleaves between the Gln and Ser/Gly/Met/Ala/Cys/His residues. It is often used for the removal of affinity purification tags such as maltose-binding protein (MBP) or poly-histidine from fusion proteins. TEV Protease has a 7xHis-tag for easy removal from a reaction using nickel affinity resins and has been engineered to improve thermal stability and decrease autolysis.

Source: Cloned from Tobacco Etch Virus and expressed in *E. coli*.

Unit Definition: 1 unit of TEV Protease will cleave 2 µg of MBP-fusion protein, MBP5-TEV-paramyosin ΔSal, to 95% completion in a total reaction volume of 10 µl in 1 hour at 30°C in 50 mM Tris-HCl (pH 7.5 @ 25°C) with 0.5 mM EDTA and 1 mM DTT.

Molecular Weight: 28 kDa.

Concentration: 10,000 units/ml

Factor Xa Protease

#P8010S 50 µg
#P8010L 250 µg

Ile-Glu/Asp-Gly-Arg▼

Description: Factor Xa cleaves after the arginine residue in its preferred cleavage site Ile-(Glu or Asp)-Gly-Arg. It will sometimes cleave at other basic residues, depending on the substrate conformation. The most common secondary site, among those that have been sequenced, is Gly-Arg. There seems to be a correlation between proteins that are unstable in *E. coli* and those that are cleaved by Factor Xa at secondary sites; this may indicate that these proteins are in a partially unfolded state. Factor Xa will not cleave a site followed by proline or arginine.

Source: Factor Xa Protease is purified from bovine plasma and activated by treatment with the activating enzyme from Russell's viper venom.

Unit Definition: 1 µg of Factor Xa will cleave 50 µg of MBP-fusion protein test substrate, MBP-FXa-Paramyosin-ΔSal to 95% completion in a total reaction volume of 50 µl in 6 hours or less at 23°C in 20 mM Tris-HCl (pH 8.0 @ 25°C) with 100 mM NaCl and 2 mM CaCl2.

Molecular Weight: 43 kDa.

Concentration: 1 mg/ml

Removal: Factor Xa will bind specifically to benzamidine-agarose.

Enterokinase, light chain

#P8070S 480 units
#P8070L 2,560 units

Companion Product:

K. lactis Protein Expression Kit
#E1000S 1 set

Asp-Asp-Asp-Asp-Lys▼

Description: Enterokinase is a specific protease that cleaves after the lysine at its cleavage site, Asp-Asp-Asp-Asp-Lys. It will sometimes cleave at other basic residues, depending on the conformation of the protein substrate. Enterokinase will not cleave a site followed by proline.

Source: This preparation is purified from *Pichia pastoris* containing a clone of the light chain of the bovine enterokinase gene.

Unit Definition: 1 unit is defined as the amount of enzyme required to cleave 25 µg of a MBP-EK-paramyosin-ΔSal substrate to 95% completion in 16 hours at 25°C in a total reaction volume of 25 µl.

Molecular Weight: 26 kDa, Apparent Molecular Weight: 31 kDa.

Concentration: 16,000 units/ml

Removal: Enterokinase will bind specifically to trypsin inhibitor agarose.

Furin

#P8077S 50 units
#P8077L 250 units

Arg-X-X-Arg▼

Description: Furin is an ubiquitous subtilisin-like proprotein convertase. It is the major processing enzyme of the secretory pathway and is localized in the trans-golgi network. Substrates of Furin include blood clotting factors, serum proteins and growth factor receptors such as the insulin-like growth factor receptor. The minimal cleavage site is Arg-X-X-Arg. However, the enzyme prefers the site Arg-X-(Lys/Arg)-Arg. An additional arginine at the P6 position appears to enhance cleavage. Furin is inhibited by EGTA, α1-Antitrypsin Portland and polyarginine compounds.

Note: The ability to cleave a particular substrate appears to depend on its tertiary structure as well as on the amino acids immediately surrounding the cleavage site.

Source: Isolated from *Spodoptera frugiperda* (Sf9) cells infected with recombinant baculovirus carrying truncated human furin.

Unit Definition: 1 unit is defined as the amount of enzyme required to cleave 25 µg of a MBP-FN-paramyosin-ΔSal substrate to 95% completion in 6 hours at 25°C in a total reaction volume of 25 µl.

Molecular Weight: 52 kDa.

Concentration: 2,000 units/ml

Lambda Protein Phosphatase (Lambda PP)

#P0753S 20,000 units
#P0753L 100,000 units

Companion Products:

p-Nitrophenyl Phosphate (PNPP)
#P0757S 1 ml
#P0757L 5 ml

Sodium Orthovanadate (Vanadate)
#P0758S 1 ml
#P0758L 5 ml

Description: Lambda Protein Phosphatase (Lambda-PP) is a Mn^{2+}-dependent protein phosphatase with activity towards phosphorylated serine, threonine and tyrosine residues. Lambda-PP is active on phosphorylated histidine residues.

Source: Isolated from a strain of *E. coli* that carries the bacteriophage lambda ORF221 open reading frame under the control of a T7 expression system.

Reaction Conditions: NEBuffer Pack for Protein MetalloPhosphatases (PMP), 30°C. Supplement with 1 mM MnCl2. Heat inactivation: 65°C for 60 minutes.

Reagents Supplied:

- NEBuffer Pack for Protein MetalloPhosphatases (PMP)
- $MnCl_2$

Unit Definition: One unit is defined as the amount of enzyme that hydrolyzes 1 nmol of *p*-Nitrophenyl Phosphate (50 mM) (NEB #P0757) in 1 minute at 30°C in a total reaction volume of 50 µl.

Molecular Weight: 25 kDa.

Concentration: 400,000 units/ml

Protein Kinases

Protein phosphorylation plays a key role in cell signaling, regulating various cellular processes. As the number of protein kinases grows, identifying their substrates has become more challenging. Consensus phosphorylation motifs, derived from amino acid sequences of known substrates, aid in predicting kinase-substrate interactions. However, these motifs oversimplify kinase specificity, ignoring complex 3D interactions and secondary/tertiary structural elements. Not all residues in these motifs contribute equally to kinase recognition, so caution is needed. Despite this, consensus motifs are valuable for predicting phosphorylation sites and serve as useful substrates for kinase assays. The table below summarizes protein kinase specificity motifs for protein kinases available from NEB, with interchangeable amino acids separated by slashes (/) and weakly recognized residues marked with "X."

Product	NEB #	Size	Recognition Determinant
cAMP-dependent Protein Kinase (PKA), catalytic subunit	P6000S P6000L	100,000 units 500,000 units	R-R-X-S/T Y
Casein Kinase II (CK2)	P6010S P6010L	10,000 units 50,000 units	S-X-X-E/D

D = aspartic acid, E = glutamic acid, R = arginine, S = serine, T = threonine, Y = tyrosine/hydrophobic residue, X = any amino acid
Note: More specific information on recognition determinants for each kinase can be found on the corresponding product page at www.neb.com.

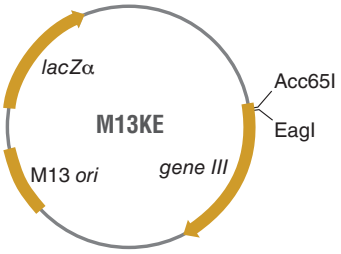
Ph.D.™ Peptide Display Cloning System

#E8101S 20 µg

Description: The Ph.D. Peptide Display Cloning System facilitates the display of custom peptide libraries on the surface of bacteriophage M13 as coat protein fusions, creating a physical linkage between each displayed peptide and its encoding DNA sequence. Peptide ligands for a variety of targets can then be selected by the straightforward method of panning. The supplied display vector M13KE is an M13 derivative with cloning sites engineered for N-terminal pIII fusion, resulting in a valency of 5 displayed peptides per virion. The use of a phage vector, rather than a phagemid, simplifies the intermediate amplification steps, since neither antibiotic selection nor helper phage superinfection are required. Since displayed proteins longer than 20–30 amino acids have a deleterious effect on the infectivity function of pIII in phage vectors, **this vector is suitable only for the display of short peptides.** Included with the cloning vector is an insert extension primer as well as a detailed protocol for cloning a peptide library into M13KE.

Kit Includes:

- M13KE gIII Cloning Vector
- M13 Extension Primer



Ph.D.™ Phage Display Peptide Library Kits

Ph.D.-7 Phage Display Peptide Library Kit v2
#E8211S 1 set

Ph.D.-12 Phage Display Peptide Library Kit v2
#E8210S 1 set

Ph.D.-C7C Phage Display Peptide Library Kit v2
#E8212S 1 set

Companion Products:

Ph.D. Peptide Display Cloning System
#E8101S 20 µg

Ph.D.-12 Phage Display Peptide Library
#E8111L 50 panning experiments

Protein G Magnetic Beads
#S1430S 1 ml

Description: Phage display describes a selection technique in which a library of peptide or protein variants is expressed on the outside of a phage virion, while the genetic material encoding each variant resides on the inside. This creates a physical linkage between each variant protein sequence and the DNA encoding it, which allows rapid partitioning based on binding affinity to a given target molecule (antibodies, enzymes, cell-surface receptors, etc.) by an *in vitro* selection process called panning. In its simplest form (Figure 1), panning is carried out by incubating a library of phage-displayed peptides with a plate (or bead) coated with the target, washing away the unbound phage, and eluting the specifically-bound phage. The eluted phage is then amplified and taken through additional binding/amplification cycles to enrich the pool in favor of binding sequences. After 3–4 rounds, individual clones are characterized by DNA sequencing and ELISA. The Ph.D. v2 kits have been updated with a new control panning target for an optional epitope mapping experiment.

NEB offers 3 pre-made random peptide libraries, as well as the cloning vector M13KE for construction of custom libraries. The pre-made libraries consist of linear heptapeptide (Ph.D.-7) and dodecapeptide (Ph.D.-12) libraries, as well as a disulfide-constrained heptapeptide (Ph.D.-C7C) library. All of the libraries have complexities in excess of 2 billion independent clones. The randomized peptide sequences in all three libraries are expressed at the N-terminus of the minor coat protein pIII, resulting in a valency of 5 copies of the displayed peptide per virion.

The Ph.D. libraries have been used for myriad applications, including epitope mapping (Figure 2), identification of protein-protein contacts and enzyme inhibitors and discovery of peptide ligands for GroEL, HIV, semiconductor surfaces and small-molecule fluorophores and drugs.

The Ph.D. Kits Include:

- Sufficient Phage Display Library for 10 separate panning experiments, complexity of 10^9 clones
- -96 gIII Sequencing Primer (500 pmol)
- Host *E. coli* K12 strain ER2738
- Monoclonal antibody (DYKDDDDK) and Protein G Magnetic Beads included for new control panning experiment
- Detailed Protocols

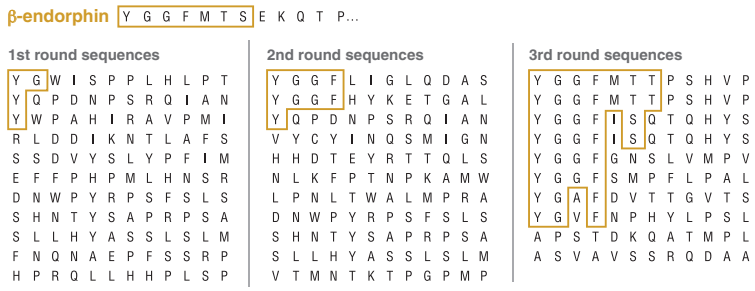


Figure 1: *Routine Phage Display Workflow. Round 1: Incubate 1011 pfu Ph.D. library + target incubation, wash away non-binders, elute bound phage, enrich selected phage with amplification in E. coli. Carry out 3-4 rounds of selection and then proceed with sequencing and/or phage-ELISA.*

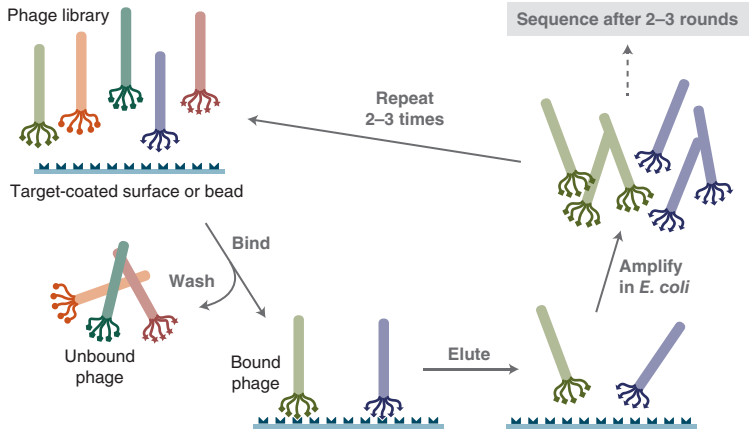


Figure 2: *Epitope mapping of an anti-β-endorphin monoclonal antibody with the Ph.D.-12 library. The Ph.D.-12 library was panned against anti-β-endorphin antibody 3-E7 in solution (10 nM antibody), followed by affinity capture of the antibody-phage complexes onto Protein A-agarose (rounds 1 and 3) or Protein G-agarose (round 2). Bound phage were eluted with 0.2 M glycine-HCl, pH 2.2. Selected sequences from each round are shown aligned with the first 12 residues of β-endorphin; consensus elements are boxed. The results clearly show that the epitope for this antibody spans the first 7 residues of β-endorphin, and that the bulk of the antibody-antigen binding energy is contributed by the first 4 residues (YGGF), with some flexibility allowed in the third position. Additionally, the conserved position of the selected sequences within the 12 residue window indicates that the free ?-amino group of the N-terminal tyrosine is part of the epitope.*

Gal Glc Man GalNAc GlcNAc Fuc NeuAc **R** = any sugar

Interested in
becoming a mentor?

LISTEN

Person in wheelchair in office
Credit: Halfpoint, Adobe Stock

Empowering Inclusive STEM Communities with DisabledInSTEM

Growing up in a rural community, Alyssa Paparella had not initially envisioned a career in STEM. It wasn't until college that she discovered a passion for biochemistry and genetics, driven by a desire to understand her own disability. Navigating the scientific world, Paparella grappled with the unique challenges of being a disabled scientist in laboratories that often lacked accessibility.

Despite having mentors who supported her academic growth, Paparella lacked role models who understood the intersection of disability and STEM, making it difficult to find guidance. Recognizing others faced similar struggles, she was motivated to create a community providing the representation and support she sought. *"I launched the DisabledInSTEM platform because I didn't want people to feel alone,"* Paparella explained. *"Disabled scientists do exist, and they belong in STEM."*

In 2020, Paparella launched the DisabledInSTEM Mentorship Program amid the global pandemic. Utilizing social media, the program connects disabled individuals across all career stages in STEM fields with mentors for a year-long journey of guidance and encouragement. So far, the program has supported 380 participants, offering a lifeline to those who might otherwise feel isolated in their academic and professional endeavors.

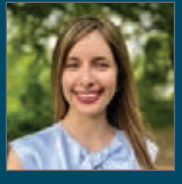
Initially structured as one-on-one pairings, the program transitioned to mentoring pods based on participant feedback. These pods, comprising multiple mentors and mentees — typically around eight individuals — foster a dynamic and supportive environment. Participants can interact with a broader community, share experiences, and build networks beyond their immediate circles.

The impact of the program is significant. Participants describe positive experiences, from gaining acceptance into graduate programs to feeling empowered to discuss their disabilities openly within their institutions. Some mentees have returned as mentors in subsequent years, eager to give back and support the next generation of disabled scientists. The program also welcomes allies as mentors, providing them with valuable insights into accessible practices and fostering a more inclusive STEM environment.

Paparella's initiative addresses a critical gap: despite one in four adults in the U.S. being disabled, less than 10% of individuals in STEM fields identify as such. By creating an inclusive, international community — the program is expanding globally with the launch of an Australian pod — DisabledInSTEM empowers individuals and enriches the field through increased diversity and inclusion.

Looking ahead, Paparella aims to expand the program further by forming a dedicated support team to sustain and grow the initiative. Despite the challenges of managing the program alongside her graduate studies, she remains driven by the impact she sees: participants feeling less alone, more confident and better equipped to navigate their STEM careers.

Paparella's journey from a student uncertain about her place in science to a leader advocating for inclusivity exemplifies the profound impact one individual can have. By addressing the unique barriers faced by disabled individuals in STEM, she is changing lives and contributing to a more diverse and innovative scientific community. The DisabledInSTEM Mentorship Program illustrates how representation and community collaboration can break down barriers, making STEM fields accessible and welcoming to all.



Alyssa Paparella
Howard Hughes Medical Institute
Chevy Chase, MD, USA
2024 Passion in Science
Science Mentorship and Advocacy Award

Epigenetics

Simplify your epigenetics research.

Epigenetics is the study of heritable changes in the phenotype of a cell or organism that are not encoded in the DNA of the genome. The molecular basis of an epigenetic profile arises from covalent modifications of the protein and DNA components of chromatin. The epigenetic profile of a cell often dictates cell memory and cell fate and, thus influences mammalian development.

The epigenetic code is hypothesized to be the combined effects of histone modifications and DNA methylation on gene expression. While the genetic code for an individual is the same in every cell, the epigenetic code is tissue- and cell-specific, and may change over time as a result of aging, disease or environmental stimuli (e.g., nutrition, life style, toxin exposure). Cross-talk between histone modifications, DNA methylation or RNAi pathways are being studied in such areas as cancer, X chromosome inactivation, and imprinting.

For over 50 years, New England Biolabs has been committed to understanding the mechanisms of restriction and methylation of DNA. This expertise in enzymology has led to the development of a suite of validated products for epigenetics research. These unique solutions to study DNA and histone modifications are designed to address some of the challenges of the current methods. All NEB® products pass stringent quality control assays to ensure the highest level of functionality and purity.

Featured Products

295 **Methylation-dependent Restriction Enzymes**

297 **NEBNext® Enzymatic Methyl-seq Products**

Featured Tools & Resources

Videos of NEB Scientists Discussing Epigenetics

Feature Articles

Epigenetics-related FAQs

Visit **www.EpiMark.com** to view an interactive tutorial explaining the phenomenon of epigenetics at the molecular level.

Find an interactive tutorial on epigenetics.

RI Recombinant Enzyme

T4 Phage β-glucosyltransferase (T4-BGT)

NEB4 RR dilB 37° 65° Epi

#M0357S 500 units
#M0357L 2,500 units

- *Glucosylation of 5-hydroxymethylcytosine in DNA*
- *Immunodetection of 5-hydroxymethylcytosine in DNA*
- *Labeling of 5-hydroxymethylcytosine residues by incorporation of [^{3}H]- or [^{14}C]-glucose into 5-hmC-containing DNA acceptor after incubation with [^{3}H]- or [^{14}C]-UDP-Glc*
- *Detection of 5-hydroxymethylcytosine in DNA by protection from endonuclease cleavage*

Description: T4 Phage β-glucosyltransferase specifically transfers the glucose moiety of uridine diphosphoglucose (UDP-Glc) to the 5-hydroxymethylcytosine (5-hmC) residues in double-stranded DNA, making beta-glucosyl-5-hydroxymethylcytosine.

Reaction Conditions: NEBuffer 4, 37°C. Supplement with 40 µM Uridine Diphosphate Glucose. Heat inactivation: 65°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 0.5 µg T4gt-DNA against cleavage by MfeI restriction endonuclease.

Concentration: 10,000 units/ml

Reagents Supplied:

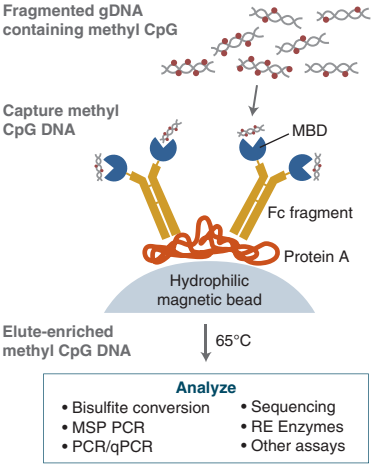
- NEBuffer 4
- Uridine Diphosphate Glucose

EpiMark® Methylated DNA Enrichment Kit

#E2600S 25 reactions

- *High-affinity binding provides greater sensitivity*
- *Elution in a small volume simplifies downstream applications*
- *Easy-to-follow protocol yields enriched fractions in less than 2 hours*
- *Enriched methylated DNA fragments can be easily ligated to double-stranded adaptors for next generation sequencing*
- *Highly pure product from a wide range of input DNA concentrations*

Description: The EpiMark Methylated DNA Enrichment Kit enables the enrichment of double-stranded CpG methylated DNA based on CpG methylation density. It utilizes the methyl-CpG binding domain of human MBD2a protein as a capture agent. The protein is fused to the Fc tail of human IgG1 (MBD2a-Fc), which is coupled to Protein A Magnetic Beads (MBD2a-Fc/Protein A Bead). This stable complex will selectively bind double-stranded methylated CpG containing DNA. The high binding affinity of the MBD2a-Fc coupled beads and optimized reagents increases sensitivity and accuracy. This kit contains all the individual components necessary to achieve enrichment in less than two hours using a four step process.



EpiMark® N6-Methyladenosine Enrichment Kit

Epi

#E1610S 20 reactions

- *Enrichment for m6A modified RNA in immunoprecipitation protocols*
- *Enriched RNA can be used directly for next gen sequencing or RT-qPCR*

Description: The EpiMark N6-Methyladenosine Enrichment Kit contains a rabbit monoclonal antibody specific for N6-Methyladenosine (m6A). The kit also contains two control RNAs, one with m6A modification (*Gaussia* luciferase) and one without (*Cypridina* luciferase) to monitor enrichment and depletion. The GLuc RNA control was transcribed in the presence of 20% m6ATP and 80% ATP.

This kit can be used to enrich m6A modified RNA in immunoprecipitation protocols for downstream analysis by next-generation RNA sequencing or RT-qPCR.

Modified RNA is isolated from a fragmented RNA sample by binding to the N6-Methyladenosine antibody attached to Protein G Magnetic Beads. After multiple wash and clean-up steps, the enriched RNA is eluted in nuclease-free water and is ready for further analysis.

Kit Includes:

- N6-Methyladenosine Antibody
- m6A Control RNA
- Unmodified Control RNA

EpiMark® Hot Start *Taq* DNA Polymerase

#M0490S 100 reactions
#M0490L 500 reactions

- *Ideal for use on bisulfite-converted DNA and AT-rich templates*
- *Specially-formulated reaction buffer system*

Description: EpiMark Hot Start *Taq* DNA Polymerase is an excellent choice for use on bisulfite-converted DNA. It is a mixture of *Taq* DNA Polymerase and a temperature-sensitive, aptamer-based inhibitor. The inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 45°C, but releases the enzyme during normal PCR cycling conditions. This permits room temperature reaction assembly with no separate high-temperature incubation step to activate the enzyme.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 15 nmol dNTP into acid insoluble material in 30 minutes at 75°C.

Concentration: 5,000 units/ml

5-methyl-dCTP

#N0356S 1 µmol

Description: Cytosine modification at carbon 5 (C5) represents an important epigenetic modification. It is also believed to be the starting substrate for the TenEleven Translocation (TET) family of enzymes and their associated oxidation pathways. 5-methyl-dCTP offers the ability to enzymatically make defined fully methylated cytosine-substituted DNA, which can be used for a variety of biochemical and cellular applications.

5-methyl-dCTP (2´-deoxy-5-methylcytidine 5´-triphosphate) is supplied as a 10 mM solution at pH 7. Nucleotide concentration is determined by measurements of absorbance at 260 nm.

Formula: $C_{10}H_{15}N_3O_{13}P_3$ (free acid)

Diluent Compatibility: Can be diluted using sterile distilled water, preferably Milli-Q® water, or can be diluted using sterile TE [10 mM Tris-HCl, 1 mM EDTA (pH 7.5)]

Concentration: 10 mM

Methylation-Dependent Restriction Enzymes

- *Specificity to epigenetically-relevant DNA modifications (5-mC and 5-hmC)*
- *Easy-to-follow protocols*
- *Less harsh than bisulfite conversion*
- *Simplified data analysis*

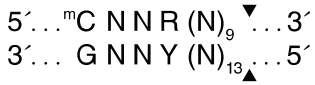
The EpiMark Suite of products has been validated for use in epigenetics applications. Visit **EpiMark.com** for more information.

Many restriction enzymes are sensitive to DNA methylation states. Cleavage can be blocked or impaired when a particular base in the recognition site is modified. The MspJI family of restriction enzymes are dependent on methylation and hydroxymethylation for cleavage to occur (1). These enzymes excise 32-base pair DNA fragments containing a centrally located 5-hmC or 5-mC modified residue that can be extracted and sequenced. Due to the known position of this epigenetic modification, bisulfite conversion is not required prior to downstream analysis. These EpiMark validated, methylation-dependent restriction enzymes expand the potential for mapping epigenetic modifications and simplify the study of DNA methylation. Additionally, they provide an opportunity to better understand the role of 5-hydroxymethylcytosine in the genome.

(1) Cohen-Karni, D. et al. (2011) *PNAS*, 108, 11040–11045.

MspJI

#R0661S 200 units
#R0661L 1,000 units



Description: MspJI is a modification-dependent endonuclease that recognizes ^{m}CNNR sites and generates a double-stranded DNA break on the 3´ side of the modified cytosine at N_9/N_{13}. The recognized cytosine modifications include C5-methylation (5-mC) and C5-hydroxymethylation (5-hmC).

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with 1X Enzyme Activator Solution. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Reagents Supplied:

- rCutSmart
- Enzyme Activator Solution

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

EPIGENETICS

Single Letter Code:
R = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

Additional Restriction Enzymes for Epigenetic Analysis

DpnI
#R0176S 1,000 units
#R0176L 5,000 units

DpnII
#R0543S 1,000 units
#R0543L 5,000 units

for high (5X) concentration
#R0543T 1,000 units
#R0543M 5,000 units

HpaII
#R0171S 2,000 units
#R0171L 10,000 units

for high (5X) concentration
#R0171M 10,000 units

MspI
#R0106S 5,000 units
#R0106L 25,000 units

for high (5X) concentration
#R0106T 5,000 units
#R0106M 25,000 units

Methylation sensitive restriction enzymes can be used to generate fragments for further analysis. When used in conjunction with an isoschizomer that has the same recognition site, but is methylation insensitive, information about methylation status can be obtained.

See the Technical Reference section for more information about Dam, Dcm and CpG methylation.

DNA Methyltransferases

CpG Methyltransferase (M.SssI)
#M0226S 100 units
#M0226L 500 units

for high (5X) concentration
#M0226M 500 units

GpC Methyltransferase (M.CviPI)
#M0227S 200 units
#M0227L 1,000 units

AluI Methyltransferase
#M0220S 100 units

BamHI Methyltransferase
#M0223S 100 units

dam Methyltransferase
#M0222S 500 units
#M0222L 2,500 units

EcoGII Methyltransferase
#M0603S 200 units

EcoRI Methyltransferase
#M0211S 10,000 units

HaeIII Methyltransferase
#M0224S 500 units

HhaI Methyltransferase
#M0217S 1,000 units

HpaII Methyltransferase
#M0214S 100 units

MspI Methyltransferase
#M0215S 100 units

*Taq*I Methyltransferase
#M0219S 1,000 units

NEB offers a selection of DNA methyltransferases that can be used in epigenetics research. More information on these products can be found in the DNA Modifying Enzymes & Cloning Technologies chapter or at www.neb.com.

NEBNext Reagents for ChIP-Seq Library Preparation Epi

NEBNext reagents are a series of highly pure reagents that facilitate library preparation of DNA or RNA for downstream applications, such as next generation sequencing and expression library construction. These reagents undergo stringent quality controls and functional validation, ensuring maximum yield, convenience and value.

For sample preparation of a ChIP-Seq DNA library, NEB offers kits, oligos and modules that support fast workflows. To decide which products to choose, use the selection chart below. For more information, see our NEBNext Reagents for Library Preparation.

ChIP

- **Kits**
  - **NEBNext Ultra II DNA Library Prep Kit +/– Sample Purification Beads** (#E7645/#E7103)
- **Modules**
  - **NEBNext Ultra II End Repair/dA-Tailing Module** (#E7546)
  - **NEBNext Ultra II Ligation Module** (#E7595)
  - **NEBNext Ultra II Q5 Master Mix** (#M0544)

Multiplex Oligos

Single Index	Dual Index	Unique Dual Index	Unique Dual Index UMIs
Set 1 (#E7335), Set 2 (#E7500), Set 3 (#E7710), Set 4 (#E7730), 96 Index Primers (#E6609)	Set 1(#E7600), Set 2 (#E7780)	Set 1 (#E6440), Set 2 (#E6442), Set 3 (#E6444), Set 4 (#E6446), Set 5 (#E6448), Primer Pairs for EM-seq (#E7140)	Set 1(#E7395), Set 2 (#E7874), Set 3 (#E7876), Set 4 (#E7878)

NEBNext Enzymatic Methyl-seq v2 (EM-seq™)

NEW
NEBNext Enzymatic Methyl-seq v2 Kit
#E8015L 96 reactions
#E8015S 24 reactions

NEW
NEBNext Enzymatic Methyl-seq v2 Conversion Module
#E8020S 24 reactions
#E8020L 96 reactions

Companion Products:

NEBNext Enzymatic Methyl-seq Kit
#E7120S 24 reactions
#E7120L 96 reactions

NEBNext UltraShear
#M7634S 24 reactions
#M7634L 96 reactions

NEBNext LV Unique Dual Index Primers Set 2A
#E3390S 24 reactions

NEBNext LV Unique Dual Index Primers Set 2B
#E3392S 24 reactions

NEBNext LV Unique Dual Index Primers Set 1
#E3400S 96 reactions

NEBNext LV Unique Dual Index Primers Set 2
#E3402S 96 reactions

NEBNext LV Unique Dual Index Primers Set 3
#E3404S 96 reactions

NEBNext LV Unique Dual Index Primers Set 4
#E3406S 96 reactions

NEBNext LV Unique Dual Index Primers Set 5
#E3408S 96 reactions

NEBNext Magnetic Separation Rack
#S1515S 24 tubes

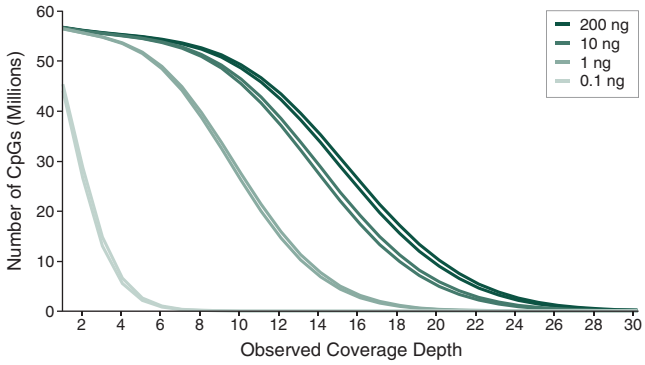
- *Superior sensitivity of detection of 5mC and 5hmC*
- *0.1 ng - 200 ng input range*
- *Detection of more CpGs with fewer sequencing reads*
- *Even GC coverage*
- *High performance library preparation and larger library insert sizes*
- *Index primers supplied separately*
- *Enzymatic fragmentatin of DNA compatible with EM-seq workflows can be achieved using NEBNext UltraShear® (NEB #M7634)*

NEBNext Enzymatic Methyl-seq is an enzymatic alternative to bisulfite conversion with superior performance. For more information, including extensive performance data, visit **NEBNext.com.**

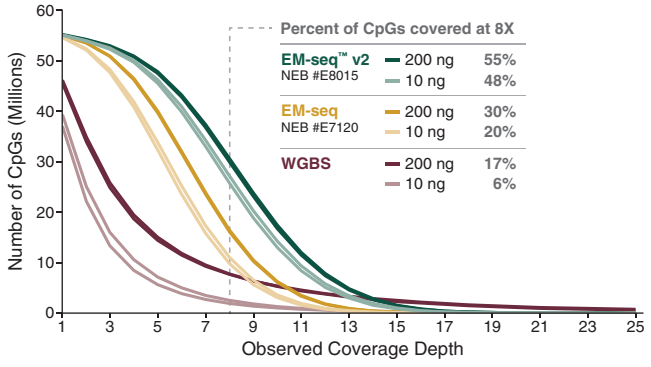
NEBNext Enzymatic Methyl-seq (EM-seq™) is a high-performance enzyme-based alternative to bisulfite conversion for the identification of 5mC and 5hmC. Unlike bisulfite conversion, this highly efficient method minimizes DNA damage, resulting in superior detection of mthylated cytosines, with fewer sequencing reads.

The new NEBNext Enzymatic Methyl-seq v2 Kit has a wider input range (as low as 100 ng) and a faster, more streamlined workflow than the original EM-seq kit (NEB #E7120).

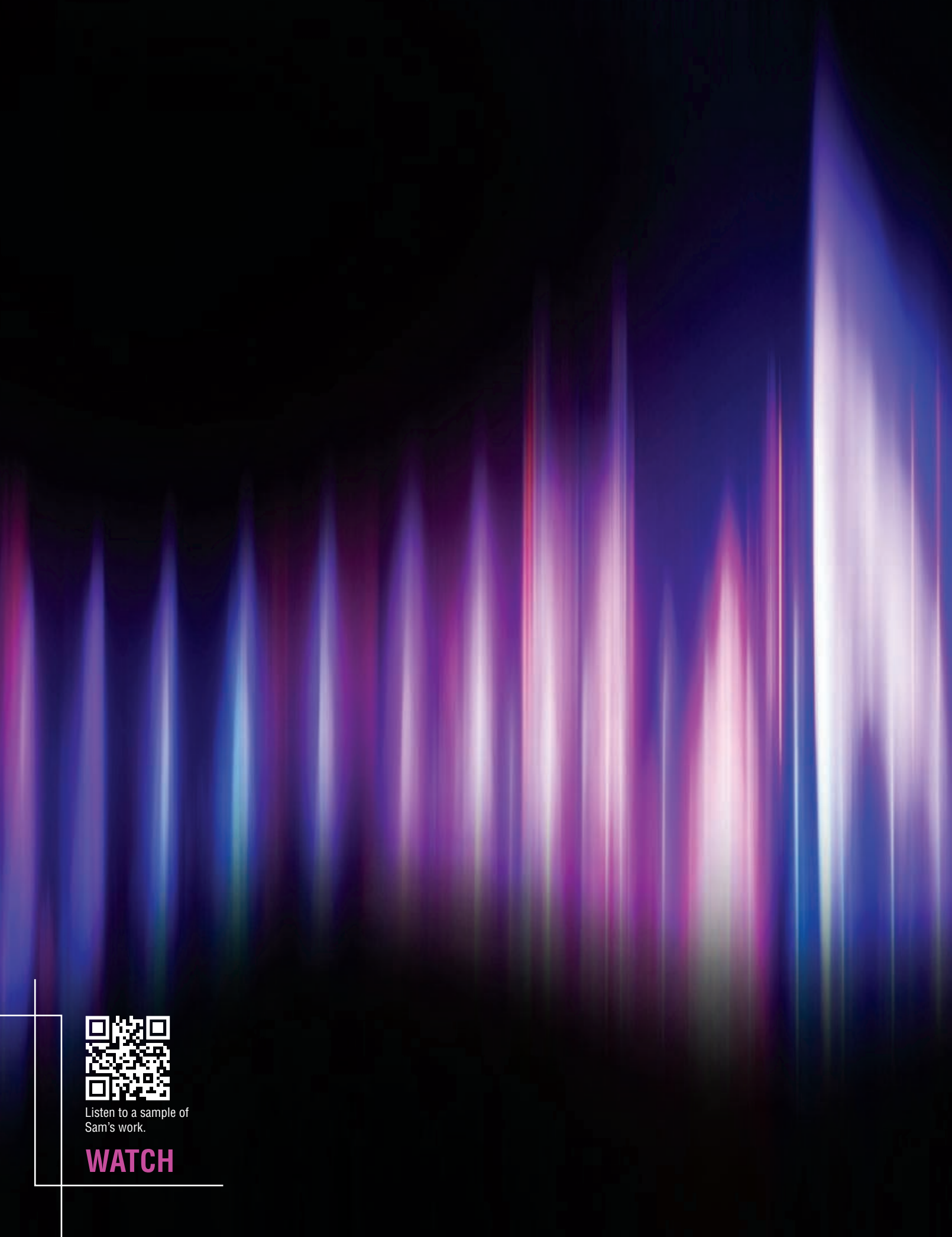
The NEBNext Enzymatic Methyl-seq v2 Kit includes conversion reagents, library prep reagents and the EM-seq Adaptor. Multiole sets of the required index primers (NEBNext LV Unique Dual Index Primers) are available separately, enabling greater flexibility in multiplexing.



EM-seq™ v2 exhibits high CpG coverage across a range of inputs. *EM-seq™ v2 libraries were prepared from 200–0.1 ng of NA12878 DNA (sheared to 350 bp using Covaris® ME220), spiked with unmethylated lambda and CpG-methylated pUC19. Libraries were sequenced on an Illumina® NovaSeq® 6000 (2 x 150 bases). Approximately 910 million reads for each library were aligned to a composite human T2T, lambda and pUC19 reference genome using bwa-meth. The T2T genome covers a maximum of 67.8 million CpGs when the top and bottom strands are counted independently. EM-seq covered over 56 million CpG sites for 200–1 ng inputs and roughly 45 million CpG sites for 0.1 ng input libraries.*

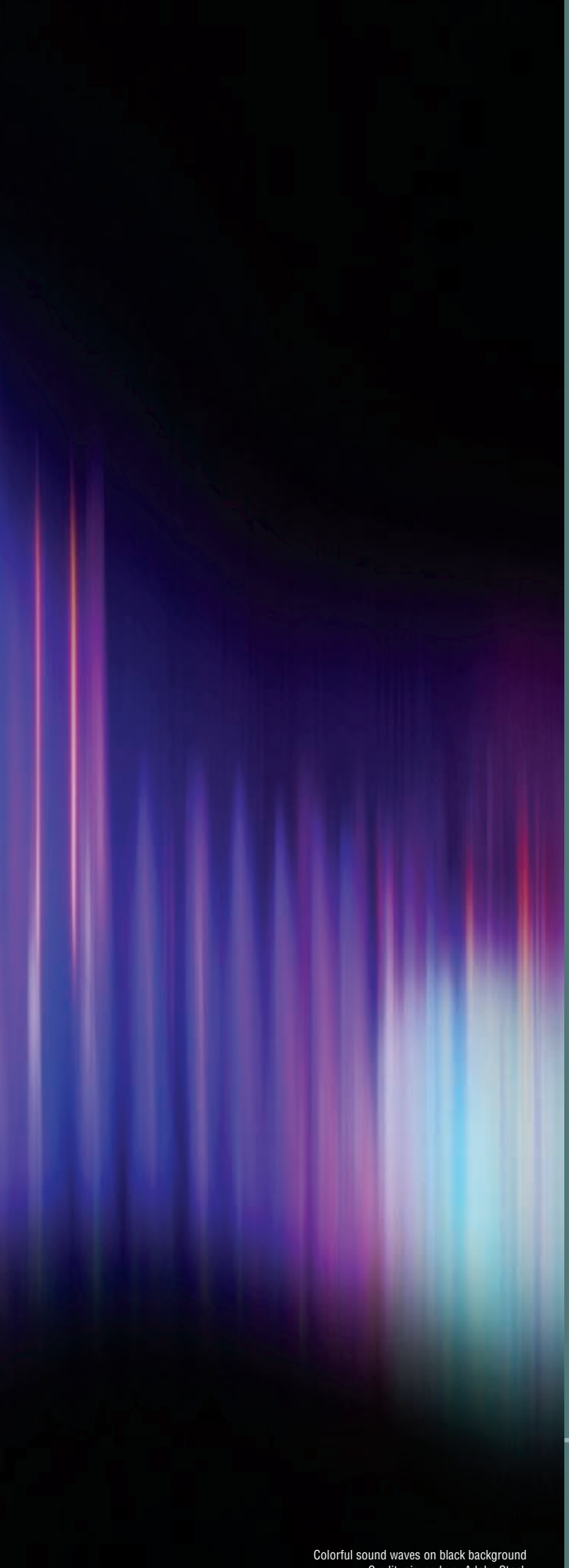


NEBNext EM-seq™ v2 identifies more CpGs than WGBS and the original EM-seq, at lower sequencing coverage depth. *EM-seq™ v2 (NEB #E8015), EM-seq (NEB #E7120) and WGBS libraries were prepared from 200 ng and 10 ng of NA12878 DNA (sheared to ~350 bp), spiked with unmethylated lambda and CpG-methylated pUC19. Libraries were sequenced on an Illumina NovaSeq 6000. For accurate comparison of the original EM-seq and WGBS data with EM-seq v2 data, we evaluated data from approximately 625 million 100 base reads for each library aligned to a composite human T2T, lambda and pUC19 reference genome using bwa-meth. The T2T genome covers a maximum of 67.8 million CpGs when the top and bottom strands are counted independently. EM-seq v2 and EM-seq covered over 54 million CpG sites for both 200 ng and 10 ng inputs; however, WGBS libraries covered only 46 million and 39 million for 200 ng and 10 ng inputs respectively at 1X coverage. The dashed lines represent coverage of 8X. The table lists the percentage of CpG sites covered by different libraries at 8X coverage level.*



Listen to a sample of Sam's work.

WATCH



Colorful sound waves on black background
Credit: nicvandum, Adobe Stock

Amplifying the Sound of Science

Mass spectrometry for proteomics generates vast, complex datasets traditionally analyzed using statistical methods and graphical representations. Recognizing how daunting these datasets can be, Sam Siljee embarked on an innovative project to sonify raw mass spectrometry data, creating an immersive audio experience.

Siljee's project stems from the idea that data interpretation can engage more than just the visual senses. By converting complex waveforms from mass spectrometry into unique tones, he provides an alternative avenue for scientists to connect with their data. He used principles from Fourier's Theorem, which is a mathematical technique that transforms time-based data into frequency-based data. He mapped the mass-to-charge ratio and intensity of spectral peaks to frequency and amplitude, respectively. Each spectrum, composed of thousands of peaks, becomes a distinctive tone generated by adding the thousands of sine waves corresponding to the data points.

Siljee emphasizes the importance of honoring the raw data without imposing arbitrary coercions, such as predefined musical scales or synthesized sounds. He ensures that the soundscapes are derived directly from the data to preserve its integrity. The timing, tone and pitch in his compositions come solely from the raw data, avoiding cultural biases inherent in traditional musical structures.

Siljee's sonification of mass spectrometry data not only makes complex datasets more comprehensible and accessible to a wider audience, including scientists with vision impairments, but also serves as an engaging educational tool. By translating technical data into audible formats, he illustrates key principles like dynamic range and peak width, enhancing understanding and stimulating broader conversations on the complexities of science. This integration of accessibility and education fosters a unique dialogue between science and art, inviting diverse groups to explore and discuss scientific challenges more interactively.

Coming from an artistic family, Siljee naturally blends art and science. His upbringing encouraged creative responses to scientific inquiries, which is evident in his project. *"I hold the philosophy that science and art naturally align, as both are methodologies to explore and explain the world around us,"* said Siljee. This fusion contributes to his understanding of proteomics data and enriches his lung cancer research by offering new perspectives and analytical methods. *"This project has emerged as my creative response to my biology project and an essential component of my Ph.D. journey,"* he said.

Looking ahead, Siljee plans to further develop his project by collaborating with artists; he envisions creating performances or interactive installations to share this experience with a broader audience. Committed to open science, his code is freely available, enabling others to audibly explore their own findings and improve accessibility through a user-friendly web application.

By sonifying mass spectrometry data, Siljee offers a unique contribution to both scientific and artistic communities. His work illustrates the potential of interdisciplinary approaches — not only in enhancing data interpretation and education but also in fostering public engagement — reminding us all that science and art together can profoundly deepen our understanding of the world.

Dr. Sam Siljee
Gillies McIndoe Research Institute, Te Herenga Waka – Victoria University of Wellington, Wellington, New Zealand
2024 Passion in Science
Arts and Creativity Award

Cellular Analysis

Novel tools to study expression & function of proteins.

Cell imaging analysis can use fluorescent dyes, fluorophore-labeled molecules or recombinant protein plasmid systems. Recombinant protein labeling systems and bioluminescent reporter systems are among the most sensitive fluorescence methods for imaging expression, transport, co-localization and degradation in either fixed or living cells. Protein labeling systems offer many advantages. For example, color changes can be easily implemented by using different substrates. Protein labeling systems can involve the use of tag-specific antibodies or antibodies to separate epitopes engineered into a plasmid tag system for detection. Protein labeling systems can be used with non-cell permeable substrates to enable the specific imaging of cell surface targets. This strategy is not possible with bioluminescent recombinant systems. In living cells, protein labeling substrates can be introduced and followed in cells over time. Two separate cellular targets can also be imaged simultaneously, using protein labeling systems with mutually exclusive, tag-specific fluorescent substrates.

Studies of protein expression, interactions and structure, often use reporter systems to introduce and select for gene targets in cells. Reporter genes confer drug resistance, bioluminescence or fluorescence properties in the cells into which they are introduced. Typical reporter studies link reporter genes directly to a promoter region of interest, the function of which can be monitored by the reporter activity. Protein fusion tagging is used to detect subcellular localization, degradation, protein-protein interactions, etc. Typical fusion tags are fluorescent proteins (e.g., eGFP) or small protein epitopes (e.g., FLAG, Myc HA) which can be detected by fluorescence FACS or western blots. New generations of reporter gene systems expand the range of applications and enhance experimental possibilities.

Featured Products

303 SNAP-Cell® 647-SiR

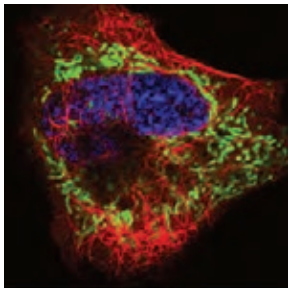
303 SNAP-Surface® 649

Featured Tools & Resources

369 Troubleshooting Guide for SNAP-tag Technology

Application Notes

Videos and Tutorials: SNAP-tag Technology



Live HeLa cell transfected with pSNAP$_f$-tubulin and pCLIP$_f$-Cox8A (mitochondrial cytochrome oxidase 8A). *Cells were labeled with 3 µM SNAP-Cell TMR-Star (red) and 5 µM CLIP-Cell 505 (green) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.*

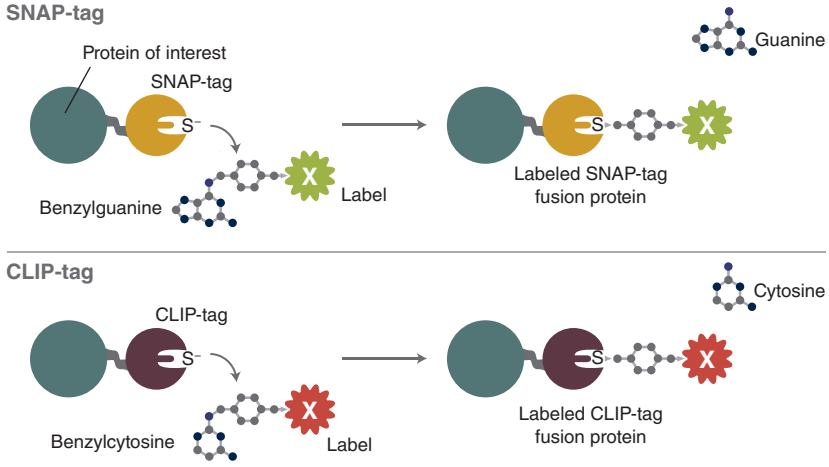
Features of SNAP-tag and CLIP-tag:

- *Clone and express once, then use with a variety of substrates*
- *Non-toxic to living cells*
- *Wide selection of fluorescent substrates*
- *Highly specific covalent labeling*
- *Simultaneous dual labeling*

Applications of SNAP-tag and CLIP-tag:

- *Simultaneous dual protein labeling inside or on the surface of live cells*
- *Protein localization and translocation*
- *Pulse-chase experiments*
- *Receptor internalization studies*
- *Selective cell surface labeling*
- *Protein pull down assays*
- *Protein detection in SDS-PAGE*
- *Flow cytometry*
- *High throughput binding assays in microtiter plates*
- *Biosensor interaction experiments*
- *FRET-based binding assays*
- *Single-molecule labeling*
- *Super-resolution microscopy*
- *Live animal imaging*

New England Biolabs offers an innovative technology for studying the function and localization of proteins in live and fixed cells. Covalent protein labeling brings simplicity and versatility to the imaging of mammalian proteins in live cells, as well as the ability to capture proteins *in vitro*. The creation of a single genetic construct generates a fusion protein which, when covalently attached to a variety of fluorophores, biotin or beads, provides a powerful tool for studying protein dynamics. For added flexibility, NEB offers two systems in which the protein is labeled by a self-labeling fusion protein (SNAP-tag® and CLIP-tag™).



Protein labeling with SNAP-tag and CLIP-tag. *The SNAP- or CLIP-tag is fused to the protein of interest. Labeling occurs through covalent attachment to the tag, releasing either a guanine or a cytosine moiety.*

SNAP-tag and CLIP-tag – Self-Labeling Tag Technology

The SNAP- and CLIP-tag protein labeling systems enable the specific, covalent attachment of virtually any molecule to a protein of interest. There are two steps to using this system: cloning and expression of the protein of interest as a SNAP-tag fusion, and labeling of the fusion with the SNAP-tag substrate of choice. The SNAP-tag is based on the human O6-alkylguanine-DNA-alkyltransferase (hAGT), a DNA repair protein. SNAP-tag substrates are fluorophores, biotin or beads conjugated to guanine or chloropyrimidine leaving groups via a benzyl linker. In the labeling reaction, the substituted benzyl group of the substrate is covalently attached to the SNAP-tag. CLIP-tag is a modified version of SNAP-tag, engineered to react with benzylcytosine rather than benzylguanine derivatives. When used in conjunction with SNAP-tag, CLIP-tag enables the orthogonal and complementary labeling of two proteins simultaneously in the same cells.

SNAP-Cell®: SNAP-Cell labels are cell-permeant and uniquely suited for the labeling of SNAP-tag fusion proteins inside living or fixed cells, on cell surfaces or *in vitro*. These labels are spread across the visible spectrum, ranging from blue to red. Non-fluorescent cell-permeable blocking agent is also available.

SNAP-Surface®: SNAP-Surface labels are non-cell-permeant and routinely used to label SNAP-tag fusion proteins on the surface of living cells, in fixed cells or *in vitro*. These labels are spread across the visible spectrum and include the photostable AlexaFluor® dyes and a variety of other commonly used fluorophores.

CLIP-Cell™: CLIP-Cell labels are cell-permeant and uniquely suited for the labeling of CLIP-tag fusion proteins inside living or fixed cells, on cell surfaces or *in vitro*. The CLIP-tag is a derivative of the SNAP-tag that reacts with orthogonal substrates, allowing simultaneous labeling of two expressed proteins with different fluorophores.

CLIP-Surface™: CLIP-Surface labels are non-cell permeant and routinely used to label CLIP-tag fusion proteins on the surface of living cells, in fixed cells or *in vitro*. The CLIP-tag is a derivative of the SNAP-tag that reacts with orthogonal substrates, allowing simultaneous labeling of two expressed proteins with different fluorophores. The labels include fluorophores at commonly used areas of the visible spectrum, such as 488, 547 and 647 nm.

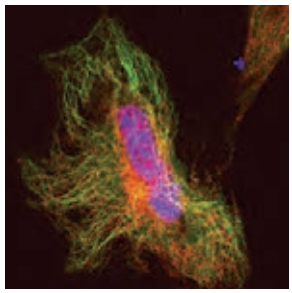
Find an overview of SNAP-tag labeling.

Comparison of SNAP-tag/CLIP-tag Technologies to GFP

While SNAP/CLIP-tag technologies are complementary to Green Fluorescent Protein (GFP), there are several applications for which SNAP- and CLIP-tag self-labeling technologies are advantageous.

Application	SNAP-tag/CLIP-tag	GFP and Other Fluorescent Proteins
Time-resolved fluorescence	Fluorescence can be initiated upon addition of label	Color is genetically encoded and always expressed. Photoactivatable fluorescent proteins require high intensity laser light, which may activate undesired cellular pathways (e.g., apoptosis)
Pulse-chase analysis	Labeling of newly synthesized proteins can be turned off using available blocking reagents (e.g., SNAP-Cell® Block)	Fluorescence of newly synthesized proteins cannot be specifically quenched to investigate dynamic processes
Ability to change colors	A single construct can be used with different fluorophore substrates to label with multiple colors	Requires separate cloning and expression for each color
Surface specific labeling	Can specifically label subpopulation of target protein expressed on cell surface using non-cell permeant substrates	Surface subpopulation cannot be specifically visualized
Single molecule detection	Conjugation with high quantum yield and photostable fluorophores	Fluorescent proteins are generally less bright and photobleach quicker than most organic fluorophores
Visualizing fixed cells	Resistant to fixation; strong labeling	Labile to fixation; weak labeling
Pull-down studies	"Bait" proteins can be covalently captured on BG beads	Requires anti-GFP antibody to non-covalently capture "bait" protein, complicating downstream analysis
Live animal imaging	Cell permeable far-red dye available, permitting deep tissue visualization	Signal is easily quenched by fixation (whole-mount specimens or thick sections); limited spectral flexibility and weaker fluorescence

Fluorescent Substrates for Protein Labeling



Live HeLa cell transfected with pSNAP$_f$-ER (endoplasmic reticulum) and pCLIP$_f$-tubulin.
Cells were labeled with 3 µM SNAP-Cell TMR-Star (red) and 5 µM CLIP-Cell 505 (green) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.

- *Fluorescently label SNAP-tag or CLIP-tag fusions for cellular imaging*
- *Labels span fluorescent imaging spectrum from aqua (430 nm) to far-red (647+ nm) wavelengths*
- *Cell-permeable and non-cell-permeable labels available*

NEB offers a large selection of fluorescent labels (substrates) for SNAP-tag and CLIP-tag fusion proteins. Cell-permeable substrates (SNAP- and CLIP-Cell) are suitable for both intracellular and cell-surface labeling, whereas non-cell-permeable substrates (SNAP- and CLIP-Surface) are specific for fusion proteins expressed on the cell surface only. The labeling reaction is specific for fusion proteins expressed on the cell surface.

Fluorescent substrates for SNAP-tag and CLIP-tag

Self-Labeling Tag	Applications	NEB #	Excitation*	Emission*[1]	Size
SNAP-tag	**Cell-permeable**				
	SNAP-Cell 430	S9109S	421	444,484	50 nmol
	SNAP-Cell 505-Star	S9103S	504	532	50 nmol
	SNAP-Cell Oregon Green®	S9104S	490	514	50 nmol
	SNAP-Cell TMR-Star	S9105S	554	580	30 nmol
	SNAP-Cell 647-SiR	S9102S	645	661	30 nmol
	Non-cell-permeable				
	SNAP-Surface Alexa Fluor® 488	S9129S	496	520	50 nmol
	SNAP-Surface 488	S9124S	506	526	50 nmol
	SNAP-Surface Alexa Fluor 546	S9132S	558	574	50 nmol
	SNAP-Surface 549	S9112S	560	575	50 nmol
	SNAP-Surface 594	S9134S	606	626	50 nmol
	SNAP-Surface Alexa Fluor 647	S9136S	652	670	50 nmol
	SNAP-Surface 649	S9159S	655	676	50 nmol
CLIP-tag	**Cell-permeable**				
	CLIP-Cell 505	S9217S	504	532	50 nmol
	CLIP-Cell TMR-Star	S9219S	554	580	30 nmol
	Non-cell-permeable				
	CLIP-Surface 488	S9232S	506	526	50 nmol
	CLIP-Surface 547	S9233S	554	568	50 nmol
	CLIP-Surface 647	S9234S	660	673	50 nmol

* Excitation and emission values determined experimentally for labeled protein tag.
[1] Colors are based on the electromagnetic spectrum. Actual color visualization may vary.

Blocking Agents

- *Irreversible blocking*
- *Ideal for pulse-chase applications*

Blocking agents are non-fluorescent substrates that block the reactivity of the SNAP-tag intracellularly (SNAP-Cell Block) or on the surface of live and fixed cells (SNAP-Surface Block). They can be used to generate inactive controls in live and fixed cells, as well as in *in vitro* labeling experiments performed with SNAP-tag fusion proteins.

SNAP-Cell Block is highly membrane permeant and once inside the cell reacts with the SNAP-tag, irreversibly inactivating it for subsequent labeling steps.

SNAP-Surface Block also reacts with the SNAP-tag irreversibly, inactivating it for subsequent labeling steps. This blocker is largely membrane impermeant essentially limiting blocking to cell surface-exposed SNAP-tags.

Product	NEB #	Application	Size
SNAP-Cell Block	S9106S	Block SNAP-tag inside live cells, fixed cells and *in vitro*	100 nmol
SNAP-Surface Block	S9143S	Block SNAP-tag on the surface of live cells, fixed cells and *in vitro*	200 nmol

Anti-SNAP-tag® Antibody (Polyclonal)

#P9310S 100 µl

Description: The Anti-SNAP-tag Antibody (Polyclonal) can be used in Western blots with SNAP-tag and CLIP-tag proteins. Polyclonal antibodies are produced from the immunization of rabbit with purified recombinant SNAP-tag protein and affinity purified using SNAP-BG resin.

Sensitivity: 5 ng of SNAP-tag per load in Western blotting.

Recommended Dilution: 1:1000

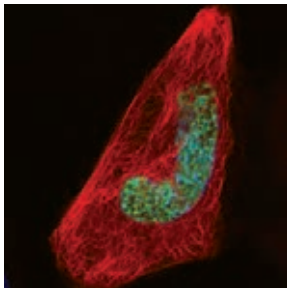
SNAP-tag® Purified Protein

#P9312S 50 µg

Description: SNAP-tag Purified Protein can be used as a positive control for *in vitro* labeling with various SNAP-tag fluorescent substrates. The coding sequence of SNAP-tag was cloned into a pTXB1 derived *E. coli* T7 expression vector. SNAP-tag protein was expressed and purified according to the instructions in the IMPACT™ kit manual (NEB #E6901). The purified SNAP-tag protein was dialyzed into 1X phosphate buffered saline (PBS) solution containing 1 mM DTT at 1 mg/ml (50 µM) and stored at -80°C.

Molecular Weight: 19,694 Da

Cloning Vectors



Live HeLa cell transfected with pSNAP$_f$-tubulin and pCLIPf-H2B constructs generated using pSNAP$_f$ and pCLIP$_f$ vectors. *Cells were labeled with 3 µM SNAP-Cell TMR-Star (red) and 5 µM CLIP-Cell 505 (green) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.*

- *Vectors for mammalian and bacterial expression available*

Vectors are available for SNAP-tag and CLIP-tag fusion protein expression and labeling in mammalian and bacterial systems. The mammalian SNAPf and CLIPf vectors express faster-reacting variants of the SNAP- and CLIP-tags than previously available vectors. Improved polylinker sequences both upstream and downstream from the tag allow expression of the tag on either end of the protein of interest, under control of the CMV promoter. SNAPf-tag and CLIPf-tag expression vectors contain a neomycin resistance (NeoR) gene for selection of stable transfectants, together with an IRES element for efficient expression of both the fusion protein and NeoR. Codon usage has been optimized for mammalian expression. Control plasmids encoding fusion proteins that are localized to the nucleus (H2B), mitochondria (Cox8A) and cell surface (ADRβ2, NK1R) are also available through Addgene.

The bacterial expression vector pSNAP-tag(T7)-2 includes cloning sites both upstream and downstream from the SNAP-tag, which is under the control of the T7 promoter. Codon usage in the SNAP-tag gene has been optimized for *E. coli* expression.

Source: Isolated from an *E. coli* strain by a standard plasmid purification procedure.

Concentration: 500 µg/ml

Restriction Map: The restriction map for pSNAPf Vector can be found in the Technical Reference section. Additional sequence and map files for expression and control plasmids can be found at www.neb.com.

Product	NEB #	Features	Size
pSNAP$_f$ Vector	N9183S	Stable and transient mammalian expression	20 µg
pSNAP-tag (T7)-2 Vector	N9181S	Bacterial expression under T7 control	20 µg
pCLIP$_f$ Vector	N9215S	Stable and transient mammalian expression	20 µg

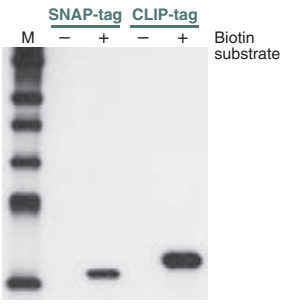
Biotin Labels

SNAP-Biotin
#S9110S 50 nmol

CLIP-Biotin
#S9221S 50 nmol

- *Label SNAP-tag and CLIP-tag fusions with biotin*
- *Compatible with a variety of streptavidin conjugates*
- *Attach to streptavidin surfaces on microtiter plates*

For optimal flexibility with existing technologies, biotinylated labels are available for studies using streptavidin platforms. Cell-permeant (SNAP-Biotin and CLIP-Biotin) substrates are suitable for applications such as biotinylation of fusion proteins for detection with streptavidin fluorophore conjugates or labeling in solution for analysis by SDS-PAGE/ Western blot. Biotin labels are also used for capture with streptavidin surfaces for binding and interaction studies.



Western blot analysis of biotin labeling reactions using anti-Biotin Antibody (CST #7075). *SNAP-tag and CLIP-tag (5 µM) labeled with a biotin-containing substrate (10 µM). Marker M is Biotinylated Protein Ladder (CST #7727).*

SNAP-Capture Magnetic Beads

#S9145S 2 ml

- *Selectively capture SNAP-tag fusion proteins from solution*
- *Ideal for protein pull-down experiments or proteomic analysis*

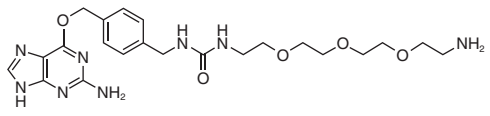
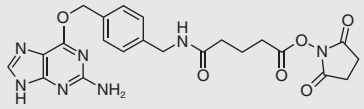
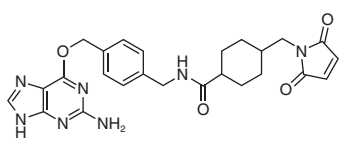
SNAP-Capture Magnetic Beads are magnetic agarose beads coupled to a benzylguanine substrate, used to selectively capture and immobilize SNAP-tag fusion proteins from solution. These beads have a high loading capacity for SNAP-tag fusion proteins and show very low non-specific adsorption of proteins from a complex lysate, making them especially suitable for pull-down applications.

Building Blocks

- *Synthesize new SNAP-tag and CLIP-tag substrates*
- *Make surfaces for protein immobilization*
- *Attach novel molecules or ligands to proteins*
- *Create custom substrates for protein labeling*

For advanced users with novel probes interested in working with SNAP-tag labeling technologies, building blocks are available for linkage of the core benzylguanine (BG) moiety to activated esters, primary amines and thiol groups. A variety of functional groups allows the choice of chemical coupling approaches to suit the molecule or surface to be coupled. Couple onto surfaces such as Biacore® chips or microarrays for specific protein immobilization. Couple onto peptides, proteins and DNA oligomers. Couple onto new fluorophores or affinity reagents for specific protein labeling. Labeling is gentle, precise, and versatile: one label is covalently bound under biological conditions in a defined position.

BIACORE® is a registered trademark of GE Healthcare Life Sciences

Product	NEB #	Structure	Application	Size
BG-PEG-NH$_2$	S9150S		SNAP-tag substrate. PEG-linker gives superior flexibility. Particularly suited for immobilization on solid surfaces.	2 mg
BG-GLA-NHS	S9151S		SNAP-tag substrate. Activated as NHS ester. Reacts with primary amines.	2 mg
BG-Maleimide	S9153S		SNAP-tag substrate. Activated as maleimide. Reacts with thiols.	2 mg

View a collection of our Passion in Science winners' bacterial art.

WATCH

A selection of bacterial art generated by our Passion in Science and Golden Butterfly winners
Credit: Mileidy Rodriguez, New England Biolabs

Revealing the Beauty of Bacteria Through Art

Bacteria, ubiquitous and ancient, are among the most misunderstood organisms on Earth. Often linked to disease, decay and contamination, their reputation is shaped by societal germophobia and media coverage focused on bacterial dangers. Yet, this perspective overlooks the reality that most bacterial species are harmless, beneficial or essential to life. These microscopic architects appeared on Earth over 3.5 billion years ago, oxygenating the atmosphere and laying the groundwork for ecosystems to thrive. Today, they continue to sustain the planet by recycling nutrients, decomposing organic matter and supporting life — from enabling human digestion to fueling deep-sea ecosystems.

Scientific advancements have revealed the profound impact of bacteria on human health and the environment. Research into the gut-brain axis, probiotics and microbiomes underscores the intricate relationship between microbes and human physiology, influencing digestion, immunity and mental health. Yet, despite their importance, public understanding of bacteria remains limited. This gap is where bacterial art emerges — a unique medium that reframes bacteria as subjects of beauty, wonder and creativity rather than fear and infection.

Bacterial art uses living microorganisms as dynamic "paints" to form intricate, evolving designs on nutrient-rich agar plates. This can be seen as far back as the early 20th century when Sir Alexander Fleming famously painted with pigmented bacteria to highlight biodiversity and integrate scientific inquiry with artistic expression. Strains like *Serratia marcescens* (red), *Deinococcus radiodurans* (pink), and various strains of *Nesterenkonia* (golden) provide vibrant palettes that bloom into natural patterns over time, celebrating bacteria's hidden beauty.

The 2024 Passion in Science Awardees experienced bacterial art firsthand during a workshop at New England Biolabs. Led by NEB Senior Scientist Dr. Mehmet Berkmen, the awardees used colorful bacterial strains to craft microbial masterpieces. Over several days, their designs grew and evolved, transforming static ideas into living works of art. The hands-on experience showcased the creativity inherent in bacteria and revealed how science and art intersect. Like scientific discovery, creating bacterial art involves curiosity, experimentation and a willingness to embrace the unexpected. Berkmen describes bacteria as co-artists, their natural behaviors influencing the final patterns. This creative process mirrors the scientific method, where careful planning meets moments of serendipity.

Unlike scientific jargon, which can alienate non-specialists, art invites curiosity and emotional connection. Through bacterial art, complex scientific concepts become accessible, transcending cultural and educational barriers. For example, time-lapse animations of bacterial growth illustrate microbial motility, cooperation and competition — concepts central to understanding ecosystems and antibiotic resistance.

Bacterial art makes the invisible visible, reframing bacteria as life's vibrant collaborators rather than adversaries. It opens doors to new narratives that celebrate both the wonder of life and the boundless possibilities of human creativity. As this field evolves, it has the potential to deepen our appreciation of the microbial world — an appreciation vital for a sustainable and interconnected future.

Mehmet Berkman
Thank you to Dr. Mehmet Berkman for sharing his love of microbes with our Passion in Science winners. Learn more about Memo's work with bacterial art at **www.bacterialart.com**

Reference Appendix

Technical Support – for scientists, by scientists

As a partner to the scientific community, New England Biolabs is committed to providing top quality tools and scientific expertise. This philosophy has led to long-standing relationships with many of our fellow scientists. NEB's commitment to scientists is the same regardless of whether or not they purchase products from NEB; their ongoing research is supported by our catalog, website and technical staff.

NEB's technical support model is unique as it utilizes most of our scientists. Several of our product lines have designated technical support scientists assigned to serving customers in those application areas. Questions regarding a product could be dealt with by one of the technical support scientists, the product manager who manufactures it, the product development scientist who optimizes it, or a researcher who uses the product in their daily research. As such, customers are supported by scientists and experts in the product or its application.

To access technical support:

- Call 1-800-632-7799 (Monday – Friday: 9:00 am - 6:00 pm EST)
- Submit an online form at www.neb.com/techsupport
- Email info@neb.com
- International customers can contact a local NEB subsidiary or distributor. For more information see inside back cover.

Featured Tools & Resources

Visit the **Tools & Resources tab** at **www.neb.com** to find additional online tools, video tech tips and tutorials to help you in your research.

Learn more about NEB's tech support program.

Online Interactive Tools, Databases & Mobile Apps

Use the Tools & Resources tab at www.neb.com to access our growing selection of interactive technical tools. These tools can also be accessed directly by visiting **www.neb.com/nebtools**.

NEB scientists are often involved in the development of online tools that will aid in their research. We are now making these tools and in some circumstances, the source code, available for you to evaluate. To learn more, visit **www.neb.com/NEBetaTools**.

Online Tools

Competitor Cross-Reference Tool

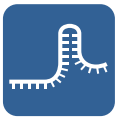
Use this tool to select another company's product and find out which NEB product is compatible. Choose either the product name or catalog number from the available selections, and this tool will identify the recommended NEB product.

DNA Sequences and Maps Tool

With the DNA Sequences and Maps Tool, find the nucleotide sequence files for commonly used molecular biology tools, including plasmid, viral and bacteriophage vectors.

Double Digest Finder

Use this tool to guide your reaction buffer selection when setting up double digests, a common timesaving procedure. Choosing the right buffers will help you to avoid star activity and loss of product.



EnGen sgRNA Template Oligo Designer

EnGen sgRNA Template Oligo Designer can be used to design target-specific DNA oligos for use with the EnGen sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322).

Enzyme Finder

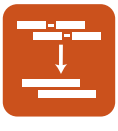
Use this tool to select restriction enzymes by name, sequence, overhang or type. Enter your sequence using single letter code nomenclature, and Enzyme Finder will identify the right enzyme for the job.

Exo Selector

Use this tool to simplify the process of selecting the appropriate exonucleases for use in your nucleic acid digestion workflows. The tool guides you to product recommendations based on your answers to a few simple questions.

Glycan Analyzer

Use this tool to interpret ultra or high pressure liquid chromatography (UPLC/HPLC) N-glycan profiles following exoglycosidase digestions.



NEBridge® Ligase Fidelity Tools

These tools can be used to design your Golden Gate Assemblies – visualize overhang ligation preferences with the Ligase Fidelity Viewer®, Predict high-fidelity junction sets with GetSet®, and split DNA sequences for scarless high-fidelity assembly with SplitSet®.

NEBridge® Golden Gate Assembly Tool

Use this tool to assist with in silico DNA construct design for Golden Gate DNA assembly. It enables the accurate design of primers with appropriate Type IIS restriction sites and overlaps, quick import of sequences in many formats and export of the final assembly, primers and settings.

NEB LAMP Primer Design Tool

NEB LAMP Primer Design Tool can be used to design primers for your Loop-mediated Isothermal Amplification. Fixed primers can be specified for the design of LAMP primers, and subsequent Loop primers are then designed based on LAMP primer selection.

NEBaseChanger®

NEBaseChanger can be used to design primers specific to the mutagenesis experiment you are performing using the Q5® Site-Directed Mutagenesis Kit. This tool will also calculate a recommended custom annealing temperature based on the sequence of the primers by taking into account any mismatches.

NEBcloner®

Use this tool to find the right products and protocols for each step (digestion, end modification, ligation, transformation and mutagenesis) of your next traditional cloning experiment. Also, find other relevant tools and resources to enable protocol optimization.

NEBcutter® V3.0

Identify the restriction sites within your DNA sequence using NEBcutter. Choose between Type II and commercially available Type III restriction enzymes to digest your DNA. NEBcutter will indicates cut frequency and methylation-state sensitivity.

NEBioCalculator®

Use this tool for your scientific calculations and conversions for DNA and RNA. Options include conversion of mass to moles, ligation amounts, conversion of OD to concentration, dilution and molarity. Additional features include sgRNA template oligo design and qPCR library quantification.

NEBNext Custom RNA Depletion Design Tool

This tool designs probes to be used with the NEBNext RNA Depletion Core Reagent Set or to supplement an existing NEBNext Depletion Kit for the depletion of unwanted RNA species.

NEBNext Selector

Use this tool to guide you through the selection of NEBNext reagents for next generation sequencing sample preparation.

Online Interactive Tools, Databases & Mobile Apps (continued)

NEBuilder® Assembly Tool

Use this tool to design primers for your DNA assembly reaction, based on the entered fragment sequences and the polymerase being used for amplification.

NEBuilder® Protocol Calculator

Use this tool to calculate the optimal amounts of input DNA sequences for the NEBuilder® HiFi Assembly reaction given the length & concentration of each input fragment.

PCR Fidelity Estimator

Estimate the percentage of correct DNA copies (those without base substitution errors) per cycle of PCR for selected DNA polymerases.

PCR Selector

Use this tool to help select the right DNA polymerase for your PCR setup. Whether your amplicon is long, complex, GC-rich or present in a single copy, the PCR selection tool will identify the perfect DNA polymerase for your reaction.

Read Coverage Calculator

This tool allows for easy calculation of values associated with read coverage in NGS protocols.

Thermostable Ligase Reaction Temperature Calculator

This tool will help you estimate an optimal reaction temperature to minimize mismatch for thermostable ligation of two adjacent ssDNA probes annealed to a template.

Tm Calculator

Determine the optimal annealing temperature for your amplicon with our Tm Calculator. Simply input your DNA polymerase, primer concentration and your primer sequence, and the Tm Calculator will guide you to successful reaction conditions.

Additional Databases

Polbase®

Polbase is a repository of biochemical, genetic and structural information about DNA Polymerases.

REBASE®

Use this tool as a guide to the ever-changing landscape of restriction enzymes. REBASE, the Restriction Enzyme DataBASE, is a dynamic, curated database of restriction enzymes and related proteins.

Additional Tools

NEBnow Locator

Use this tool to find an NEBnow Freezer Program near you. Search by city, company or institution.

NEBeta™ Tools

View our selection of online tools currently in development. These tools are not yet optimized for design and usability – we are looking for feedback on functionality and utility to improve them for future use.

Mobile App

NEB Tools for iPhone®, iPad® or Android®

NEB Tools brings New England Biolabs' most popular web tools to your iPhone, iPad or Android devices.

- Use Enzyme Finder to select a restriction enzyme by category or recognition sequence, or search by name to find information on any NEB enzyme. Sort your results so they make sense to you, then email them to your inbox or connect directly to www.neb.com.
- Use Double Digest Finder to determine buffer and reaction conditions for experiments requiring two restriction enzymes.
- Use Tm Calculator to calculate annealing temperatures for your PCR reaction.
- Also included are several popular calculators from the NEBioCalculator web app.

Optimizing Restriction Enzyme Reactions

While standard recommended reaction conditions are a good place to start, in some cases, optimization may be necessary to achieve the best results. Depending on the enzyme(s) being used, variables such as incubation time and number of enzyme units used can be tested to find the optimal reaction conditions for your substrate DNA and enzyme(s) of choice.

Protocol Restriction Enzyme Reactions

	Standard Protocol	Time-Saver Protocol
DNA	up to 1 µg	up to 1 µg
10X Buffer	5 µl (1X)	5 µl (1X)
Restriction Enzymes	10 units*	1 µl
Total Volume	50 µl	50 µl
Incubation Temperature	Enzyme-dependent	Enzyme-dependent
Incubation Time	60 minutes	5–15 minutes**

* Sufficient to digest all types of DNAs.
** Time-Saver qualified enzymes can also be incubated overnight with no star activity.

Tools & Resources

Visit NEBRestrictionEnzymes.com to find:

- Online tutorials for setting up restriction enzyme digests
- Tips to avoid Star activity
- Restriction Enzyme Performance Chart
- Troubleshooting Guide
- Access to NEB's online tools, including: **Enzyme Finder, DoubleDigest Finder** and **NEBcloner**

Tips for Optimization

Enzyme

- Keep on ice when not in the freezer
- Should be the last component added to reaction
- Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube. Follow with a quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.
- In general, we recommend 5–10 units of enzyme per µg DNA, and 10–20 units per µg of genomic DNA in a 1 hour digest
- NEB has introduced a line of High-Fidelity (HF®) enzymes for added flexibility
- Some restriction enzymes require more than one recognition site to cleave efficiently. These are designated with the "multi-site" icon 2+site. Please review recommendations on working with these enzymes at www.neb.com.

Star Activity

- Unwanted cleavage that can occur when enzyme is used under sub-optimal conditions, such as:
  - Too much enzyme present
  - Too long of an incubation time
  - Using a non-recommended buffer
  - Glycerol concentrations above 5%
- Star activity can be reduced by using a High-Fidelity (HF®) enzyme, reducing incubation time, using a Time-Saver™ enzyme or increasing reaction volume

DNA

- Should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents and salts. Extra wash steps during purification are recommended.
- Methylation of DNA can affect digestion with certain enzymes. For more information about methylation visit www.neb.com/methylation

Buffer

- Use at a 1X concentration
- Supplement with rAlbumin, DTT or Activator to recommended concentration if required
- Recombinant Albumin is included in NEBuffer r1.1, r2.1, r3.1 and rCutSmart™ Buffer.

Reaction Volume

- A 50 µl reaction volume is recommended for digestion of up to 1 µg of substrate. This helps maintain salt levels introduced by miniprepped DNA low enough that they don't affect enzyme activity.
- Enzyme volume should not exceed 10% of the total reaction volume to prevent star activity due to excess glycerol
- Additives in the restriction enzyme storage buffer (e.g., glycerol, salt), as well as contaminants found in the substrate solution (e.g., salt, EDTA or alcohol), can be problematic in smaller reaction volumes.

Incubation Time

- Incubation time for the Standard Protocol is 1 hour. Incubation for the Time-Saver Protocol is 5–15 minutes.
- Visit www.neb.com/timesaver for list of Time-Saver qualified enzymes
- It is possible, with many enzymes, to use fewer units and digest for up to 16 hours. For more information, visit www.neb.com

Stopping a Reaction

- If no further manipulation of DNA is required:
  - Terminate with a stop solution (10 µl per 50 µl rxn) [1x: 2.5% Ficoll®-400, 10mM EDTA, 3.3 mM Tris-Hcl, 0.08% SDS, 0.02% Dye 1, 0.001% Dye 2, pH 8.0@25°C] (e.g., NEB #B7024)
- When further manipulation of DNA is required:
  - Heat inactivation can be used for some samples
  - Remove enzyme by using a spin column (NEB #T1130) or phenol/chloroform extraction

Storage

- Storage at –20°C is recommended for most restriction enzymes. For a few enzymes, storage at–80°C is recommended. Visit www.neb.com for storage information.
- 10X NEBuffers should be stored at –20°C

Stability

- The expiration date is found on the label
- Long term exposure to temperatures above –20°C should be minimized whenever possible

Learn more about optimizing restriction endonuclease reactions

Double Digestion

Digesting a DNA substrate with two restriction enzymes simultaneously (double digestion) is a common timesaving procedure. Over 210 restriction enzymes are 100% active in rCutSmart Buffer, making double digestion simple. If you are using an enzyme that is not supplied with rCutSmart Buffer, the Performance Chart for Restriction Enzymes rates the percentage activity of each restriction endonuclease in the four standard NEBuffers.

Setting up a Double Digestion

- Double digests with CutSmart restriction enzymes can be set up in rCutSmart Buffer. Otherwise, choose an NEBuffer that results in the most activity for both enzymes. If star activity is a concern, consider using one of our High-Fidelity (HF) enzymes.
- Set up reaction according to recommended protocol. The final concentration of glycerol in any reaction should be less than 5% to minimize the possibility of star activity. For example, in a 50 µl reaction, the total amount of enzyme added should not exceed 5 µl. NEBcloner can also be used to determine recommended double-digest conditions.
- If two different incubation temperatures are necessary, choose the optimal reaction buffer and set up reaction accordingly. Add the first enzyme and incubate at the desired temperature. Then, heat inactivate the first enzyme, if it can be heat inactivated, add the second enzyme and incubate at the recommended temperature.
- Depending on an enzyme's activity rating in a non-optimal NEBuffer, the number of units or incubation time may be adjusted to compensate for the slower rate of cleavage. The Performance Chart for Restriction Enzymes indicates if star activity is an issue with sub-optimal buffers.

Setting up a Double Digestion with a Unique Buffer (designated "U")

- NEB currently supplies two enzymes with unique buffers: EcoRI and DpnII. In most cases, DpnII requires a sequential digest. Note that EcoRI has an HF version (NEB #R3101) which is supplied with rCutSmart Buffer.

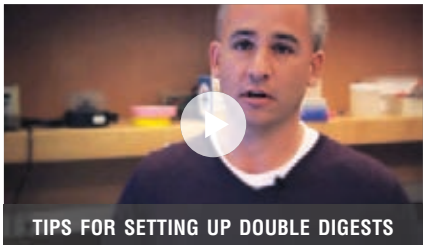
Setting up a Sequential Digestion

- If there is no buffer in which the two enzymes exhibit > 50% activity, a sequential digest can be performed.
- Set up a reaction using the restriction endonuclease that has the lowest salt concentration in its recommended buffer and incubate to completion.
- Adjust the salt concentration of the reaction (using a small volume of a concentrated salt solution) to approximate the reaction conditions of the second restriction endonuclease.
- Add the second enzyme and incubate to complete the second reaction.
- Alternatively, a spin column can be used to isolate the DNA prior to the second reaction. NEB recommend's using our Monarch Nucleic Acid Purification Kits (see the Nucleic Acid Purification chapter or visit **NEBmonarch.com**).

Tools & Resources

Visit www.neb.com/nebtools for:

- Help choosing double digest conditions using NEB's **DoubleDigest Finder** and **NEBcloner®**



Types of Restriction Enzymes

Restriction enzymes are traditionally classified into four types on the basis of subunit composition, cleavage position, sequence specificity and cofactor requirements. However, amino acid sequencing has uncovered extraordinary variety among restriction enzymes and revealed that at the molecular level there are many more than four different types.

Type I Enzymes are complex, multisubunit, combination restriction-and-modification enzymes that cut DNA at random far from their recognition sequences. Type I enzymes are of considerable biochemical interest, but they have little practical value since they do not produce discrete restriction fragments or distinct gel-banding patterns.

Type II Enzymes cut DNA at defined positions close to or within their recognition sequences. They produce discrete restriction fragments and distinct gel banding patterns, and they are the predominant class used in the laboratory for DNA analysis and gene cloning. Type IIS restriction enzymes recognize asymmetric DNA sequences and cleave outside of their recognition sequences. They are useful for many applications, including Golden Gate Assembly.

Rather than forming a single family of related proteins, Type II enzymes are a collection of unrelated proteins of many different sorts. Type II enzymes frequently differ greatly in amino acid sequence from one another, and indeed from every other known protein, that they exemplify the class of rapidly evolving proteins that are often indicative of involvement in host-parasite interactions.

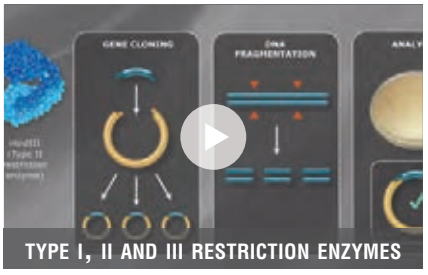
Type III Enzymes are also large combination restriction-and-modification enzymes. They cleave outside of their recognition sequences and require two such sequences in opposite orientations within the same DNA molecule to accomplish cleavage; they rarely yield complete digests.

Type IV Enzymes recognize modified, typically methylated DNA and are exemplified by the McrBC and Mrr systems of *E. coli*.

Tools & Resources

Visit the video library at www.neb.com to find:

- Tutorials on Type I, II and III restriction enzymes



View double digest protocol.

Restriction Enzyme Troubleshooting Guide

Problem	Cause	Solution
Few or no transformants	Restriction enzyme(s) didn't cleave completely	• Check the methylation sensitivity of the enzyme(s) to determine if the enzyme is blocked by methylation of the recognition sequence • Use the recommended buffer supplied with the restriction enzyme • Clean up the DNA to remove any contaminants that may inhibit the enzyme • When digesting a PCR fragment, make sure to have at least 6 nucleotides between the recognition site and the end of the DNA molecule
The digested DNA ran as a smear on an agarose gel	The restriction enzyme(s) is bound to the substrate DNA	• Lower the number of units • Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the DNA or use Gel Loading Dye, Purple (6X) (NEB #B7024)
	Nuclease contamination	• Use fresh, clean running buffer and a fresh agarose gel • Clean up the DNA. We recommend the Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130).
Incomplete restriction enzyme digestion	Cleavage is blocked by methylation	• Check the methylation sensitivity of the enzyme(s) to determine if the enzyme is blocked by methylation of the recognition sequence • DNA isolated from a bacterial source may be blocked by Dam and Dcm methylation • If the enzyme is inhibited by Dam or Dcm methylation, grow the plasmid in a dam-/dcm- strain (NEB #C2925) • DNA isolated from eukaryotic source may be blocked by CpG methylation
	Salt inhibition	• Enzymes that have low activity in salt-containing buffers (NEBuffer r3.1) may be salt sensitive, so clean up the DNA (NEB #T1130) prior to digestion • Some DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. To prevent this, DNA solution should be no more than 25% of total reaction volume.
	Inhibition by PCR components	• Clean up the PCR fragment prior to restriction digest (NEB #T1130)
	Using the wrong buffer	• Use the recommended buffer supplied with the restriction enzyme
	Too few units of enzyme used	• Use at least 3–5 units of enzyme per µg of DNA
	Incubation time was too short	• Increase the incubation time
	Digesting supercoiled DNA	• Some enzymes have a lower activity on supercoiled DNA. Increase the number of enzyme units in the reaction.
	Presence of slow sites	• Some enzymes can exhibit slower cleavage towards specific sites. Increase the incubation time, 1–2 hours is typically sufficient.
	Two sites required	• Some enzymes require the presence of two recognition sites to cut efficiently
	DNA is contaminated with an inhibitor	• Assay substrate DNA in the presence of a control DNA. Control DNA will not cleave if there is an inhibitor present. Miniprep DNA is particularly susceptible to contaminants. • Clean DNA with a spin column or increase volume to dilute contaminant. We recommend the Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130).
Extra bands in the gel	If larger bands than expected are seen in the gel, this may indicate binding of the enzyme(s) to the substrate	• Lower the number of units in the reaction • Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the substrate, or use Gel Loading Dye, Purple (6X) (NEB #B7024)
	Star activity	• Use the recommended buffer supplied with the restriction enzyme • Decrease the number of enzyme units in the reaction • Make sure the amount of enzyme added does not exceed 10% of the total reaction volume. This ensures that the total glycerol concentration does not exceed 5% v/v. • Decrease the incubation time. Using the minimum reaction time required for complete digestion will help prevent star activity. • Try using a High-Fidelity (HF) restriction enzyme. HF enzymes have been engineered for reduced star activity.
	Partial restriction enzyme digest	• Enzymes that have low activity in salt-containing buffers (e.g., NEBuffer r3.1) may be salt sensitive. Make sure to clean up the DNA prior to digestion. • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. To prevent this, DNA solution should be no more than 25% of total reaction volume. • Clean-up the PCR fragment prior to restriction digest • Use the recommended buffer supplied with the restriction enzyme • Use at least 5–10 units of enzyme per µg of DNA and digest the DNA for 1–2 hours

Performance Chart for Restriction Enzymes

New England Biolabs supplies > 210 restriction enzymes that are 100% active in rCutSmart Buffer. This results in increased efficiency, flexibility and ease-of-use, especially when performing double digests.

This performance chart summarizes the activity information of NEB restriction enzymes. To help select the best conditions for double digests, this chart shows the optimal (supplied) NEBuffer and approximate activity in the four standard NEBuffers for each enzyme. In addition, this performance chart shows recommended reaction temperature, heat-inactivation temperature, recommended diluent buffer, methylation sensitivity and whether the enzyme is Time-Saver qualified (e.g., cleaves substrate in 5–15 minutes under recommended conditions, and can be used overnight without degradation of DNA), and whether the enzyme works better in a substrate with multiple sites.

Chart Legend

- **U** — Supplied with a unique reaction buffer that is different from the four standard NEBuffers. The compatibility with the four standard NEBuffers is indicated in the chart.
- RR — Recombinant
- e — Engineered enzyme for maximum performance
- Time-Saver qualified
- 2*site — Indicates that the restriction enzyme requires two or more sites for cleavage
- dcm — dcm methylation sensitivity
- dam — dam methylation sensitivity
- CpG — CpG methylation sensitivity

Activity Notes (see last column)

FOR STAR ACTIVITY

1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.
2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.

* May exhibit star activity in this buffer.

\+ For added flexibility, NEB offers an isoschizomer or HF enzyme, supplied with rCutSmart Buffer.

FOR LIGATION AND RECUTTING

a. Ligation is less than 10%

b. Ligation is 25% – 75%

c. Recutting after ligation is < 5%

d. Recutting after ligation is 50% – 75%

e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or the recognition sequence contains variable sequences.

Performance Chart for Restriction Enzymes

			Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1		Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity		Notes
RR	e		AatII	rCutSmart	<10	50*	50	100	37°	80°	B	λ DNA		CpG	
RR	e		Acc65I	r3.1	10	75*	100	25	37°	65°	A	pBC4 DNA	dcm	CpG	
RR	e		AccI	rCutSmart	50	50	10	100	37°	80°	A	λ DNA		CpG	
RR	e		AciI	rCutSmart	<10	25	100	100	37°	65°	A	λ DNA		CpG	
RR	e		AclI	rCutSmart	<10	<10	<10	100	37°	No	B	λ DNA		CpG	
RR	e		AcuI	rCutSmart	50	100	50	100	37°	65°	B	λ DNA			1, b, d
RR			AfeI	rCutSmart	25	100	25	100	37°	65°	B	pXba DNA		CpG	
RR	e		AflII	rCutSmart	50	100	10	100	37°	65°	A	ΦX174 RF I DNA			
RR	e		AflIII	r3.1	10	50	100	50	37°	80°	B	λ DNA			
RR	e	e	AgeI-HF	rCutSmart	100	50	10	100	37°	65°	A	λ DNA		CpG	
RR	e		AhdI	rCutSmart	25	25	10	100	37°	65°	A	λ DNA		CpG	a
RR		e	AleI-v2	rCutSmart	<10	<10	<10	100	37°	65°	B	λ DNA		CpG	
RR	e		AluI	rCutSmart	25	100	50	100	37°	80°	B	λ DNA			b
RR			AlwI	rCutSmart	50	50	10	100	37°	No	A	λ DNA (dam-)	dam		1, b, d
RR	e		AlwNI	rCutSmart	10	100	50	100	37°	80°	A	λ DNA	dcm		
RR	e		ApaI	rCutSmart	25	25	<10	100	37°	65°	A	pXba DNA	dcm	CpG	
RR	e		ApaLI	rCutSmart	100	100	10	100	37°	No	A	λ DNA (HindIII digest)		CpG	
RR	e		ApeKI	r3.1	25	50	100	10	75°	No	B	λ DNA		CpG	
RR	e	e	ApoI-HF	rCutSmart	10	100	10	100	37°	80°	B	λ DNA			
RR	e		AscI	rCutSmart	<10	10	10	100	37°	80°	A	λ DNA		CpG	
RR	e		AseI	r3.1	<10	50*	100	10	37°	65°	B	λ DNA			3
RR			AsiSI	rCutSmart	100	100	25	100	37°	80°	B	XhoI digested pXba		CpG	2, b
RR	e		AvaI	rCutSmart	<10	100	25	100	37°	80°	A	λ DNA		CpG	
RR	e		AvaII	rCutSmart	50	75	10	100	37°	80°	A	λ DNA	dcm	CpG	
RR	e		AvrII	rCutSmart	100	50	50	100	37°	No	B	λ DNA (HindIII digest)			

Performance Chart for Restriction Enzymes (Continued)

Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1		Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity	Notes
BaeGI	r3.1	75	75	100	25	37°	80°	A	λ DNA		
BaeI	rCutSmart	50	100	50	100	37°	65°	A	λ DNA	CpG	e
BamHI	r3.1	75*	100*	100	100*	37°	No	A	λ DNA		3
BamHI-HF	rCutSmart	100	50	10	100	37°	No	A	λ DNA		
BanI	rCutSmart	10	25	<10	100	37°	65°	A	λ DNA	dcm CpG	1
BanII	rCutSmart	100	100	50	100	37°	80°	A	λ DNA		2
BbsI	r2.1	100	100	25	75	37°	65°	B	λ DNA		
BbsI-HF	rCutSmart	10	10	10	100	37°	65°	B	λ DNA		
BbvCI	rCutSmart	10	100	50	100	37°	No	B	λ DNA	CpG	1, a
BbvI	rCutSmart	100	100	25	100	37°	65°	B	pBR322 DNA		3
BccI	rCutSmart + DTT	100	50	10	100	37°	65°	A	pXba DNA		3, b
BceAI	r3.1	100*	100*	100	100*	37°	65°	A	pBR322 DNA	CpG	1
BcgI	r3.1	10	75*	100	50*	37°	65°	A	λ DNA	dam CpG	e
BciVI	rCutSmart	100	25	<10	100	37°	80°	C	λ DNA		b
BclI	r3.1	50	100	100	75	37°	No	A	λ DNA (dam-)	dam	
BclI-HF	rCutSmart	100	100	10	100	37°	65°	B	λ DNA (dam-)	dam	
BcoDI	rCutSmart	50	75	75	100	37°	No	B	λ DNA	CpG	
BfaI	rCutSmart	<10	10	<10	100	37°	80°	B	λ DNA		2, b
BfuAI	r3.1	<10	25	100	10	50°	65°	B	λ DNA	CpG	3
BglI	r3.1	10	25	100	10	37°	65°	B	λ DNA	CpG	
BglII	r3.1	10	10	100	<10	37°	No	A	λ DNA		
BlpI	rCutSmart	50	100	10	100	37°	No	A	λ DNA		d
BmgBI	r3.1	<10	10	100	10	37°	65°	B	λ DNA	CpG	3, b, d
BmrI	r2.1	75	100	75	100*	37°	65°	B	λ DNA (HindIII digest)		b
BmtI-HF	rCutSmart	50	100	10	100	37°	65°	B	pXba DNA		
BpmI	r3.1	75	100	100	100*	37°	65°	B	λ DNA		2
Bpu10I	r3.1	10	25	100	25	37°	80°	B	λ DNA		3, b, d
BpuEI	rCutSmart	50*	100	50*	100	37°	65°	B	λ DNA		d
BsaAI	rCutSmart	100	100	100	100	37°	No	C	λ DNA	CpG	
BsaBI	rCutSmart	50	100	75	100	60°	80°	B	λ DNA (dam-)	dam CpG	2
BsaHI	rCutSmart	50	100	100	100	37°	80°	C	λ DNA	dcm CpG	
BsaI-HFv2	rCutSmart	100	100	100	100	37°	80°	B	pXba DNA	dcm CpG	
BsaJI	rCutSmart	50	100	100	100	60°	80°	A	λ DNA		
BsaWI	rCutSmart	10	100	50	100	60°	80°	A	λ DNA		
BsaXI	rCutSmart	50*	100*	10	100	37°	No	C	λ DNA		e
BseRI	rCutSmart	100	100	75	100	37°	80°	A	λ DNA		d
BseYI	r3.1	10	50	100	50	37°	80°	B	λ DNA	CpG	d
BsgI	rCutSmart	25	50	25	100	37°	65°	B	λ DNA		d
BsiEI	rCutSmart	25	50	<10	100	60°	No	A	λ DNA	CpG	
BsiHKAI	rCutSmart	25	100	100	100	65°	No	A	λ DNA		
BsiWI	r3.1	25	50*	100	25	55°	80°	B	ΦX174 DNA	CpG	

a. Ligation is less than 10%
b. Ligation is 25% – 75%
c. Recutting after ligation is < 5%
d. Recutting after ligation is 50% – 75%
e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or the recognition sequence contains variable sequences.

Performance Chart for Restriction Enzymes (Continued)

Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1	100	Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity		Notes
BsiWI-HF	rCutSmart	50	100	10	100	37°	80°	B	ΦX174 DNA		CpG	
BslI	rCutSmart	50	75	100	100	37°	No	A	λ DNA	dcm	CpG	b
BsmAI	rCutSmart	50	100	100	100	37°	No	B	λ DNA		CpG	
BsmBI-v2	r3.1	<10	50	100	25	55°	80°	B	λ DNA		CpG	
BsmFI	rCutSmart	25	50	50	100	37°	80°	A	pBR322 DNA	dcm	CpG	1
BsmI	rCutSmart	25	100	<10	100	65°	80°	A	λ DNA			
BsoBI	rCutSmart	25	100	100	100	37°	80°	A	λ DNA			
Bsp1286I	rCutSmart	25	25	25	100	37°	65°	A	λ DNA			3
BspCNI	rCutSmart	100	75	10	100	37°	80°	A	λ DNA			b
BspDI	rCutSmart	25	75	50	100	37°	80°	A	λ DNA	dam	CpG	
BspEI	r3.1	<10	10	100	<10	37°	80°	B	λ DNA (dam-)	dam	CpG	
BspHI	rCutSmart	10	50	25	100	37°	80°	A	λ DNA	dam		
BspMI	r3.1	10	50*	100	10	37°	65°	B	λ DNA			
BspQI	r3.1	100*	100*	100	100*	50°	80°	B	λ DNA			3
BspQI-HF	rCutSmart	10	50	25	100	37°	80°	B	λ DNA			
BsrBI	rCutSmart	50	100	100	100	37°	80°	A	λ DNA		CpG	d
BsrDI	r2.1	10	100	75	25	37°	80°	A	λ DNA			3, d
BsrFI-v2	rCutSmart	25	25	0	100	37°	No	C	pBR322 DNA		CpG	
BsrGI-HF	rCutSmart	10	100	100	100	37°	80°	A	λ DNA			
BsrI	r3.1	<10	50	100	10	65°	80°	B	ΦX174 DNA			b
BssHII	rCutSmart	100	100	100	100	37°	65°	B	λ DNA		CpG	
BssSI-v2	rCutSmart	10	25	<10	100	37°	No	B	λ DNA			
BstAPI	rCutSmart	50	100	25	100	60°	80°	A	λ DNA		CpG	b
BstBI	rCutSmart	75	100	10	100	65°	No	A	λ DNA		CpG	
BstEII-HF	rCutSmart	<10	10	<10	100	37°	No	A	λ DNA			
BstNI	r3.1	10	100	100	75	60°	No	A	λ DNA			a
BstUI	rCutSmart	50	100	25	100	60°	No	A	λ DNA		CpG	b
BstXI	r3.1	<10	50	100	25	37°	80°	B	λ DNA	dcm		3
BstYI	rCutSmart	25	100	75	100	60°	No	A	λ DNA			
BstZ17I-HF	rCutSmart	100	100	10	100	37°	No	A	λ DNA		CpG	
Bsu36I	rCutSmart	25	100	100	100	37°	80°	C	λ DNA (HindIII digest)			b
BtgI	rCutSmart	50	100	100	100	37°	80°	B	pBR322 DNA			
BtgZI	rCutSmart	10	25	<10	100	60°	80°	A	λ DNA		CpG	3, b, d
BtsCI	rCutSmart	10	100	25	100	50°	80°	B	λ DNA			
BtsI-v2	rCutSmart	100	100	25	100	37°	No	A	λ DNA			1
BtsIMutI	rCutSmart	100	50	10	100	55°	80°	A	pUC19 DNA			b
Cac8I	rCutSmart	50	75	100	100	37°	65°	B	λ DNA		CpG	b
ClaI	rCutSmart	10	50	50	100	37°	65°	A	λ DNA (dam-)	dam	CpG	
CspCI	rCutSmart	10	100	10	100	37°	65°	A	λ DNA			e
CviKI-1	rCutSmart	25	100	100	100	37°	No	A	pBR322 DNA			1, b
CviQI	r3.1	75	100*	100	75*	25°	No	C	λ DNA			b
DdeI	rCutSmart	75	100	100	100	37°	65°	B	λ DNA			

1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.
2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.

* May exhibit star activity in this buffer.
+ NEB isoschizomer or HF enzyme supplied with rCutSmart Buffer.

Performance Chart for Restriction Enzymes (Continued)

Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1		Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity		Notes
DpnI	rCutSmart	100	100	75	100	37°	80°	B	pBR322 DNA (dam methylated)		CpG	b
DpnII	U	25	25	100*	25	37°	65°	B	λ DNA (dam-)	dam		
DraI	rCutSmart	75	75	50	100	37°	65°	A	λ DNA			
DraIII-HF	rCutSmart	<10	50	10	100	37°	No	B	λ DNA		CpG	b
DrdI	rCutSmart	25	50	10	100	37°	65°	A	pUC19 DNA		CpG	3
EaeI	rCutSmart	10	50	<10	100	37°	65°	A	λ DNA	dcm	CpG	b
EagI-HF	rCutSmart	25	100	100	100	37°	65°	B	pXba DNA		CpG	
EarI	rCutSmart	50	10	<10	100	37°	65°	B	λ DNA		CpG	b, d
EciI	rCutSmart	100	50	50	100	37°	65°	A	λ DNA		CpG	2
Eco53kI	rCutSmart	100	100	<10	100	37°	65°	A	pXba DNA		CpG	3, b
EcoNI	rCutSmart	50	100	75	100	37°	65°	A	λ DNA			b
EcoO109I	rCutSmart	50	100	50	100	37°	65°	A	λ DNA (HindIII digest)	dcm		3
EcoP15I	U + ATP	75	100	100	100	37°	65°	A	pUC19 DNA			e
EcoRI	U	25	100*	50	50*	37°	65°	C	λ DNA		CpG	
EcoRI-HF	rCutSmart	10	100	<10	100	37°	65°	C	λ DNA		CpG	
EcoRV	r3.1	10	50	100	10	37°	80°	A	λ DNA		CpG	
EcoRV-HF	rCutSmart	25	100	100	100	37°	65°	B	λ DNA		CpG	
Esp3I	rCutSmart	100	100	<10	100	37°	65°	B	λ DNA		CpG	
FatI	r2.1	10	100	50	50	55°	80°	A	pUC19 DNA			
FauI	rCutSmart	100	50	10	100	55°	65°	A	λ DNA		CpG	3, b, d
Fnu4HI	rCutSmart	<10	<10	<10	100	37°	No	A	λ DNA		CpG	a
FokI	rCutSmart	100	100	75	100	37°	65°	A	λ DNA	dcm	CpG	3, b, d
FseI	rCutSmart	100	75	<10	100	37°	65°	B	pBC4 DNA	dcm	CpG	
FspI	rCutSmart	10	100	10	100	37°	No	C	λ DNA		CpG	b
HaeII	rCutSmart	25	100	10	100	37°	80°	A	λ DNA		CpG	
HaeIII	rCutSmart	50	100	25	100	37°	80°	A	λ DNA			
HgaI	r1.1	100	100	25	100*	37°	65°	A	ΦX174 DNA		CpG	1
HhaI	rCutSmart	25	100	100	100	37°	65°	A	λ DNA		CpG	
HinP1I	rCutSmart	100	100	100	100	37°	65°	A	λ DNA		CpG	
HincII	rCutSmart	25	100	100	100	37°	65°	B	λ DNA		CpG	
HindIII	r2.1	25	100	50	50	37°	80°		λ DNA			
HindIII-HF	rCutSmart	10	100	10	100	37°	80°	B	λ DNA			
HinfI	rCutSmart	50	100	100	100	37°	80°	A	λ DNA		CpG	
HpaI	rCutSmart	<10	75*	25	100	37°	No	A	λ DNA		CpG	1
HpaII	rCutSmart	100	50	<10	100	37°	80°	A	λ DNA		CpG	
HphI	rCutSmart	50	50	<10	100	37°	65°	B	λ DNA	dcm		1, b, d
Hpy166II	rCutSmart	100	100	50	100	37°	65°	C	pBR322 DNA		CpG	
Hpy188I	rCutSmart	25	100	50	100	37°	65°	A	pBR322 DNA	dam		1, b
Hpy188III	rCutSmart	100	100	10	100	37°	65°	B	pUC19 DNA	dam	CpG	3, b
Hpy99I	rCutSmart	50	10	<10	100	37°	65°	A	λ DNA		CpG	
HpyAV	rCutSmart	100	100	25	100	37°	65°		λ DNA		CpG	3, b, d

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d. Recutting after ligation is 50% – 75%
e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or the recognition sequence contains variable sequences.

Performance Chart for Restriction Enzymes (Continued)

Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1		Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity		Notes
HpyCH4III	rCutSmart	100	25	<10	100	37°	65°	A	λ DNA			b
HpyCH4IV	rCutSmart	100	50	25	100	37°	65°	A	pUC19 DNA		CpG	
HpyCH4V	rCutSmart	50	50	25	100	37°	65°	A	λ DNA			
I-CeuI	rCutSmart	10	10	10	100	37°	65°	B	pBHS ScaI-linearized Control Plasmid			
I-SceI	rCutSmart	10	50	25	100	37°	65°	B	pGPS2 NotI-linearized Control Plasmid			
KasI	rCutSmart	50	100	50	100	37°	65°	B	pBR322 DNA		CpG	3
KpnI-HF	rCutSmart	100	25	<10	100	37°	No	A	pXba DNA			
MboI	rCutSmart	75	100	100	100	37°	65°	A	λ DNA (dam-)	dam		
MboII	rCutSmart	100*	100	50	100	37°	65°	C	λ DNA (dam-)	dam		b
MfeI-HF	rCutSmart	75	25	<10	100	37°	No	A	λ DNA			
MluCI	rCutSmart	100	10	10	100	37°	No	A	λ DNA			
MluI-HF	rCutSmart	25	100	100	100	37°	No	A	λ DNA		CpG	
MlyI	rCutSmart	50	50	10	100	37°	65°	A	λ DNA			b, d
MmeI	rCutSmart	50	100	50	100	37°	65°	B	ΦX174 RF I DNA		CpG	b, c
MnlI	rCutSmart	75	100	50	100	37°	65°	B	λ DNA			b
MscI	rCutSmart	25	100	100	100	37°	80°	C	λ DNA	dcm		
MseI	rCutSmart	75	100	75	100	37°	65°	A	λ DNA			
MslI	rCutSmart	50	50	<10	100	37°	80°	A	λ DNA			
MspA1I	rCutSmart	10	50	10	100	37°	65°	B	λ DNA		CpG	
MspI	rCutSmart	75	100	50	100	37°	No	A	λ DNA			
MspJI	rCutSmart + Enz. Activ.	<10	<10	<10	100	37°	65°	B	pBR322 (dcm+) DNA			1, e
MwoI	rCutSmart	<10	100	100	100	60°	No	B	λ DNA		CpG	
NaeI	rCutSmart	25	25	<10	100	37°	No	A	pXba DNA		CpG	b
NarI	rCutSmart	100	100	10	100	37°	65°	A	pXba DNA		CpG	
Nb.BbvCI	rCutSmart	25	100	100	100	37°	80°	A	supercoiled plasmid DNA			e
Nb.BsmI	r3.1	<10	50	100	10	65°	80°	A	supercoiled plasmid pBR322 DNA			e
Nb.BsrDI	rCutSmart	25	100	100	100	37°	80°	A	supercoiled pUC19 DNA			e
Nb.BssSI	r3.1	10	100	100	25	37°	No	B	supercoiled pUC19 DNA			e
Nb.BtsI	rCutSmart	75	100	75	100	37°	80°	A	supercoiled pUC101 DNA (dam-/dcm-)			e
NciI	rCutSmart	100	25	10	100	37°	No	A	λ DNA		CpG	b
NcoI	r3.1	100	100	100	100	37°	80°	A	λ DNA			
NcoI-HF	rCutSmart	50	100	10	100	37°	80°	B	λ DNA			
NdeI	rCutSmart	75	100	100	100	37°	65°	A	λ DNA			
NgoMIV	rCutSmart	100	50	10	100	37°	No	A	pXba DNA		CpG	1
NheI-HF	rCutSmart	100	25	10	100	37°	80°	C	λ DNA (HindIII digest)		CpG	
NlaIII	rCutSmart	<10	<10	<10	100	37°	65°	B	ΦX174 RF I DNA			
NlaIV	rCutSmart	10	10	10	100	37°	65°	B	pBR322 DNA	dcm	CpG	
NmeAIII	rCutSmart	10	10	<10	100	37°	65°	B	ΦX174 RF I DNA			c
NotI	r3.1	<10	50	100	25	37°	65°	C	pBC4 DNA		CpG	
NotI-HF	rCutSmart	25	100	25	100	37°	65°	A	pBC4 DNA		CpG	

1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.
2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.

* May exhibit star activity in this buffer.
+ NEB isoschizomer or HF enzyme supplied with rCutSmart Buffer.

Performance Chart for Restriction Enzymes (Continued)

Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1		Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity		Notes
NruI-HF	rCutSmart	0	25	50	100	37°	No	A	λ DNA	dam	CpG	
NsiI	r3.1	10	75	100	25	37°	65°	B	λ DNA			
NsiI-HF	rCutSmart	<10	20	<10	100	37°	80°	B	λ DNA			
NspI	rCutSmart	100	100	<10	100	37°	65°	A	λ DNA			
Nt.AlwI	rCutSmart	10	100	100	100	37°	80°	A	pUC101 DNA (dam-/dcm-)	dam		e
Nt.BbvCI	rCutSmart	50	100	10	100	37°	80°	A	supercoiled plasmid DNA		CpG	e
Nt.BsmAI	rCutSmart	100	50	10	100	37°	65°	A	supercoiled plasmid DNA		CpG	e
Nt.BspQI	r3.1	<10	25	100	10	50°	80°	B	supercoiled pUC19 DNA			e
Nt.BstNBI	r3.1	0	10	100	10	55°	80°	A	T7 DNA			e
Nt.CviPII	rCutSmart	10	100	25	100	37°	65°	A	pUC19 DNA		CpG	e
PI-PspI	U + rAlbumin	10	10	10	10	65°	No	B	pAKR7 XmnI-linearized Control Plasmid			
PI-SceI	U + rAlbumin	10	10	10	10	37°	65°	B	pBSvdeX XmnI-linearized Control Plasmid			
PacI	rCutSmart	100	75	10	100	37°	65°	A	pNEB193 DNA			
PaeR7I	rCutSmart	25	100	10	100	37°	No	A	λ DNA (HindIII digest)		CpG	
PaqCI	rCutSmart + PaqCI Activator	10	100	10	100	37°	65°	B	λ DNA		CpG	1
PciI	r3.1	50	75	100	50*	37°	80°	B	pXba DNA			
PflFI	rCutSmart	25	100	25	100	37°	65°	A	pBC4 DNA			b
PflMI	r3.1	0	100	100	50	37°	65°	A	λ DNA	dcm		3, b, d
PleI	rCutSmart	25	50	25	100	37°	65°	A	λ DNA		CpG	b, d
PluTI	rCutSmart	100	25	<10	100	37°	65°	A	pXba DNA		CpG	b
PmeI	rCutSmart	<10	50	10	100	37°	65°	A	λ DNA		CpG	
PmlI	rCutSmart	100	50	<10	100	37°	65°	A	λ DNA (HindIII digest) DNA		CpG	
PpuMI	rCutSmart	<10	<10	<10	100	37°	No	B	λ DNA (HindIII digest)	dcm		
PshAI	rCutSmart	25	50	10	100	37°	65°	A	λ DNA		CpG	
PsiI-v2	rCutSmart	25	50	10	100	37°	65°	B	λ DNA			3
PspGI	rCutSmart	25	100	50	100	75°	No	A	T7 DNA	dcm		3
PspOMI	rCutSmart	10	10	<10	100	37°	65°	B	pXba DNA	dcm	CpG	
PspXI	rCutSmart	<10	100	25	100	37°	No	B	λ DNA (HindIII digest)		CpG	
PstI	r3.1	75	75	100	50*	37°	80°	C	λ DNA			
PstI-HF	rCutSmart	10	75	50	100	37°	No	C	λ DNA			
PvuI-HF	rCutSmart	25	100	100	100	37°	No	B	pXba DNA		CpG	
PvuII	r3.1	50	100	100	100*	37°	No	B	λ DNA			
PvuII-HF	rCutSmart	<10	<10	<10	100	37°	No	B	λ DNA			
RsaI	rCutSmart	25	50	<10	100	37°	No	A	λ DNA		CpG	
RsrII	rCutSmart	25	75	10	100	37°	65°	C	λ DNA		CpG	
SacI-HF	rCutSmart	10	50	<10	100	37°	65°	A	λ DNA (HindIII digest)		CpG	
SacII	rCutSmart	10	100	10	100	37°	65°	A	pXba DNA		CpG	
SalI	r3.1	<10	<10	100	<10	37°	65°	A	λ DNA (HindIII digest)		CpG	
SalI-HF	rCutSmart	10	100	100	100	37°	65°	A	λ DNA (HindIII digest)		CpG	
SapI	rCutSmart	75	50	<10	100	37°	65°	B	λ DNA			

a. Ligation is less than 10%
b. Ligation is 25% – 75%
c. Recutting after ligation is < 5%
d. Recutting after ligation is 50% – 75%
e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or the recognition sequence contains variable sequences.

Performance Chart for Restriction Enzymes (Continued)

Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1		Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity		Notes
Sau3AI	r1.1	100	50	10	100	37°	65°	A	λ DNA		CpG	b
Sau96I	rCutSmart	50	100	100	100	37°	65°	A	λ DNA	dcm	CpG	
SbfI-HF	rCutSmart	50	25	<10	100	37°	80°	B	λ DNA			
ScaI-HF	rCutSmart	100	100	10	100	37°	80°	B	λ DNA			
ScrFI	rCutSmart	100	100	100	100	37°	65°	C	λ DNA	dcm	CpG	2, a
SexAI	rCutSmart	100	75	50	100	37°	65°	A	pBC4 DNA (dcm-)	dcm		3, b, d
SfaNI	r3.1	<10	75	100	25	37°	65°	B	ΦX174 RF I DNA		CpG	3, b
SfcI	rCutSmart	75	50	25	100	37°	65°	B	λ DNA			3
SfiI	rCutSmart	25	100	50	100	50°	No	C	pXba DNA	dcm	CpG	
SfoI	rCutSmart	50	100	100	100	37°	No	B	λ DNA (HindIII digest)	dcm	CpG	
SgrAI	rCutSmart	100	100	10	100	37°	65°	A	λ DNA		CpG	1
SmaI	rCutSmart	<10	<10	<10	100	37°	65°	B	λ DNA (HindIII digest)		CpG	b
SmlI	rCutSmart	25	75	25	100	55°	No	A	λ DNA			b
SnaBI	rCutSmart	50*	50	10	100	37°	80°	A	T7 DNA		CpG	1
SpeI-HF	rCutSmart	25	50	10	100	37°	80°	C	pXba-XbaI DNA			
SphI	r2.1	100	100	50	100	37°	65°	B	λ DNA			2
SphI-HF	rCutSmart	50	25	10	100	37°	65°	B	λ DNA			
SrfI	rCutSmart	10	50	0	100	37°	65°	B	pNEB193-SrfI DNA		CpG	
SspI-HF	rCutSmart	25	100	<10	100	37°	65°	B	λ DNA			
StuI	rCutSmart	50	100	50	100	37°	No	A	λ DNA	dcm		
StyD4I	rCutSmart	10	100	100	100	37°	65°	B	λ DNA	dcm	CpG	
StyI-HF	rCutSmart	25	100	25	100	37°	65°	A	λ DNA			
SwaI	r3.1	10	10	100	10	25°	65°	B	pXba DNA			b, d
TaqI-v2	rCutSmart	50	100	50	100	65°	No	B	λ DNA	dam		
TfiI	rCutSmart	50	100	100	100	65°	No	C	λ DNA		CpG	
TseI	rCutSmart	75	100	100	100	65°	No	B	λ DNA		CpG	3
Tsp45I	rCutSmart	100	50	<10	100	65°	No	A	λ DNA			
TspMI	rCutSmart	50*	75*	50*	100	75°	No	B	pBC4 DNA		CpG	d
TspRI	rCutSmart	25	50	25	100	65°	No	B	λ DNA			
Tth111I	rCutSmart	25	100	25	100	65°	No	B	pBC4 DNA			b
WarmStart Nt.BstNBI	r3.1	0	10	100	25	55°	80°	A	T7 DNA			
XbaI	rCutSmart	<10	100	75	100	37°	65°	A	λ DNA (dam-/Hind III digest)	dam		
XcmI	r2.1	10	100	25	100*	37°	65°	C	λ DNA			2
XhoI	rCutSmart	75	100	100	100	37°	65°	A	λ DNA (HindIII digest)		CpG	b
XmaI	rCutSmart	25	50	<10	100	37°	65°	A	pXba DNA		CpG	3
XmnI	rCutSmart	50	75	<10	100	37°	65°	A	λ DNA			b
ZraI	rCutSmart	100	25	10	100	37°	80°	B	λ DNA		CpG	

1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.
2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.

* May exhibit star activity in this buffer.
+ NEB isoschizomer or HF enzyme supplied with rCutSmart Buffer.

Activity at 37°C for Restriction Enzymes with Alternate Incubation Temperatures

Listed below is the percentage of activity exhibited at 37°C for enzymes that have an optimal incubation temperature higher (thermophiles) or lower (25°C) than 37°C.

Enzyme	Optimal Temp. (°C)	% Activity at 37°C
ApeKI	75°	10
BfuAI	50°	25
BsaBI	60°	25
BsaJI	60°	25
BsaWI*	60°	50
BsiEI	60°	10
BsiHKAI	65°	10
BsiWI	55°	25
BsmBI-v2	55°	10
BsmI	65°	10
BspQI	50°	50
BsrI	65°	10
BstAPI	60°	25
BstBI	65°	10

Enzyme	Optimal Temp. (°C)	% Activity at 37°C
BstNI	60°	25
BstUI	60°	10
BstYI	60°	10
BtgZI	60°	50
BtsCI	50°	25
BtsIMutI	55°	50
CviQI	25°	25
FatI	55°	100
FauI	55°	50
MwoI	60°	25
Nb.BsmI	65°	N/A
Nt.BspQI	50°	50
Nt.BstNBI	55°	50
PI-PspI	65°	10

Enzyme	Optimal Temp. (°C)	% Activity at 37°C
PspGI	75°	25
SfiI	50°	10
SmlI	55°	10
SwaI	25°	25
TaqI-v2	65°	10
TfiI	65°	10
TseI	65°	10
Tsp45I	65°	10
TspMI	75°	10
TspRI	65°	10
Tth111I	65°	10
WarmStart® Nt. BstNBI	55°	0

*An HF version is available.

Activity of DNA Modifying Enzymes in rCutSmart Buffer

A selection of DNA modifying enzymes were assayed in rCutSmart Buffer, in lieu of their supplied buffers. Functional activity was compared to the activity in its supplied buffer, plus required supplements. Reactions were set up according to the recommended reaction conditions, with rCutSmart Buffer replacing the supplied buffer.

Enzyme	Activity in rCutSmart	Required Supplements
Antarctic Phosphatase	+++	Requires Zn^{2+}
Authenticase	++	
Bst DNA Polymerase	+++	
CpG Methyltransferase (M.SssI)	+++	
DNA Polymerase I	+++	
DNA Polymerase I, Large (Klenow) Fragment	+++	
DNA Polymerase Klenow Exo⁻	+++	
DNase I (RNase-free)	+++	Requires Ca^{2+}
DNase I-XT	+++	Requires Ca^{2+}
Duplex DNase	+	
E. coli DNA Ligase	+++	Requires NAD
Endonuclease III (Nth), recombinant	+++	
Endonuclease VIII	+++	
Exonuclease I	+++	
Exonuclease III	+++	
Exonuclease VII	+++	
Exonuclease V (Rec BCD)	+++	Requires ATP
Fpg	+++	
GpC Methyltransferase (M.CviPI)	+	Requires DTT
Hi-T4 DNA Ligase	+++	Requires ATP
Lambda Exonuclease	++	
Micrococcal Nuclease	+++	Requires Ca^{2+}
Mismatch Endonuclease I	++	

Enzyme	Activity in rCutSmart	Required Supplements
phi29 DNA Polymerase	+++	Requires DTT
Quick CIP	+++	
$RecJ_f$	+++	
Salt-T4 DNA Ligase	+	Requires ATP
Shrimp Alkaline Phosphatase (rSAP)	+++	
T3 DNA Ligase	+++	Requires ATP + PEG
T4 DNA Ligase	+++	Requires ATP
T4 DNA Polymerase	+++	
T4 Phage β-glucosyltransferase (T4-BGT)	+++	
T4 Polynucleotide Kinase	+++	Requires ATP + DTT
T4 PNK (3´ phosphatase minus)	+++	Requires ATP + DTT
T5 Exonuclease	+++	
T7 DNA Ligase	+++	Requires ATP + PEG
T7 DNA Polymerase (unmodified)	+++	
T7 Exonuclease	+++	
Thermolabile Exonuclease I	+++	
Thermolabile USER II Enzyme	+++	
Thermostable USER III Enzyme	++	
Thermostable Endonuclease Q	++	
Thermostable OGG	+++	
USER Enzyme, recombinant	+++	
WarmStart Afu Uracil-DNA Glycosylase (UDG)	+	

+++ full functional activity ++ 50–100% functional activity + 0–50% functional activity

Tips for Avoiding Star Activity

Under non-standard reaction conditions, some restriction enzymes are capable of cleaving sequences which are similar but not identical to their defined recognition sequence. This altered specificity has been termed "star activity". Although the propensity for star activity varies, the vast majority of enzymes from New England Biolabs will not exhibit star activity when used under recommended conditions in their supplied NEBuffers. If an enzyme has been reported to exhibit star activity, it will be indicated in the product entry found in the catalog, on the supplied card and on our website.

Conditions That Contribute to Star Activity	Steps That Can Be Taken to Inhibit Star Activity
High glycerol concentration (> 5% v/v)	• Restriction enzymes are stored in 50% glycerol, therefore the amount of enzyme added should not exceed 10% of the total reaction volume. • Use the standard 50 µl reaction volume to reduce evaporation during incubation.
High concentration of enzyme/µg of DNA ratio (varies with each enzyme, usually 100 units/µg)	• Use the fewest units possible to achieve digestion. This avoids overdigestion and reduces the final glycerol concentration in the reaction.
Non-optimal buffer	• Whenever possible, set up reactions in the recommended buffer. Buffers with differing ionic strengths and pHs may contribute to star activity.
Prolonged reaction time	• Use the minimum reaction time required for complete digestion. Prolonged incubation may result in increased star activity, as well as evaporation.
Presence of organic solvents [DMSO, ethanol (1), ethylene glycol, dimethylacetamide, dimethylformamide, sulphalane (2)]	• Make sure the reaction is free of any organic solvents, such as alcohols, that might be present in the DNA preparation.
Substitution of Mg^{2+} with other divalent cations (Mn^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+})	• Use Mg^{2+} as the divalent cation. Other divalent cations may not fit correctly into the active site of the restriction enzyme, possibly interfering with proper recognition.

Note: The relative significance of each of these altered conditions will vary from enzyme to enzyme.

New England Biolabs recommends setting up restriction enzyme digests in a 50 µl reaction volume. However, different methods may require smaller reaction volumes. When performing restriction enzyme digests in smaller reaction volumes, extra care must be taken to follow the steps listed above to avoid star activity. Alternatively, using our line of **High Fidelity (HF) restriction enzymes** will allow greater flexibility in reaction setup.

Reference:
(1) Nasri, M. and Thomas, D. (1987) *Nucleic Acids Res.* 15, 7677. PMID: 2823216
(2) Tikchinenko, T.I. et al (1978) *Gene* 4, 195–212. PMID: 33871

Tools & Resources

Visit NEBRestrictionEnzymes.com to find:

- Online tutorials for setting up restriction enzyme digests
- Access to troubleshooting guides & usage guidelines

High-Fidelity (HF) Restriction Enzymes

As part of our ongoing commitment to the study and improvement of restriction enzymes, NEB offers a line of High-Fidelity (HF) restriction enzymes. These engineered enzymes have the same specificity as the native enzymes, are all active in rCutSmart or CutSmart Buffer and have reduced star activity. Star activity, or off-target cleavage, is an intrinsic property of restriction enzymes. Most restriction enzymes will not exhibit star activity when used under recommended reaction conditions. However, for enzymes that have reported star activity, extra caution must be taken to set up reactions according to the recommended conditions to avoid unwanted cleavage.

Many techniques such as cloning, genotyping, mutational analysis, mapping, probe preparation, sequencing and methylation detection employ a wide range of reaction conditions and require the use of enzymes under suboptimal conditions. HF enzymes with reduced star activity offer increased flexibility to reaction setup and help maximize results under a wide range of conditions.

In addition to reduced star activity, all of these engineered enzymes work optimally in rCutSmart or CutSmart Buffer, which has the highest level of enzyme compatibility and will simplify double digest reactions. They are all Time-Saver qualified and digest substrate DNA in 5–15 minutes, and can also be incubated overnight without degradation of DNA. HF enzymes are available at the same price as the native enzymes and are supplied with purple loading dye.

Visit **www.neb.com/HF** to learn more about HF enzymes.

Tools & Resources

Visit www.neb.com/HF

- The full list of HF enzymes available
- Online tutorials on how to avoid star activity and setting up digests using the Time-Saver protocol

Learn how star activity is reduced with HF enzymes.

Isoschizomers

Restriction enzymes that recognize the same sequence are isoschizomers. The first example discovered is called a prototype, and all subsequent enzymes that recognize the same sequence are isoschizomers of the prototype.

NEB provides a list of isoschizomers for commercially-available restriction endonucleases at **www.neb.com/isoschizomers**. This table also specifies which isoschizomer is available from New England Biolabs.

All recognition sequences are written 5´ to 3´ using the single letter code nomenclature with the point of cleavage indicated by a "/".

Numbers in parentheses indicate point of cleavage for non-palindromic enzymes.
For example, GGTCTC(1/5) indicates cleavage at:

5´ ...GGTCTCN/...3´
3´ ...CCAGAGNNNNN/...5´

Isoschizomers with alternative cleavage sites (neoschizomers) are indicated with a "^". Enzymes that are not currently commercially available are indicated with a "⊗".

For more information on isoschizomers, visit **REBASE.neb.com**

Survival in a Reaction

Restriction enzymes vary with respect to their ability to maintain activity in a reaction over an extended period of time.

+ + +	Enzyme is active > 8 hours	N/A	Not Available
+ +	Enzyme is active 4–8 hours		
+	Enzyme is active 2–4 hours		
–	No benefit from digesting over 1 hour		

While most routine restriction digests are incubated for one hour or less at 37°C, there are certain applications that require the addition of less than 1 unit/µg of DNA and increasing the reaction time beyond one hour. The table below can be used as a guide when low levels of enzyme and extended reaction times are needed.

For example, 1 unit of AatII can be used to digest 8 µg of DNA in a 16 hour digest (+ + +).

Extended activity was determined by performing the restriction endonuclease unit assay, using a 16 hour incubation in place of the standard 1 hour digestion. After the 16 hour digestion, extended activity enzymes (+ + +) required only 0.13 units to completely digest 1 µg of DNA. Intermediate activity enzymes required either 0.25 (+ +) or 0.50 (+) units for complete digestion over this extended incubation time. Finally, enzymes marked (–) required 1.0 unit for complete digestion, the same amount of enzyme required for a 1 hour digestion.

Note: Reaction temperature is 37°C, unless otherwise noted.

Enzyme	Survival
AatII	+ + +
AbaSI @25°C	N/A
AccI	+ + +
Acc65I	+
AciI	–
AclI	+
AcuI	–
AfeI	+ +
AflII	+ + +
AflIII	+ + +
AgeI-HF	+ +
AhdI	+ + +
AleI-v2	+ + +
AluI	+ +
AlwI	+
AlwNI	+ + +
ApaI @25°C	+ + +
ApaLI	+ + +
ApeKI @75°C	+ + +
ApoI-HF	+ + +
AscI	+ + +
AseI	+ + +
AsiSI	+ + +
AvaI	+ +
AvaII	+ +
AvrII	+ + +
BaeI @25°C	+
BaeGI	+
BamHI	+
BamHI-HF	+
BanI	+ + +
BanII	+
BbsI	+ +
BbsI-HF	–
BbvI	–
BbvCI	+ + +

Enzyme	Survival
BccI	+
BceAI	+ + +
BcgI	+
BciVI	–
BclI @50°C	+
BclI-HF	N/A
BcoDI	+ + +
BfaI	+
BfuAI @50°C	+ +
BglI	+ + +
BglII	+ +
BlpI	+
BmgBI	–
BmrI	–
BmtI-HF	+ + +
BpmI	–
Bpu10I	+
BpuEI	–
BsaI-HFv2	+ + +
BsaAI	+ +
BsaBI @60°C	+
BsaHI	+ + +
BsaJI @60°C	+ + +
BsaWI @60°C	+ + +
BsaXI	+ +
BseRI	+
BseYI	+ +
BsgI	+
BsiEI @60°C	+ +
BsiHKAI @65°C	–
BsiWI @55°C	+ + +
BsiWI-HF	+ + +
BsmI @55°C	+ + +
BsmI @65°C	+
BsmAI @55°C	+ +
BsmBI-v2 @55°C	+

Enzyme	Survival
BsmFI @65°C	+ + +
BsoBI	+ + +
Bsp1286I	+
BspCNI @25°C	–
BspDI	+ +
BspEI	+ + +
BspHI	+ + +
BspMI	+ +
BspQI @50°C	–
BspQI-HF	+ + +
BsrI @65°C	+ +
BsrBI	+
BsrDI @65°C	+
BsrFI-v2	+ + +
BsrGI-HF	+ + +
BssHII @50°C	+
BssSI-v2	+ + +
BstAPI @60°C	+ +
BstBI @65°C	+ + +
BstEII-HF	–
BstNI @60°C	–
BstUI @60°C	+ + +
BstXI @55°C	+ + +
BstYI @60°C	+ + +
BstZ17I-HF	+ +
Bsu36I	+ + +
BtgI	+
BtgZI @60°C	–
BtsI-v2 @55°C	+ + +
BtsMutI @55°C	+
BtsCI @50°C	+
Cac8I	+ +
ClaI	+
CspCI	+
CviKI-1	–
CviQI @25°C	+ +

Enzyme	Survival
DdeI	+ + +
DpnI	+ + +
DpnII	+ + +
DraI	+ +
DraIII-HF	+ + +
DrdI	+ + +
EaeI	+ + +
EagI-HF	+ + +
EarI	+ + +
EciI	–
Eco53kI	+ +
EcoNI	+ + +
Eco0109I	+ + +
EcoP15I	–
EcoRI	+ + +
EcoRI-HF	+ + +
EcoRV	+
EcoRV-HF	+
Esp3I	+ + +
FatI @55°C	+
FauI @55°C	–
Fnu4HI	+ +
FokI	–
FseI	–
FspI	+ + +
HaeII	–
HaeIII	+ +
HgaI	–
HhaI	+ +
HincII	+ + +
HindIII	+ + +
HindIII-HF	+ + +
HinfI	+ +
HinP1I	+ +
HpaI	+ +
HpaII	+ +

Survival in a Reaction (continued)

Enzyme	Survival
HphI	++
Hpy99I	–
Hpy166II	+++
Hpy188I	–
Hpy188III	++
HpyAV	–
HpyCH4III	+++
HpyCH4IV	++
HpyCH4V	++
I-CeuI	++
I-SceI	++
KasI	–
KpnI-HF	+
MboI	+
MboII	–
MfeI-HF	++
MluI-HF	+++
MluCI	–
MlyI	–
MmeI	–
MnlI	++
MscI	+
MseI	+++
MslI	–
MspI	+
MspA1I	++
MspJI	+++
MwoI @60°C	+++
NaeI	+
NarI	–
Nb.BbvCI	+++

Enzyme	Survival
Nb.BsmI @65°C	++
Nb.BsrDI @65°C	++
Nb.BssSI	+++
Nb.BtsI	++
NciI	+
NcoI	++
NcoI-HF	++
NdeI	+++
NgoMIV	+++
NheI-HF	+++
NlaIII	+
NlaIV	+
NmeAIII	–
NotI	++
NotI-HF	+++
NruI-HF	+++
NsiI	++
NsiI-HF	N/A
NspI	++
Nt.AlwI	+++
Nt.BbvCI	+++
Nt.BsmAI	+++
Nt.BspQI @50°C	++
Nt.BstNBI @55°C	+
Nt.CviPII	–
PacI	+++
PaeR7I	+++
PaqCI	++
PciI	++
PflFI	+++
PflMI	+

Enzyme	Survival
PI-PspI @65°C	+++
PI-SceI	+++
PleI	+
PluTI	+
PmeI	–
PmlI	+
PpuMI	+++
PshAI	–
PsiI-v2	+++
PspGI @75°C	++
PspOMI	+++
PspXI	+++
PstI	+
PstI-HF	+
PvuI-HF	+++
PvuII	+++
PvuII-HF	–
RsaI	++
RsrII	++
SacI-HF	+++
SacII	+++
SalI	+++
SalI-HF	++
SapI	–
Sau3AI	+
Sau96I	++
SbfI-HF	–
ScaI-HF	++
ScrFI	++
SexAI	++
SfaNI	+

Enzyme	Survival
SfcI	–
SfiI @50°C	++
SfoI	–
SgrAI	–
SmaI @25°C	+++
SmlI @55°C	++
SnaBI	+
SpeI-HF	+
SphI	+++
SphI-HF	–
SrfI	+++
SspI-HF	+
StuI	++
StyI-HF	++
StyD4I	+++
SwaI @25°C	++
TaqI-v2 @65°C	+
TfiI @65°C	++
TseI @65°C	+
Tsp45I @65°C	+
TspMI @75°C	+++
TspRI @65°C	+++
Tth111I @65°C	++
WarmStart Nt. BstNBI	+
XbaI	+++
XcmI	+++
XhoI	+++
XmaI	+
XmnI	++
ZraI	+

Gina and Patricia are members of our HR Team. Gina (left) is the Senior Manager of HR Operations and is actively involved in our DE&I Committee. Patricia (right) is the Senior Benefits Specialist and participates in our 401K Committee. Combined, they have been supporting NEB employees for over 10 years.

Compatible Cohesive Ends and Generation of New Restriction Sites

Restriction enzymes that produce compatible cohesive ends often produce recleavable ligation products. The combinations listed were identified by computeranalysis, and have not necessarily been confirmed by experimentation.

Where isoschizomers exist, only one member of each set is listed. A selection of enzymes available from New England Biolabs has been listed. For a more complete listing visit our website, **www.neb.com**

Enzyme	Ligated To	Recleaved By
Acc65I (G/GTACC)	BanI (G/GTACC) BsiWI, BsrGI	Acc65I, BanI, KpnI, NlaIV, RsaI RsaI
AccI (GT/CGAC) (GT/CGAC)	AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI ClaI, BstBI, TaqI-v2	 — TaqI-v2
AciI (C/CGC)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI-v2 BsaHI (GR/CGCC), HinP1I, NarI HpaII	— AciI HpaII
AclI (AA/CGTT)	AccI (GT/CGAC), AciI, ClaI, BstBI, HinP1I, HpaII, NarI, TaqI-v2	 —
AgeI* (A/CCGGT)	AvaI (C/CCGGG), XmaI BsaWI, BspEI BsrFI (A/CCGGT), SgrAI (CA/CCGGTG) NgoMIV	HpaII, NciI, ScrFI BsaWI, HpaII AgeI, BsaWI, BsrFI, HpaII BsrFI, HpaII
ApaI (GGGCC/C)	BanII (GGGCC/C), Bsp1286I (GGGCC/C)	ApaI, BanII, Bsp120I, Bsp1286I, HaeIII, NlaIV, Sau96I
ApaLI (G/TGCAC)	SfcI (C/TGCAG)	BsgI
ApoI* (A/AATTY) (G/AATTY) (R/AATTY)	 EcoRI EcoRI MfeI, Tsp509I	 ApoI, Tsp509I ApoI, EcoRI, Tsp509I Tsp509I
AscI (GG/CGCGCC)	AflIII (A/CGCGT), MluI BssHII	BstUI, HhaI BssHII, BstUI, Cac8I, HhaI
AseI (AT/TAAT)	BfaI, Csp6I, NdeI MseI	— MseI
AsiSI (GCGAT/CGC)	BsiEI (CGAT/CG) PacI PvuI	DpnII, PvuI MseI DpnII, PvuI
AvaI (C/CCGGG) (C/TCGAG) (C/TCGAG) (C/CCGGG)	AgeI, BsaWI, BspEI, BsrFI (R/CCGGY), NgoMIV, SgrAI (CR/CCGGYG) XhoI SalI XmaI	HpaII, NciI, ScrFI AvaI, TaqI-v2, XhoI TaqI-v2 AvaI, BsaJI, HpaII, NciI, ScrFI, SmaI
AvaII (G/GWCC)	PpuMI (RG/GACCY) RsrII PpuMI (RG/GTCCY)	AvaII, NlaIV, Sau96I AvaII, Sau96I AvaII, BsmFI, NlaIV, Sau96I
AvrII (C/CTAGG)	NheI, SpeI, XbaI StyI (C/CTAGG)	BfaI AvrII, BfaI, BsaJI, StyI
BamHI* (G/GATCC)	BclI, DpnII BglII, BstYI (R/GATCY) BstYI (G/GATCC)	AlwI, DpnII AlwI, BstYI, DpnII AlwI, BamHI, BstYI, DpnII, NlaIV
BanI (G/GTACC) (G/GCGCC) (G/GTACC)	Acc65I KasI BsiWI, BsrGI	Acc65I, BanI, KpnI, NlaIV, RsaI BanI, BsaHI, HaeII, HhaI, KasI, NarI, NlaIV RsaI
BanII (GGGCC/C) (GAGCT/C)	ApaI, Bsp1286I (GGGCC/C) Bsp1286I (GAGCT/C), SacI	ApaI, BanII, Bsp1286I, HaeIII, NlaIV, Sau96I AluI, BanII, BsiHKAI, Bsp1286I, SacI
BclI* (T/GATCA)	BamHI, BstYI (R/GATCY) BglII, MboI	AlwI, DpnII DpnII
BfaI (C/TAG)	AseI, Csp6I, MseI, NdeI	—

For enzymes that have degenerate recognition sequences* (i.e., recognize more than one sequence), a specific sequence is listed in the first column titled "Enzyme" only if a non-degenerate equivalent does not exist. Be aware that these degenerate enzymes will cleave sequences in addition to the one listed. Please note that enzyme containing "N" in their recognition sequence are not listed.
A "–" denotes a ligation product that cannot be recleaved.

*See single letter code.

Enzyme	Ligated To	Recleaved By
BglII (A/GATCT)	BamHI, BstYI (R/GATCY) BclI, DpnII	AlwI, BstYI, DpnII DpnII
BsaHI (GR/CGYC) (GA/CGYC) (GG/CGYC) (GG/CGYC) (GA/CGYC) (GG/CGYC)	 AccI (GT/CGAC), ClaI, BstBI, TaqI-v2 AciI, HinP1I AciI, HinP1I HpaII NarI NarI	 — HgaI HhaI AciI BsaHI, HgaI BanI, BsaHI, HaeII, HhaI, NarI, NlaIV
BsaWI (W/CCGGW)	AgeI, BsrFI (R/CCGGY), SgrAI (CR/ CCGGYG) AvaI (C/CCGGG), XmaI BspEI BsrFI (R/CCGGY), NgoMIV NgoMIV	AgeI, BsaWI, BsrFI, HpaII HpaII, NciI, ScrFI BsaWI, BspEI, HpaII BsrFI, HpaII HpaII
BsiEI (CGAT/CG) (CGAT/CG) (CGGC/CG)	 PacI PvuI SacII	 MseI BsiEI, DpnII, PvuI AciI
BsiHKAI (GTGCA/C)	Bsp1286I (GTGCA/C) Bsp1286I (GAGCA/C) Bsp1286I (GAGCT/C), SacI NsiI PstI, SbfI	BsiHKAI, Bsp1286I BsiHKAI, Bsp1286I AluI, BanII, BsiHKAI, Bsp1286I, SacI — BsgI
BsiWI* (C/GTACG)	Acc65I, BanI (G/GTACC), BsrGI	RsaI
Bsp1286I (GGGCC/C) (GTGCA/C) (GGGCC/C) (GAGCT/C) (GWGCW/C) (GTGCA/C) (GTGCA/C)	 ApaI, BanII (GGGCC/C) BsiHKAI BanII (GGGCC/C) BanII (GAGCT/C), BsiHKAI, SacI BsiHKAI NsiI PstI, SbfI	 ApaI, BanII, Bsp1286I, HaeIII, NlaIV, Sau96I ApaLI, BsiHKAI, Bsp1286I BanII, Bsp1286I AluI, BanII, BsiHKAI, Bsp1286I, SacI BsiHKAI, Bsp1286I — BsgI
BspEI (T/CCGGA)	AgeI, BsaWI, BsrFI (R/CCGGY), SgrAI (CR/CCGGYG) AvaI (C/CCGGG), XmaI BsaWI BsrFI (R/CCGGY), NgoMIV	BsaWI, HpaII HpaII, NciI, ScrFI BsaWI, BspEI, HpaII HpaII
BspHI (T/CATGA)	FatI, NcoI, PciI	FatI, NlaIII
BsrFI (A/CCGGY) (G/CCGGY) (R/CCGGY) (A/CCGGY) (R/CCGGY) (G/CCGGY) (CR/CCGGYG)	 AgeI, BsaWI AgeI, BsaWI, NgoMIV AvaI (C/CCGGG), XmaI BsaWI, BspEI BsaWI, BspEI NgoMIV SgrAI	 AgeI, BsaWI, BsrFI, HpaII BsrFI, HpaII HpaII, NciI, ScrFI BsaWI, HpaII HpaII BsrFI, Cac8I, HpaII, NaeI BsrFI, HpaII
BsrGI* (T/GTACA)	Acc65I, BanI (G/GTACC), BsiWI	RsaI
BssHII (G/CGCGC)	MluI AscI	BstUI, HhaI BssHII, BstUI, Cac8I, HhaI
BstBI (TT/CGAA)	AccI (GT/CGAC), ClaI, TaqI-v2 AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	TaqI-v2 —

Compatible Cohesive Ends and Generation of New Restriction Sites (continued)

Enzyme	Ligated To	Recleaved By
BstYI (A/GATCY) (G/GATCY) (R/GATCY) (G/GATCY) (A/GATCY)	 BamHI, BglII BamHI BclI, DpnII BclI, DpnII BglII	 AlwI, BstYI, DpnII AlwI, BamHI, BstYI, DpnII, NlaIV DpnII AlwI, DpnII BglII, BstYI, DpnII
ClaI (AT/CGAT)	AccI (GT/CGAC), BstBI, TaqI-v2 AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	TaqI-v2 —
DpnII/MboI/ Sau3AI (/GATC)	BamHI, BstYI (R/GATCC) BclI, BglII, BstYI (R/GATCY)	AlwI, DpnII DpnII
EaeI (Y/GGCCR) (C/GGCCR) (T/GGCCR) (C/GGCCR) (T/GGCCR)	 PspOMI EagI EagI NotI NotI	 HaeIII, Sau96I BsiEI, EaeI, EagI, HaeIII EaeI, HaeIII AciI, BsiEI, EaeI, EagI, Fnu4HI, HaeIII AciI, EaeI, Fnu4HI, HaeIII
EagI* (C/GGCCG)	PspOMI EaeI (Y/GGCCR) EaeI (C/GGCCG) NotI	HaeIII, Sau96I EaeI, HaeIII BsiEI, EaeI, EagI, HaeIII AciI, BsiEI, EaeI, EagI, Fnu4HI, HaeIII
EcoRI* (G/AATTC)	ApoI (G/AATTC) ApoI (R/AATTY) MfeI, Tsp509I	ApoI, EcoRI, Tsp509I ApoI, Tsp509I Tsp509I
FatI (/CATG)	BspHI, NcoI, PciI	FatI, NlaIII
HinP1I (G/CGC)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI-v2 AciI, BsaHI (GR/CGCC), NarI BsaHI (GR/CGTC) HpaII	— HhaI HgaI AciI
HpaII/MspI (C/CGG)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI-v2 AciI, BsaHI (GR/CGCC), HinP1I, NarI	— AciI
KasI (G/GCGCC)	BanI (G/GCGCC)	BanI, BsaHI, HaeII, HhaI, KasI, NarI, NlaIV
MfeI* (C/AATTG)	ApoI (R/ATTTY), EcoRI, Tsp509I	Tsp509I
MluI (A/CGCGT)	AscI, BssHII	BstUI, HhaI
MseI (T/TAA)	AseI BfaI, Csp6I, NdeI	MseI —
NarI (GG/CGCC)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI-v2 AciI, HinP1I BsaHI (GR/CGCC) BsaHI (GR/CGTC) HpaII	— HhaI BanI, BsaHI, HaeII, HhaI, NarI, NlaIV BsaHI, HgaI AciI
NcoI* (C/CATGG)	BspHI, FatI, PciI	FatI, NlaIII
NdeI (CA/TATG)	AseI, BfaI, Csp6I, MseI	—
NgoMIV (G/CCGGC)	AgeI, BsaWI, BsrFI (R/CCGGY), SgrAI AvaI (C/CCGGG), XmaI BsaWI, BspEI BsrFI (R/CCGGC), SgrAI	BsrFI, HpaII HpaII, NciI, ScrFI HpaII BsrFI, Cac8I, HpaII, NaeI
NheI* (G/CTAGC)	AvrII, SpeI, StyI (C/CTAGG), XbaI	BfaI
NlaIII (CATG/)	SphI, NspI	NlaIII
NotI* (GC/GGCCGC)	PspOMI EagI EaeI (Y/GGCCR)	AciI, EaeI, Fnu4HI, HaeIII AciI, BsiEI, EaeI, EagI, Fnu4HI, HaeIII AciI, BsiEI, EaeI, Fnu4HI, HaeIII

Enzyme	Ligated To	Recleaved By
NsiI* (ATGCA/T)	BsiHKAI (GTGCA/C), Bsp1286I (GTGCA/C), PstI, SbfI	—
NspI (RCATG/Y)	NlaIII, SphI	NlaIII, NspI
PacI (TTAAT/TAA)	AsiSI, BsiEI (CGAT/CG), PvuI	MseI
PciI (A/CATGT)	BspHI, FatI, NcoI	FatI, NlaIII
PpuMI (RG/GWCCY) (GG/GTCCY) (GG/GACCY)	 AvaII, RsrII AvaII, RsrII AvaII, RsrII	 AvaII, Sau96I AvaII, BsmFI, NlaIV, Sau96I AvaII, NlaIV, Sau96I
PspOMI (G/GGCCC)	EaeI (Y/GGCCR), EagI NotI	HaeIII, Sau96I AciI, Fnu4HI, HaeIII, Sau96I
PspXI (VC/TCGAGB)	XhoI, TliI SalI	XhoI, TliI TaqI-v2
PstI* (CTGCA/G)	BsiHKAI, Bsp1286I (GTGCA/C) NsiI SbfI	BsgI — PstI
PvuI* (CGAT/CG)	AsiSI PacI BsiEI (CGAT/CG)	DpnI, PvuI MseI BsiEI, DpnII, PvuI
RsrII (CG/GWCCG)	AvaII, PpuMI (RG/GACCY) PpuMI (RG/GACCY) PpuMI (RG/GTCCY)	AvaII, Sau96I AvaII, NlaIV, Sau96I AvaII, BsmFI, NlaIV, Sau96I
SacI* (GAGCT/C)	BanII (GAGCT/C), BsiHKAI, Bsp1286I (GAGCT/C)	AluI, BanII, BsiHKAI, Bsp1286I, SacI
SacII (CCGC/GG)	BsiEI (CGGC/CG)	AciI
SalI* (G/TCGAC)	PspXI, XhoI	TaqI-v2
SbfI* (CCTGCA/GG)	BsiHKAI, Bsp1286I (GTGCA/C) NsiI PstI	BsgI — PstI
SfcI (C/TGCAG)	ApaLI	BsgI
SgrAI (CR/CCGGYG)	See BsrFI	
SpeI* (A/CTAGT)	AvrII, NheI, StyI (C/CTAGG), XbaI	BfaI
SphI* (GCATG/C)	NlaIII, NspI	NlaIII, NspI
StyI* (C/CTAGG) (C/CATGG)	AvrII NheI, SpeI, XbaI BspHI NcoI	AvrII, BfaI, BsaJI, StyI BfaI NlaIII BsaJI, NcoI, NlaIII, StyI
TaqI-v2 (T/CGA)	AccI (GT/CGAC), ClaI, BstBI AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	TaqI-v2 —
Tsp509I (/AATT)	ApoI (R/AATTY), EcoRI, MfeI	Tsp509I
XbaI (T/CTAGA)	AvrII, NheI, SpeI, StyI (C/CTAGG)	BfaI
XhoI (TliI) (C/TCGAG)	PspXI SalI	XhoI, TliI TaqI-v2
XmaI (C/CCGGG)	AgeI, BsaWI, BspEI, BsrFI, NgoMIV, SgrAI AvaI (C/CCGGG)	HpaII, NciI, ScrFI AvaI, BsaJI, HpaII, NciI, ScrFI, SmaI, XmaI

*HF (high fidelity) versions of these enzymes are available.

Dam (GmATC), Dcm (CmCWGG) and CpG (mCG) Methylation

DNA methyltransferases (MTases) that transfer a methyl group from S-adenosylmethionine to either adenine or cytosine residues are found in a wide variety of prokaryotes and eukaryotes. Methylation should be considered when digesting DNA with restriction endonucleases because cleavage can be blocked or impaired when a particular base in the recognition site is methylated.

Prokaryotic Methylation

In prokaryotes, MTases have most often been identified as elements of restriction/modification systems that act to protect host DNA from cleavage by the corresponding restriction endonuclease. Most laboratory strains of *E. coli* contain three site-specific DNA methyltransferases.

- Dam methyltransferases– methylation at the N^6 position of the adenine in the sequence GATC (1,2).
- Dcm methyltransferases– methylation at the C^5 position of cytosine in the sequences CCAGG and CCTGG (1,3).
- EcoKI methylase– methylation of adenine in the sequences AAC(N^6)GTGC and GCAC(N^6)GTT.

Some or all of the sites for a restriction endonuclease may be resistant to cleavage when isolated from strains expressing the Dam or Dcm MTase if the methylase recognition site overlaps the endonuclease recognition site. For example, plasmid DNA isolated from *dam*$^+$ *E. coli* is completely resistant to cleavage by MboI, which cleaves at GATC sites.

Not all DNA isolated from *E. coli* is methylated to the same extent. While pBR322 DNA is fully modified (and is therefore completely resistant to MboI digestion), only about 50% of λ DNA Dam sites are methylated, presumably because the methylase does not have the opportunity to methylate the DNA fully before it is packaged into the phage head. As a result, enzymes blocked by Dam or Dcm modification will yield partial digestion patterns with λ DNA.

Restriction sites that are blocked by Dam or Dcm methylation can be unmethylated by cloning your DNA into a *dam*$^-$, *dcm*$^-$ strain of *E. coli*, such as *dam*$^-$/*dcm*$^-$ Competent *E. coli* (NEB #C2925).

Restriction sites can also be blocked if an overlapping site is present. In this case, part of the Dam or Dcm sequence is generated by the restriction enzyme sequence, followed by the flanking sequence. This situation should also be considered when designing restriction enzyme digests.

Eukaryotic Methylation

CpG MTases, found in higher eukaryotes (e.g., Dnmt1), transfer a methyl group to the C^5 position of cytosine residues. Patterns of CpG methylation are heritable, tissue specific and correlate with gene expression. Consequently, CpG methylation has been postulated to play a role in differentiation and gene expression (4).

Note: The effects of CpG methylation are mainly a concern when digesting eukaryotic genomic DNA. CpG methylation patterns are not retained once the DNA is cloned into a bacterial host.

Methylation Sensitivity

Information on methylation sensitivity for NEB restriction enzymes can be found in the Restriction Enzymes Performance Chart, as well as at **REBASE.neb.com**.

References
(1) Marinus, M.G. and Morris, N.R. (1973) *J. Bacteriol.*, 114, 1143–1150.
(2) Geier, G.E. and Modrich, P. (1979) *J. Biol. Chem.*, 254, 1408–1413.
(3) May, M.S. and Hattman, S. (1975) *J. Bacteriol.*, 123, 768–770.
(4) Siegfried, Z. and Cedar, H. (1997) *Curr. Biol.*, 7, –307.

Meet two members of NEB's Marketing Communications Team: Laurie (left) is our Global Sales Support Manager, and Ashley (right) is our Campaign Manager.

Learn about dam, dcm and CpG methylation.

Choosing the Best Exonuclease for Your Workflow

Selecting the exonuclease that meets your application needs is easier with NEB's technical insight and user-friendly selection tools. Drawing from our long history in enzymology and solutions for basic research and applied scientific development, we're sharing answers to some common questions about exonucleases and providing the resources you can use for successful exonuclease selection.

Exonucleases are essential enzymes in many in vivo biological processes, including DNA damage repair, error correction, and recombination. Exonucleases hydrolyze phosphodiester bonds in nucleic acids, ultimately digesting the polymer into mononucleotides or short oligos, depending on the enzyme. Exonucleases require a free nucleic acid end to initiate activity, which differentiates them from endonucleases, which can initiate in the middle of a nucleic acid polymer and can even digest closed circular molecules. Exonucleases can exhibit directionality (also referred to as polarity), digesting in either the 5´ to 3´ direction, the 3´ to 5´ direction, or bifunctionally. Exonucleases can prefer single-stranded (ss) or double-stranded (ds) DNA, or can digest both with near equal activity.

In addition to their critical roles in many organisms, exonucleases are also important enzymatic tools for selectively degrading nucleic acids in many in vitro molecular biology applications, such as next-generation sequencing (NGS), plasmid cleanup, site-directed mutagenesis, and gene synthesis. Check out some frequently asked questions about working with exonucleases below.

How do I choose the right exonuclease for my workflow?

NEB has several resources to help you choose the correct exonuclease.

Selection Chart

The *Properties of Exonucleases and Nonspecific Endonucleases* chart provides an overview of the activity for each enzyme, including the polarity, activity on different forms of ssDNA and dsDNA, the expected products of digestion, and any relevant notes for each specific enzyme. This chart is a great resource to start your selection process.

Interactive Tool

The Exo Selector webtool (exoselector.neb.com) allows you to specify the different nucleic acid forms present in your reaction mixture and indicate which you want to digest and which you want to retain. The tool will then suggest the appropriate enzyme(s) to achieve your desired outcome. For example, if you are trying to remove excess ssDNA primers from a polymerase chain reaction (PCR) but retain the dsDNA PCR products, the Exo Selector tool will suggest exonucleases which meet this requirement. Exonuclease I is the enzyme typically used for this application, but the Exo Selector tool presents all options that satisfy the specified requirements, allowing you to choose the enzyme that will be most compatible with your workflow.

Webinar

To see specific examples of exonuclease use in various workflows, refer to our *Exonucleases and Endonucleases as Molecular Tools Webinar*. Here, you can learn more about a workflow using molecular inversion or padlock probes; after the circularization of a single stranded probe, you can enrich for circular ssDNA using Exonuclease VII which degrades any remaining unligated linear probe DNA.

Are exonucleases blocked by modifications? Which modifications block exonuclease progress?

Some, but not all, exonucleases are sensitive to modification of the nucleic acid substrate. In some applications, such as ssDNA production or second strand synthesis in NGS library preparation, you may wish to retain one molecule of nucleic acid while digesting another, despite both being potential substrates for your exonuclease of choice. In these cases, selective modification of a nucleic acid can prevent degradation by exonucleases. NEB scientists have evaluated the activity of exonucleases on nucleic acid substrates containing a variety of chemical modifications, including modifications to the phosphodiester backbone, the nucleobase, and the sugar moiety.

The most common modification for blocking or halting exonuclease activity is the phosphorothioate (pt) bond. The pt linkages must be installed at the end(s) where the exonuclease to be blocked initiates. A single pt bond is insufficient to block cleavage by most exonucleases. Tests confirm five consecutive pt bonds are required to effectively block degradation. Importantly, even multiple pt bonds are not sufficient to block certain exonucleases that can scan past blocking linkages to begin digestion deeper in the polymer, such as Exo VII, and some also have endonuclease activity, such as T5 Exonuclease. We also found that bulky substituents at the 2´ position of the sugar, such as a 2´-O-methoxyethyl (MOE) modification, can provide even more robust protection against the activity of many exonucleases. We recommend incorporating at least three MOEs in a row in the appropriate position to block/halt the exonuclease of interest. Both pt bonds and MOEs are standard modifications offered by many oligonucleotide synthesis companies. Modification of the nucleobase is generally not an effective strategy for blocking exonucleases.

For a comprehensive summary of our results, please see the feature article, *The effect of nucleic acid modifications on digestion by DNA exonucleases.*

Can exonucleases digest DNA/RNA hybrids?

The ability to digest DNA/RNA hybrids varies between different exonucleases. In most cases, exonucleases do not change specificity on DNA/RNA hybrid helices. ssDNA-specific exonucleases are generally blocked by hybridization to an RNA strand, while dsDNA specific exonucleases will digest the DNA strand of DNA/RNA hybrid helices and exhibit some ability to degrade the RNA strand. Several nucleases, including T7 Exonuclease, Exonuclease III, and T5 Exonuclease, have significant activity on the RNA strand of a DNA/RNA helix. If it is desirable to digest the DNA strand of a DNA/RNA helix while leaving the RNA portion completely intact, we recommend Duplex DNase.

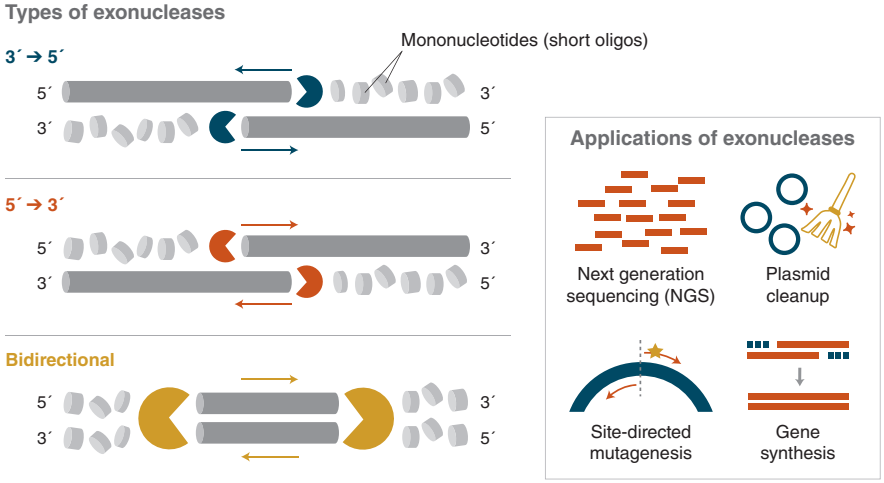
What should I do if I see unwanted digestion?

If you are seeing digestion of nucleic acids you want to retain, we recommend titering the enzyme (gradually decreasing the amount of enzyme added) to empirically determine the appropriate amount required for your particular application. In some cases, you can also modulate the amount of activity by increasing or decreasing reaction time or temperature.

It is important to note that many exonucleases have multiple activities, which can lead to unwanted digestion products. For example, in addition to exonuclease activity, Exonuclease III is reported to have RNase H, 3´ phosphatase, and AP endonuclease activities (1). Additionally, there is a wide variety of processivity among exonucleases, with some acting in an extremely processive manner and others that catalyze only a few rounds of digestion before dissociating. This processivity can be impacted by reaction conditions, such as temperature and buffer, as well as sequence specificities that are not fully characterized.

Why are exonucleases important?

Exonucleases are critical enzymes used for selective degradation of nucleic acids in a variety of applications, such as PCR, NGS, and gene synthesis. It is important to note the specific preferences of each exonuclease, as awareness of these activities and nuances will inform your choice of enzyme. Additionally, it is important to recognize that it may be necessary to empirically determine the best conditions for your experiment.



Exonucleases can digest in multiple directions and have several important applications.

(1) Rogers, G.S. and Weiss, B. (1980). L. Grossman and K. Moldave(Ed.), *Methods Enzymol.* 65, 201-211. New York: Academic Press.

General Guidelines for PCR Optimization

New England Biolabs offers a diverse group of DNA Polymerases for PCR-based applications. Specific recommendations for PCR optimization can be found in the product literature or on the individual product webpages. However, these general guidelines will help to ensure success using New England Biolabs' PCR enzymes.

Setup Guidelines

DNA Template

- Use high quality, purified DNA templates whenever possible. Please refer to specific product information for amplification from unpurified DNA (e.g., colony PCR or direct PCR).
- For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg–10 ng of DNA per 50 µl reaction
- For higher complexity templates (e.g., genomic DNA), use 1 ng–1 µg of DNA per 50 µl reaction
- Higher DNA concentrations tend to decrease amplicon specificity, particularly for high numbers of cycles

Primers

- Primers should typically be 20–30 nucleotides in length, with 40–60% GC Content
- Primer Tm values should be determined with NEB's Tm Calculator (**TmCalculator.neb.com**)
- Primer pairs should have Tm values that are within 5°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between the primers
- Higher than recommended primer concentrations may decrease specificity
- When engineering restriction sites onto the end of primers, 6 nucleotides should be added 5´ to the site
- Annealing temperatures should be determined according to specific enzyme recommendations. *Please note that Q5® and Phusion™* annealing temperature recommendations are unique.*
- Final concentration of each primer should be 0.05–1 µM in the reaction. Please refer to the more detailed recommendations for each specific enzyme.
- When amplifying products > 20 kb in size, primers should be ≥ 24 nucleotides in length with a GC content above 50% and matched Tm values above 60°C
- To help eliminate primer degradation and subsequent non-specific product formation, use a hot-start enzyme (e.g., One*Taq*® Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)

Magnesium Concentration

- Optimal Mg^{2+} concentration is usually 1.5–2.0 mM for most PCR polymerases
- Most PCR buffers provided by NEB already contain sufficient levels of Mg^{2+} at 1X concentrations.
- NEB offers a variety of Mg-free reaction buffers to which supplemental Mg^{2+} can be added for applications that require complete control over Mg^{2+} concentration
- Further optimization of Mg^{2+} concentration can be done in 0.2-1 mM increments, if necessary. For some specific applications, the enzyme may require as much as 6 mM Mg^{2+} in the reaction.
- Excess Mg^{2+} may lead to spurious amplification; Insufficient Mg^{2+} concentrations may cause reaction failure

Deoxynucleotides

- Ideal dNTP concentration is typically 200 µM of each, however, some enzymes may require as much as 400 µM each. Please refer to specific product literature for more detailed recommendations.
- Excess dNTPs can chelate Mg^{2+} and inhibit the polymerase
- Lower dNTP concentration can increase fidelity, however, yield is often reduced
- The presence of uracil in the primer, template, or deoxynucleotide mix will cause reaction failure when using archaeal PCR polymerases. Use Q5U®, One*Taq* or *Taq* DNA Polymerases for these applications.

Enzyme Concentration

- Optimal enzyme concentration in the reaction is specific to each polymerase. Please see the product literature for specific recommendations.
- In general, excess enzyme can lead to amplification failure, particularly when amplifying longer fragments

Starting Reactions

- Unless using a hot start enzyme (e.g., One*Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase), assemble all reaction components on ice
- Add the polymerase last, whenever possible
- Transfer reactions to a thermocycler that has been pre-heated to the denaturation temperature. Please note that pre-heating the thermocycler is not necessary when using a hot start enzyme (e.g., One*Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase).

Cycling Guidelines

Denaturation

- Optimal denaturation temperature ranges from 94°–98°C and is specific to the polymerase in the reaction. Please refer to product information for recommended conditions.
- Avoid longer or higher temperature incubations unless required due to high GC content of the template
- For most PCR polymerases, denaturation of 5–30 seconds is recommended during cycling
- NEB's aptamer-based hot start enzymes do not require additional denaturation steps to activate the enzymes

Annealing

- Primer Tm values should be determined using the NEB Tm Calculator (**TmCalculator.neb.com**)
- For PCR polymerases other than Q5 High-Fidelity DNA Polymerase or Phusion High-Fidelity DNA Polymerase, annealing temperatures are usually set at 2°–5°C below the lowest Tm of the primer pair
- When using Q5 High-Fidelity DNA Polymerase or Phusion High-Fidelity DNA Polymerase, annealing temperatures should be set at 0°–3°C above the lowest Tm of the primer pair. Please refer to the product literature for detailed recommendations.
- Non-specific product formation can often be avoided by optimizing the annealing temperature or by switching to a hot start enzyme (e.g., One*Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)
- Annealing temperatures can be optimized by doing a temperature gradient PCR, starting at 5°C below the lowest Tm of the primer pair
- Ideally, primer Tm values should be less than the extension temperature. However, if Tm values are calculated to be greater than the extension temperature, a two-step PCR program (combining annealing and extension into one step) can be employed.

Extension

- Extension temperature recommendations range from 65°–72°C and are specific to each PCR polymerase. Please refer to the product literature for specific recommendations.
- Extension rates are specific to each PCR polymerase. In general, extension rates range from 15–60 seconds per kb. Please refer to the recommendations for each specific product.
- Longer than recommended extension times can result in higher error rates, spurious banding patterns and/or reduction of amplicon yields

PCR Troubleshooting Guide

The following guide can be used to troubleshoot PCR reactions. Additional tips for optimizing reactions can be found in the technical reference section of our website, **www.neb.com**.

Problem	Possible Cause	Solution
Sequence errors	Low fidelity polymerase	• Choose a higher fidelity polymerase such as Q5 High-Fidelity (NEB #M0491) or Phusion (NEB #M0530)* DNA Polymerases
	Suboptimal reaction conditions	• Reduce number of cycles • Decrease extension time • Decrease Mg^{2+} concentration in the reaction
	Unbalanced nucleotide concentrations	• Prepare fresh deoxynucleotide mixes
	Template DNA has been damaged	• Start with a fresh template • Try repairing DNA template with the PreCR® Repair Mix (NEB #M0309) • Limit UV exposure time when analyzing or excising PCR product from the gel
	Desired sequence may be toxic to host	• Clone into a non-expression vector • Use a low-copy number cloning vector
Incorrect product size	Incorrect annealing temperature	• Recalculate primer Tm values using the NEB Tm calculator (TmCalculator.neb.com)
	Mispriming	• Verify that primers have no additional complementary regions within the template DNA
	Improper Mg^{2+} concentration	• Adjust Mg^{2+} concentration in 0.2–1 mM increments
	Nuclease contamination	• Repeat reactions using fresh solutions
No product	Incorrect annealing temperature	• Recalculate primer Tm values using the NEB Tm calculator (TmCalculator.neb.com) • Test an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair
	Poor primer design	• Check specific product literature for recommended primer design • Verify that primers are non-complementary, both internally and to each other • Increase length of primer
	Poor primer specificity	• Verify that oligos are complementary to proper target sequence
	Insufficient primer concentration	• Primer concentration can range from 0.05–1 µM in the reaction. Please see specific product literature for ideal conditions
	Missing reaction component	• Repeat reaction setup
	Suboptimal reaction conditions	• Optimize Mg^{2+} concentration by testing 0.2–1 mM increments • Thoroughly mix Mg^{2+} solution and buffer prior to adding to the reaction • Optimize annealing temperature by testing an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair
	Poor template quality	• Analyze DNA via gel electrophoresis before and after incubation with Mg^{2+} • Check $A_{260/280}$ ratio of DNA template
	Presence of inhibitor in reaction	• Further purify starting template by alcohol precipitation, drop dialysis or clean up kit (NEB #T1130) • Decrease sample volume
	Insufficient number of cycles	• Rerun the reaction with more cycles
	Incorrect thermocycler programming	• Check program, verify times and temperatures
	Inconsistent thermocycler block temperature	• Test calibration of heating block
	Contamination of reaction tubes or solutions	• Autoclave empty reaction tubes prior to use to eliminate biological inhibitors • Prepare fresh solutions or use new reagents & new tubes
	Complex template	• Use Q5 High-Fidelity (NEB #M0491) or One*Taq* DNA Polymerase (NEB #M0480) • For GC-rich templates, use Q5 High-Fidelity DNA Polymerase (NEB #M0491) with the High GC Enhancer or One*Taq* DNA Polymerase (NEB #M0480) with One*Taq* GC Reaction Buffer (plus One*Taq* High GC Enhancer, if necessary) • For longer templates, we recommend LongAmp® *Taq* DNA Polymerase (NEB #M0323), Q5 or Q5 Hot Start High Fidelity DNA Polymerase (NEB #M0493)
Multiple or non-specific products	Premature replication	• Use a hot start polymerase, such as Q5 Hot Start High-Fidelity (NEB #M0493) or One*Taq* Hot Start (NEB #M0481) DNA Polymerases • Set up reactions on ice using chilled components and add samples to thermocycler preheated to the denaturation temperature
	Primer annealing temperature too low	• Recalculate primer Tm values using the NEB Tm Calculator (TmCalculator.neb.com) • Increase annealing temperature
	Incorrect Mg^{2+} concentration	• Adjust Mg^{2+} in 0.2–1 mM increments
	Poor primer design	• Check specific product literature for recommended primer design • Verify that primers are non-complementary, both internally and to each other • Increase length of primer • Avoid GC-rich 3´ ends
	Excess primer	• Primer concentration can range from 0.05–1 µM in the reaction. Please see specific product literature for ideal conditions.
	Contamination with exogenous DNA	• Use positive displacement pipettes or non-aerosol tips • Set-up dedicated work area and pipettor for reaction setup • Wear gloves during reaction setup
	Incorrect template concentration	• For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg–10 ng of DNA per 50 µl reaction • For higher complexity templates (e.g., genomic DNA), use 1 ng–1 µg of DNA per 50 µl reaction

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. Phusion™ and Thermo Scientific™ are trademarks and property of Thermo Fisher Scientific.

Optimization Tips for Luna® qPCR

New England Biolabs provides Luna products for your qPCR and RT-qPCR experiments. For more information on these products, visit **LUNAqPCR.com**.
The following tips can be used to help optimize qPCR. For RT-qPCR guidelines, please see next page.

Target Selection

- Short PCR amplicons, ranging from 70 to 200 bp, are recommended for maximum PCR efficiency
- Target sequences should ideally have a GC content of 40–60%
- Avoid highly repetitive sequences when possible

DNA Template

- Use high quality, purified DNA templates whenever possible. Luna qPCR is compatible with DNA samples prepared through typical nucleic acid purification methods.
- Template dilutions should be freshly prepared in either TE or water for each qPCR experiment
- Generally, useful concentrations of standard and unknown material will be in the range of 10^6 copies to 1 copy. For gDNA samples from large genomes, (e.g., human, mouse) a range of 50–1 pg of gDNA is typical. For small genomes, adjust as necessary using 10^6–1 copy input as an approximate range. Note that for dilutions in the single-copy range, some samples will contain multiple copies and some will have none, as defined by the Poisson distribution.
- To generate cDNA, use of the LunaScript® RT SuperMix Kit (NEB #E3010) is recommended. Up to 1 µg total RNA, 1 µg mRNA or 100 ng specific RNA can be used in a 20 µl reaction.
- cDNA does not need to be purified before addition to the Luna reaction but should be diluted at least 1:20 before addition to qPCR

Primers

- Primers should typically be 15–30 nucleotides in length
- Ideal primer content is 40–60% GC
- Primer Tm should be approximately 60°C
- Primer Tm calculation should be determined with NEB's TmCalculator (**TmCalculator.neb.com**) using the Hot Start *Taq* setting
- For best results in qPCR, primer pairs should have Tm values that are within 3°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between primers
- G homopolymer repeats ≥ 4 should be avoided
- Optimal primer concentration for dye-based experiments (250 nM) is lower than for probe-based experiments (400 nM). If necessary, the primer concentration can be optimized between 100–500 nM for dye-based qPCR or 200–900 nM for probe-based experiments.
- Higher primer concentrations may increase secondary priming and create spurious amplification products
- When using primer design software, enter sufficient sequence around the area of interest to permit robust primer design and use search criteria that permit cross-reference against relevant sequence databases to avoid potential off-target amplification.
- For cDNA targets, it is advisable to design primers across known exon-exon junctions in order to prevent amplification from genomic DNA
- Primers designed to target intronic regions can ensure amplification exclusively from genomic DNA

Hydrolysis Probes

- Probes should typically be 15–30 nucleotides in length to ensure sufficient quenching of the fluorophore
- The optimal probe concentration is 200 nM but may be optimized between 100 to 500 nM
- Both single or double-quenched probes may be used
- In general, non-fluorescence quenchers result in better signal-to-noise ratio than fluorescence quenchers
- Ideal probe content is 40–60% GC
- The probe Tm should be 5–10°C higher than the Tm of the primers to ensure all targeted sequences are saturated with probe prior to amplification by the primers
- Probes may be designed to anneal to either the sense or antisense strand
- Generally, probes should be designed to anneal in close proximity to either the forward or reverse primer without overlapping
- Avoid a 5´-G base which is known to quench 5´-fluorophores

Multiplexing

- Avoid primer/probe combinations that contain complementary sequences, and ensure target sequences do not overlap
- Probes should be designed such that each amplicon has a unique fluorophore for detection
- Select fluorophores based on the detection capabilities of the available real-time PCR instrument
- The emission spectra of the reporter fluorophores should not overlap
- Test each primer/probe combination in a singleplex reaction to establish a performance baseline. Ensure C_q values are similar when conducting the multiplex qPCR.
- Pair dim fluorescence dyes with high abundance targets and bright dyes with low abundance targets
- Optimization may require lower primer/probe concentrations to be used for high copy targets along with higher concentrations for low copy targets

Cycling Conditions

- Generally, best performance is achieved using the cycling conditions provided in the manual
- Longer amplicons (> 400 bp) can be used but may require optimization of extension times
- Due to the hot start nature of the polymerase, it is not necessary to preheat the thermocycler prior to use
- Select the "Fast" ramp speed where applicable (e.g., Applied Biosystems QuantStudio®)
- Amplification for 40 cycles is sufficient for most applications, but for very low input samples 45 cycles may be used

Reaction Setup

- For best results, keep reactions on ice prior to thermocycling
- A reaction volume of 20 µl is recommended for 96-well plates while a reaction volume of 10 µl is recommended for 384-well plates
- Reactions should be carried out in triplicate for each sample
- For each amplicon, ensure to include no template controls (NTC)
- To prevent carry-over contamination, treat reactions with 0.2 units/µl Antarctic Thermolabile UDG (NEB #M0372) for 10 minutes at room temperature prior to thermocycling
- The Luna reference dye supports broad instrument compatibility (High-ROX, Low-ROX, ROX-independent) so no additional ROX is required for normalization

Assay Performance

- Ensure 90–110% PCR efficiency for the assay over at least three $\log_{10}$ dilutions of template
- Linearity over the dynamic range (R^2) should ideally be ≥ 0.99
- Target specificity should be confirmed by product size, sequencing or melt-curve analysis

Optimization Tips for Luna® One-Step RT-qPCR

New England Biolabs provides Luna products for your qPCR and RT-qPCR experiments. For more information on these products, visit **LUNAqPCR.com**. The following tips can be used to help optimize your one-step RT-qPCR. For qPCR guidelines (DNA/cDNA starting material), please see previous page.

Target Selection

- Short PCR amplicons, ranging from 70 to 200 bp, are recommended for maximum PCR efficiency
- Target sequences should ideally have a GC content of 40–60%
- Avoid highly repetitive sequences when possible
- Target sequences containing significant secondary structure should be avoided

RNA Template

- Use high quality, purified RNA templates whenever possible. Luna RT-qPCR is compatible with RNA samples prepared through typical nucleic acid purification methods.
- Prepared RNA should be stored in an EDTA-containing buffer (e.g., 1X TE) for long-term stability
- Template dilutions should be freshly prepared in either TE or water for each qPCR experiment
- Treatment of RNA samples with DNase I (NEB #M0303) may minimize amplification from genomic DNA contamination
- Generally, useful concentrations of standard and unknown material will be in the range of 10^8 copies to 10 copies. Note that for dilutions in the single-copy range, some samples will contain multiple copies and some will have none, as defined by the Poisson distribution. For total RNA, Luna One-Step Kits can provide linear quantitation over an 8-order input range of 1 µg–0.1 pg. For most targets, a standard input range of 100 ng–10 pg total RNA is recommended. For purified mRNA, input of ≤ 100 ng is recommended. For *in vitro*-transcribed RNA, input of ≤ 10^9 copies is recommended.

Primers

- Primers should typically be 15–30 nucleotides in length
- Ideal primer content is 40–60% GC
- Primer Tm should be approximately 60°C
- Primer Tm calculation should be determined with NEB's TmCalculator. (**TmCalculator.neb.com**) using the Hot Start *Taq* setting.
- For best results in qPCR, primer pairs should have Tm values that are within 3°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between primers
- G homopolymer repeats ≥ 4 should be avoided
- The optimal primer concentration for dye-based experiments and probe-based experiments is 400 nM. If necessary, the primer concentration can be optimized between 100–900 nM.
- Higher primer concentrations may increase secondary priming and create spurious amplification products
- When using primer design software, enter sufficient sequence around the area of interest to permit robust primer design and use search criteria that permit cross-reference against relevant sequence databases to avoid potential off-target amplification
- It is advisable to design primers across known exon-exon junctions in order to prevent amplification from genomic DNA

Hydrolysis Probes

- Probes should typically be 15–30 nucleotides in length to ensure sufficient quenching of the fluorophore
- The optimal probe concentration is 200 nM but may be optimized between 100 to 500 nM
- Both single or double-quenched probes may be used
- In general, non-fluorescence quenchers result in better signal-to-noise ratio than fluorescence quenchers
- Ideal probe content is 40–60% GC
- The probe Tm should be 5–10°C higher than the Tm of the primers to ensure all targeted sequences are saturated with probe prior to amplification by the primers
- Probes may be designed to anneal to either the sense or antisense strand
- Generally, probes should be designed to anneal in close proximity to either the forward or reverse primer without overlapping
- Avoid a 5´-G base which is known to quench 5´-fluorophores

Multiplexing

- Avoid primer/probe combinations that contain complementary sequences, and ensure target sequences do not overlap
- Probes should be designed such that each amplicon has a unique fluorophore for detection
- Select fluorophores based on the detection capabilities of the available real-time PCR instrument
- The emission spectra of the reporter fluorophores should not overlap
- Test each primer/probe combination in a singleplex reaction to establish a performance baseline. Ensure C_q values are similar when conducting the multiplex qPCR.
- Pair dim fluorescence dyes with high abundance targets and bright dyes with low abundance targets
- Optimization may require lower primer/probe concentrations to be used for high copy targets along with higher concentrations for low copy targets

Reverse Transcription

- The default reverse transcription temperature is 55°C
- For difficult targets, the temperature of reverse transcription may be increased to 60°C for 10 minutes
- Due to the WarmStart feature of the Luna RT, reverse transcription temperatures lower than 50°C are not recommended

Cycling Conditions

- Generally, best performance is achieved using the cycling conditions provided in the manual
- Longer amplicons (> 400 bp) can be used but may require optimization of extension times
- Due to the dual WarmStart/Hot Start feature of the Luna kits, it is not necessary to preheat the thermocycler prior to use
- Select the "Fast" ramp speed where applicable (e.g., Applied Biosystems QuantStudio®).
- Amplification for 40 cycles is sufficient for most applications, but for very low input samples 45 cycles may be used

Reaction Setup

- For best results, keep reactions on ice prior to thermocycling
- A reaction volume of 20 µl is recommended for 96-well plates while a reaction volume of 10 µl is recommended for 384-well plates
- Reactions should be carried out in triplicate for each sample
- For each amplicon, ensure to include no template controls (NTC)
- A no Luna RT control should be conducted to guarantee amplification is specific for RNA input and not due to genomic DNA contamination
- To prevent carry-over contamination, treat reactions with 0.2 units/µl Antarctic Thermolabile UDG (NEB #M0372) for 10 minutes at room temperature prior to thermocycling. Some Luna products (NEB #M3019, M3029, L4001) contain Thermolabile UDG, so no treatment is necessary.
- The Luna reference dye supports broad instrument compatibility (High-ROX, Low-ROX, ROX-independent) so no additional ROX is required for normalization
- No ROX versions (NEB #E3007, M3029) contain no reference dye and are compatible with any instrument that does not require ROX. If ROX normalization is needed, ROX can be added. Please refer to instrument manufacturer's instructions for details.

Assay Performance

- Ensure 90–110% PCR efficiency for the assay over at least three $\log_{10}$ dilutions of template.
- Linearity over the dynamic range (R^2) should ideally be ≥ 0.99
- Target specificity should be confirmed by product size, sequencing or melt-curve analysis

Luna® qPCR Troubleshooting Guide

Problem	Probable Cause(s)	Solution(s)
qPCR traces show low or no amplification	Reagent omitted from qPCR assay	• Verify all steps of the protocol were followed correctly
	Reagent added improperly to qPCR assay	
	Incorrect cycling protocol	• Refer to the proper qPCR cycling protocol in product manual
	Incorrect channel selected for the qPCR thermal cycler	• Verify correct optical settings on the qPCR instrument
	DNA template or reagents are contaminated or degraded	• Confirm the expiration dates of the kit reagents • Verify proper storage conditions provided in this user manual • Rerun the qPCR assay with fresh reagents • Confirm template input amount
Inconsistent qPCR traces for triplicate data	Improper pipetting during qPCR assay set-up	• Ensure proper pipetting techniques
	qPCR plate film has lost its seal, causing evaporation in the well. The resulting qPCR trace may show significantly different fluorescence values relative to its replicates	• Ensure the qPCR plate is properly sealed before inserting into the qPCR thermal cycler. • Exclude problematic trace(s) from data analysis.
	Poor mixing of reagents during qPCR set-up	• Make sure all reagents are properly mixed after thawing them
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler • Exclude problematic trace(s) from data analysis
DNA standard curve has a poor correlation coefficient/efficiency of the DNA standard curve falls outside the 90–110% range	Presence of outlying qPCR traces	• Omit data produced by qPCR traces that are clearly outliers caused by bubbles, plate sealing issues, or other experimental problems
	Improper pipetting during qPCR assay set-up	• Ensure that proper pipetting techniques are used
	Reaction conditions are incorrect	• Verify that all steps of the protocol were followed correctly
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler
	Poor mixing of reagents	• After thawing, make sure all reagents are properly mixed
	Threshold is improperly set for the qPCR traces	• Ensure the threshold is set in the exponential region of qPCR traces • Refer to the real-time instrument user manual to manually set an appropriate threshold
Melt curve shows different peaks for low input samples	Non-template amplification is occurring	• Compare melt curve of NTC to samples • Redesign primers with a Tm of 60°C or use our Tm calculator to determine the optimal annealing temperature of the primers • Perform a primer matrix analysis to determine optimal primer concentrations
	Infrequently, denaturation of a single species can occur in a biphasic manner, resulting in two peaks	
No template control qPCR trace shows amplification, NTC C_q is close to or overlapping lower copy standards	Reagents are contaminated with carried-over products of previous qPCR (melt curve of NTC matches melt curve of higher input standards)	• Replace all stocks and reagents • Clean equipment and setup area with a 10% chlorine bleach • Consider use of 0.2 U/µl Antarctic Thermolabile UDG to eliminate carryover products
	Primers produce non-specific amplification (melt curve of NTC does not match melt curve of higher input standards)	• Redesign primers with a Tm of 60°C or use qPCR primer design software

Luna® One-Step RT-qPCR Troubleshooting Guide

Problem	Probable Cause(s)	Solution(s)
qPCR traces show low or no amplification	Incorrect RT step temperature or RT step omitted	• For typical use, a 55°C RT step temperature is optimal for the Luna WarmStart Reverse Transcriptase.
	Incorrect cycling protocol	• Refer to the proper RT-qPCR cycling protocol in product manual
	Reagent omitted from RT-qPCR assay	• Verify all steps of the protocol were followed correctly
	Reagent added improperly to RT-qPCR assay	
	Incorrect channel selected for the qPCR thermal cycler	• Verify correct optical settings or select FAM/SYBR on the qPCR instrument
	RNA template or reagents are contaminated or degraded	• Prepare high quality RNA without RNase/DNase contamination • Confirm template input amount • Confirm the expiration dates of the kit reagents • Verify proper storage conditions provided in product manual • Rerun the RT-qPCR assay with fresh reagents
Inconsistent qPCR traces for triplicate data	Improper pipetting during qPCR assay set-up	• Ensure proper pipetting techniques
	qPCR plate film has lost its seal, causing evaporation in the well. The resulting qPCR trace may show significantly different fluorescence values relative to its replicates.	• Ensure the qPCR plate is properly sealed before inserting into the qPCR thermal cycler • Exclude problematic trace(s) from data analysis
	Poor mixing of reagents during RT-qPCR set-up	• Make sure all reagents are properly mixed after thawing them
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler • Exclude problematic trace(s) from data analysis
Standard curve has a poor correlation coefficient/ efficiency of the standard curve falls outside the 90–110% range	Cycling protocol is incorrect	• Refer to the proper RT-qPCR cycling protocol in product manual • Use a 55°C RT step temperature • For ABI instruments, use a 1 minute 60°C annealing/ extension step
	Presence of outlying qPCR traces	• Omit data produced by qPCR traces that are clearly outliers caused by bubbles, plate sealing issues, or other experimental problems
	Improper pipetting during RT-qPCR assay set-up	• Ensure that proper pipetting techniques are used
	Reaction conditions are incorrect	• Verify that all steps of the protocol were followed correctly
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler
	Poor mixing of reagents	• After thawing, make sure all reagents are properly mixed
	Threshold is improperly set for the qPCR traces	• Ensure the threshold is set in the exponential region of qPCR traces • Refer to the real-time instrument user manual to manually set an appropriate threshold
Melt curve shows different peaks for low input samples	Non-template amplification is occurring Infrequently, denaturation of a single species can occur in a biphasic manner, resulting in two peaks	• Compare melt curve of NTC to samples • Redesign primers with a Tm of 60°C or use our Tm calculator to determine the optimal annealing temperature of the primers • Perform a primer matrix analysis to determine optimal primer concentrations
No template control qPCR trace shows amplification/ NTC C_q is close to or overlapping lower copy standards	Reagents are contaminated with carried-over products of previous qPCR (Melt curve of NTC matches melt curve of higher input standards)	• Replace all stocks and reagents • Clean equipment and setup area with a 10% chlorine bleach • Consider use of 0.2 U/l Antarctic Thermolabile UDG to eliminate carryover products
	Primers produce non-specific amplification (Melt curve of NTC does not match melt curve of higher input standards)	• Redesign primers with a Tm of 60°C or use qPCR primer design software
Amplification in No-RT control	RNA is contaminated with genomic DNA	• Treat sample with DNase I • Redesign primer to span exon-exon junction

Cleavage Close to the End of DNA Fragments

Annealed 5´ FAM-labeled oligos were incubated with the indicated enzyme (10 units/ 1 pmol oligo) for 60 minutes at the recommended incubation temperature and NEBuffer. The digest was run on a TBE acrylamide gel and analyzed by fluorescent imaging. The double stranded oligos were designed to have the indicated number of base pairs from the end followed by the recognition sequence and an additional 12 bases. In some cases asymmetric cleavage was observed and interpreted as a negative result. Asymmetric cleavage decreased with increasing base pairs from the end.

Note: As a general rule and for enzymes not listed below, 6 base pairs should be added on on either side of the recognition site to cleave efficiently.

The extra bases should be chosen so that palindromes and primer dimers are not formed. In most cases there is no requirement for specific bases.

–	0%	+	0–20%
++	20–50%	+++	50–100%
NT	not tested		

Enzyme	Base Pairs From End 1 bp	2 bp	3 bp	4 bp	5 bp
AccI*	–	–	–	–	–
AciI	–	+	+	++	+++
AgeI-HF	++	+++	+++	+++	+++
AleI-v2	+++	+++	+++	+++	+++
AluI	–	+++	+++	+++	+++
ApaI	+++	+++	+++	+++	+++
AscI	+++	+++	+++	+++	+++
AvrII	++	++	+++	+++	+++
BamHI	+	++	+++	+++	+++
BamHI-HF	+	+	+++	+++	+++
BbsI-HF	+++	+++	+++	+++	+++
BclI-HF	–	–	+++	+++	+++
BglII	++	+++	+++	+++	+++
BmtI-HF	+++	+++	+++	+++	+++
BsaI-HFv2	+++	+++	+++	+++	+++
BsiWI	++	+++	+++	+++	+++
BsiWI-HF	+++	+++	+++	+++	+++
BsmBI-v2	+++	+++	+++	+++	+++
BsrGI-HF	+++	+++	+++	+++	+++
BssHII	+	+++	+++	+++	+++
BstZ17I-HF	+	+++	+++	+++	+++
ClaI	–	–	+	+++	+++
DdeI	+++	+++	+++	+++	+++
DpnI	–	++	++	NT	NT
DraIII-HF	+++	+++	+++	+++	+++
EagI-HF	+	+++	+++	+++	+++
EcoRI	+	+	++	++	+++
EcoRI-HF	+	+	++	+++	+++
EcoRV	++	++	++	++	+++
EcoRV-HF	+	++	++	++	+++
Esp3I	+++	+++	+++	+++	+++
FseI	+	++	+++	+++	+++
HindIII	–	+	+++	+++	+++
HindIII-HF	–	+	+++	+++	+++
HpaI	+++	+++	+++	+++	+++
KpnI-HF	+	+++	+++	+++	+++
MfeI-HF	+	++	+++	+++	+++
MluI-HF	+	++	+++	+++	+++
MseI	+++	+++	+++	+++	+++

*AccI requires at least 13 base pairs beyond the end of its recognition sequence to cleave efficiently.

Enzyme	Base Pairs From End 1 bp	2 bp	3 bp	4 bp	5 bp
NcoI-HF	+	++	+++	+++	+++
NdeI	+	+	+++	+++	+++
NheI-HF	++	++	+++	+++	+++
NlaIII	++	+++	+++	+++	+++
NotI	++	++	++	++	++
NotI-HF	++	++	++	++	++
NsiI	+	+	+++	+++	+++
NspI	–	–	+	+	+++
PacI	+++	+++	+++	+++	+++
PaqCI	++	+++	–	–	–
PciI	+++	+++	+++	+++	+++
PmeI	+++	+++	+++	+++	+++
PsiI-v2	+	+++	+++	+++	+++
PstI	+	+++	+++	+++	+++
PstI-HF	++	+++	+++	+++	+++
PvuI-HF	+++	+++	+++	+++	+++
PvuII	++	++	++	+++	+++
PvuII-HF	–	++	++	+++	+++
RsaI	+	+++	+++	+++	+++
SacI-HF	–	+	+++	+++	+++
SacII	+++	+++	+++	+++	+++
SalI	–	++	+++	+++	+++
SalI-HF	–	++	+++	+++	+++
SapI	+++	+++	+++	+++	+++
Sau3AI	+++	+++	+++	+++	+++
SbfI-HF	++	+++	+++	+++	+++
ScaI-HF	+	+++	+++	+++	+++
SfiI	+++	+++	+++	+++	+++
SmaI	+++	+++	+++	+++	+++
SpeI-HF	+	++	++	++	++
SphI	+++	+++	+++	+++	+++
SphI-HF	++	++	+++	+++	+++
SspI-HF	+	+++	+++	+++	+++
StuI	+++	+++	+++	+++	+++
StyI-HF	+	+++	+++	+++	+++
XbaI	++	++	++	++	++
XhoI	++	++	++	+++	+++
XmaI	+++	+++	+++	+++	+++

Activity of Restriction Enzymes in PCR Buffers

Frequently, a PCR product must be further manipulated by cleavage with restriction enzymes. This table summarizes the activity of restriction enzymes on the DNA in *Taq*, Q5, Phusion*, One*Taq* and LongAmp *Taq* PCR mixes. 50 µl reactions containing 5 units of restriction enzyme were incubated at the appropriate temperature for 1 hour in a PCR mix containing the following: 1 µg DNA, 1 unit of DNA Polymerase and 1X ThermoPol Reaction Buffer, Q5 Reaction Buffer, Phusion HF Buffer, One*Taq* Standard Reaction Buffer or LongAmp *Taq* Reaction Buffer. Reactions were supplemented with 200 µM dNTPs. Enzyme activity was analyzed by gel electrophoresis.

Notes: The polymerase is still active and can alter the ends of DNA fragments after cleavage, affecting subsequent ligation. Primers containing the restriction enzyme recognition site can act as competitive inhibitors in the cleavage reaction. The use of restriction enzymes under non-optimal conditions may increase the likelihood of star activity. If any problems are encountered, the DNA should be purified by spin column or phenol/chloroform extraction followed by alcohol precipitation.

Cleavage in extension mix with 5 units of enzyme:

+++ complete cleavage ++ ~50% cleavage
\+ ~ 25% cleavage – no cleavage

Enzyme	*Taq* in Thermopol Rxn Buffer	Q5 in Q5 Buffer**	Phusion in Phusion HF Buffer	One*Taq* in One*Taq* Rxn Buffer	LongAmp *Taq* in LongAmp *Taq* Rxn Buffer
AatII	<+++	<+	+	++	+
AccI	<+++	<+	<+	+++	+++
Acc65I	+++	<+	<+	<+	+
AciI	++	++	+++	+++	+++
AclI	+++	<+	<+	+++	+++
AcuI	+++	<+	++	+++	+++
AfeI	+++	<+	++	+++	+++
AflII	+	<+	<+	+	<+
AflIII	<+	+++	+	<+	<+
AgeI-HF	+++	<+	++	+++	+++
AhdI	<+	–	–	<+	<+
AleI-v2	–	–	–	+	+
AluI	+++	+	+++	+++	+++
AlwI	–	<+	<+	<+	<+
AlwNI	<+++	+	+++	<+	+
ApaI	+++	<+	<+	<+	–
ApaLI	+++	<+	<+	+++	+++
ApeKI @75°C	<+++	++	+++	<+	+
ApoI-HF	+++	+	++	+++	+++
AscI	+++	<+	<+	<+	–
AseI	+++	<+	+	++	++
AsiSI	+++	<+	++	+++	+++
AvaI	+++	<+	+++	+++	+
AvaII	+++	<+	++	+++	+++
AvrII	+++	<+	<+	+++	+++
BaeGI	+++	<+	+++	+++	+++
BaeI	–	<+	++	<+	<+
BamHI	+++	<+	+++	+++	+++
BamHI-HF	+++	<+	–	<+	++
BanI	+++	<+	+++	+++	+++
BanII	+++	<+	+++	+++	+++
BbsI	+++	<+	<+	+++	+++
BbsI-HF	+	–	–	–	+
BbvCI	+++	–	–	<+	<+
BbvI	+++	<+	++	+++	+++
BccI	<+	<+	<+	<+	<+
BceAI	<+	<+	++	+	<+
BcgI	<+	<+	+	++	++
BciVI	–	–	–	<+	–
BclI	+++	++	+++	+++	+++
BclI-HF	+++	–	–	+	+
BcoDI	<+	<+	+	+	<+
BfaI	–	<+	–	–	–
BfuAI @50°C	<+++	–	+	<+	–
BglI	<+	++	+	<+	<+
BglII	<+	+	++	<+	<+
BlpI	<+++	<+	<+	<+	–
BmgBI	–	++	+	<+	<+
BmrI	<+++	<+	+++	+++	+++
BmtI-HF	++	<+	+	++	+++
BpmI	<+	<+	+++	<+++	<+++
BpuEI	+++	–	++	<+++	<+++
Bpu10I	<+	<+	+++	++	+++
BsaAI	+++	++	+++	+++	+++
BsaBI @60°C	+	<+	++	++	+++
BsaHI	+++	+	+++	+++	+++
BsaI-HFv2	+	<+	+	+	++

Enzyme	*Taq* in Thermopol Rxn Buffer	Q5 in Q5 Buffer**	Phusion in Phusion HF Buffer	One*Taq* in One*Taq* Rxn Buffer	LongAmp *Taq* in LongAmp *Taq* Rxn Buffer
BsaJI @60°C	+++	<+	++	+++	+++
BsaWI @60°C	<+++	<+	++	+	+
BsaXI	<+++	<+	<+	<+	<+
BseRI	+++	<+	++	++	+
BseYI	+++	++	++	+++	+++
BsgI	<+	<+	+	<+	<+
BsiEI @60°C	+++	<+	++	++	++
BsiHKAI @65°C	–	++	+	–	–
BsiWI @55°C	+++	<+	+++	+++	+++
BsiWI-HF	–	–	–	–	–
BsII	+++	++	+++	+++	+++
BsmAI	+++	++	+++	<+	<+
BsmBI-v2 @55°C	<+++	+	++	<+	<+
BsmFI @65°C	<+	+++	++	+	+
BsmI @65°C	+++	+	<+	+++	+
BsoBI	+++	+++	+++	++	+++
BspCNI	<+	<+	+	–	–
BspDI	<+++	<+	++	+++	+++
BspEI	–	<+	<+	–	–
BspHI	+++	<+	+++	+++	+++
Bsp1286I	<+	<+	<+	<+	<+
BspMI	+++	<+	++	<+	<+
BspQI @50°C	+	++	+++	+++	+++
BspQI-HF	++	+	+	–	–
BsrBI	+++	<+	+	+++	+++
BsrDI	<+	<+	+	<+	<+
BsrFI-v2	<+	–	–	–	–
BsrI @65°C	+++	<+	+++	++	+++
BssHII	+++	<+	+	+++	+++
BssSI-v2	+++	–	+	+++	+++
BstAPI @60°C	+++	<+	++	+++	+++
BstBI @65°C	+++	++	+++	+++	+++
BstEII-HF	+++	<+	<+	++	++
BstNI @60°C	+++	<+	<+	<+	<+
BstUI @60°C	+++	<+	<+	+++	+
BstXI	<+++	+	+	+	<+
BstYI @60°C	+++	<+	<+	++	+
BstZ17I-HF	+++	–	+	+++	+++
Bsu36I	<+	<+	<+	<+	+
BtgI	+++	<+	+	<+	<+
BtgZI @60°C	+++	+	++	++	++
BtsI-v2	+++	–	+	+++	+++
BtsIMutI @55°C	++	–	–	+	+
BtsCI @50°C	+++	<+	<+	+++	+++
Cac8I	+++	<+	<+	+++	++
ClaI	++	<+	<+	<+	++
CspCI	<+	–	+	<+	<+
CviKI-1	+++	<+	++	+++	+++
CviQI @25°C	+++	+	+++	++	+++
DdeI	+++	++	+	+++	+++
DpnI	+++	++	+++	++	++
DpnII	+++	++	+++	+++	++
DraI	+++	<+	+++	+++	+++
DraIII-HF	++	++	+++	++	++
DrdI	+++	<+	+++	+++	+++
EaeI	+++	<+	–	<+	<+

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

** It has been shown that the addition of 1X Restriction Enzyme Buffer may help to improve the ability of some enzymes to cleave.

Activity of Restriction Enzymes in PCR Buffers (continued)

Enzyme	*Taq* in Thermopol Rxn Buffer	Q5 in Q5 Buffer**	Phusion in Phusion HF Buffer	One*Taq* in One*Taq* Rxn Buffer	Longamp *Taq* in Longamp *Taq* Rxn Buffer
EagI-HF	+	<+	+	++	++
EarI	+++	<+	+++	+	<+
EciI	<+	++	+++	<++	<++
Eco53kI	+++	<+	<+	+++	+++
EcoNI	+++	<+	+	+++	+++
EcoO109I	+++	<+	–	<+	+
EcoP15I	<+	<+	+	<+	+
EcoRI	+	<+	+++	–	–
EcoRI-HF	+++	<+	+	+++	+++
EcoRV	<+	<+	+	–	<+
EcoRV-HF	+	<+	<+	+	++
Esp3I	+++	-	+++	+	+++
FatI @55°C	++	<+	+++	<+	+++
FauI @55°C	+	<+	++	+++	++
Fnu4HI	+++	<+	<+	++	+
FokI	+++	+	+	+++	+++
FseI	+	<+	++	+++	–
FspI	<++	<+	+	+	+
HaeII	+++	<+	+++	+++	+++
HaeIII	+++	<+	+++	+++	+++
HgaI	<+	<+	+	<++	<++
HhaI	+++	<+	+++	+++	+++
HincII	+++	<+	<+	+++	+++
HindIII	+++	<+	+	++	+++
HindIII-HF	+++	<+	<+	+++	+++
HinfI	+++	+++	+++	+	+++
HinP1I	+++	+	+++	+++	+++
HpaI	+++	<+	+++	+++	+++
HpaII	+++	<+	<+	<+	<+
HphI	<++	<+	<+	<+	<+
HpyAV	+++	–	++	+	++
HpyCH4III	<++	<+	+	<++	<++
HpyCH4IV	+++	<+	<+	+++	+++
HpyCH4V	+++	<+	<+	+++	+++
Hpy99I	+++	–	+	<+	<+
Hpy188I	+++	<+	+	++	++
Hpy166II	+++	+	++	+++	+++
Hpy188III	+	<+	<+	+	<+
KasI	+++	<+	+++	+++	–
KpnI-HF	++	–	++	<+	<+
MboI	+++	<+	+++	+++	+++
MboII	+++	+	++	+	+
MfeI-HF	+	–	–	+++	<+
MluCI	+	<+	<+	++	+
MluI-HF	++	–	++	++	++
MlyI	+++	+	++	<+	+
MmeI	<+	–	++	<+	<+
MnlI	+++	+	+	+	+
MscI	<+	<+	+	<+	<+
MseI	<+	<+	<+	<+	<+
MslI	+++	<+	+	+++	++
MspA1I	+++	<+	+++	++	+++
MspI	+++	<+	+++	++	+++
MwoI @60°C	+++	+++	+++	++	+++
NaeI	<+	<+	+	<+	<+
NarI	–	<+	++	+++	+++
NciI	+++	<+	<+	+	<+
NcoI	+++	<+	+	++	++
NcoI-HF	+++	<+	–	++	+
NdeI	<++	++	+++	++	<+
NgoMIV	–	<+	+	<+	<+
NheI-HF	+++	<+	–	++	++
NlaIII	<+	<+	+	++	<+
NlaIV	+++	<+	+++	+++	+++
NmeAIII	<+	–	+++	<+	<+
NotI	++	<+	+	<+	<+
NotI-HF	+++	<+	<+	<+	+
NruI-HF	++	–	–	+	–

Enzyme	*Taq* in Thermopol Rxn Buffer	Q5 in Q5 Buffer**	Phusion in Phusion HF Buffer	One*Taq* in One*Taq* Rxn Buffer	Longamp *Taq* in Longamp *Taq* Rxn Buffer
NsiI	+++	+	+++	++	+
NsiI-HF	+++	++	+++	+++	+++
NspI	+++	<+	<+	+++	++
PacI	+++	<+	<+	++	+++
PaeR7I	+++	<+	<+	+++	+++
PaqCI	–	–	–	+++	++
PciI	<+	<+	–	–	–
PflFI	+++	<+	<+	<+	+
PflMI	+	<+	+++	++	+++
PleI	+++	<+	<+	<+	<+
PluTI	+++	<+	+	+++	+++
PmeI	+++	<+	<+	+++	+++
PmlI	–	–	–	+	<+
PpuMI	+++	<+	+++	+++	+++
PshAI	+++	<+	<+	<+	<+
PsiI-v2	+++	–	–	+++	+++
PspGI @75°C	+++	+++	+++	+++	+++
PspOMI	+++	<+	+	+++	+++
PspXI	+++	<+	++	+++	+++
PstI	++	+	+	<+	<+
PstI-HF	+++	<+	++	++	+
PvuI-HF	+++	<+	+++	++	+++
PvuII	+++	<+	+	+++	+++
PvuII-HF	+	–	–	<+	<+
RsaI	+++	<+	++	+++	+++
RsrII	<++	–	–	<+	<+
SacI-HF	+++	<+	<+	<+	++
SacII	+++	<+	+++	++	+
SalI	<+	+	++	–	–
SalI-HF	+	<+	+++	+	+++
SapI	<++	<+	++	++	++
Sau3AI	+++	<+	<+	<+	<+
Sau96I	<++	+	+	+++	+++
SbfI-HF	+	–	–	<+	<+
ScaI-HF	+	<+	<+	–	–
ScrFI	+++	+++	+++	+++	+++
SexAI	+++	<+	+++	+++	+++
SfaNI	–	<+	++	<++	<++
SfcI	+++	<+	<+	+	+
SfiI @50°C	+++	–	–	+++	+++
SfoI	+++	<+	+++	+	+++
SgrAI	<++	<+	++	+	+++
SmaI	+++	<+	++	+++	+++
SmlI @55°C	<+	<+	+	+	+
SnaBI	<+	<+	<+	+++	+++
SpeI-HF	+++	–	<+	+++	+++
SphI	+++	+	++	<+	<+
SphI-HF	+++	<+	+	+++	+++
SrfI	<+	<+	+++	+	++
SspI-HF	++	<+	+	+++	+++
StuI	+++	<+	<+	+++	+++
StyD4I	<++	<+	+	<+	<+
StyI-HF	+	<+	<+	++	+++
SwaI @25°C	<+	<+	<+	<+	+++
TaqI-v2 @65°C	+++	<+	+	+++	+++
TfiI @65°C	<++	<+	<+	++	++
TseI @65°C	+++	+++	+++	+++	+++
Tsp45I @65°C	+++	–	–	+	<+
TspMI @75°C	+++	<+	+	+++	+++
TspRI @65°C	+	<+	<+	+++	+++
Tth111I @65°C	+++	<+	++	<+	+
XbaI	+++	–	<+	++	++
XcmI	+++	<+	+	+++	+++
XhoI	<+	<+	+++	++	+++
XmaI	+++	<+	+	–	–
XmnI	+++	<+	<+	++	+++
ZraI	+++	<+	<+	++	+

APPENDIX

Getting Started with Molecular Cloning

Molecular cloning has traditionally used restriction enzymes to excise a fragment from source DNA, and to linearize a plasmid vector while creating compatible ends. After purification, insert and vector are ligated to form a recombinant vector, which is transformed into an *E. coli* host. Alternatively, PCR can been used to generate both the vector and insert, which can be joined using a variety of techniques, such as standard DNA ligation, enzymatic joining using a recombinase or topoisomerase, homologous recombination, or synthetic biology (see NEBuilder HiFi DNA Assembly and Gibson Assembly).

Regardless of the method chosen, the process can be made more efficient and successful by following good practices in the lab. The following tips will help improve the success of your cloning experiments.

1. Take the time to plan your experiments

Pay attention to the junction sequences and the effect on reading frames of any translated sequences. Check both the vector and insert for internal restriction sites (we recommend NEBcutter at **NEBcutter.neb.com**) prior to designing PCR primers that contain similar sites to those used for cloning. Verify that the antibiotic selective marker in the vector is compatible with the chosen host strain.

2. Start with clean DNA at the right concentration

Ensure that your source DNA is free of contaminants, including nucleases and unwanted enzymatic activities. Use commercially-available spin columns to purify starting DNA, (e.g., Monarch Spin Plasmid Miniprep Kit, NEB #T1110 for DNA plasmids, Monarch Spin PCR & DNA Cleanup Kit, NEB #T1130 for DNA Fragments). Completely remove solvents, such as phenol, chloroform and ethanol, prior to manipulation of the DNA. Elute DNA from the spin columns with salt-free buffer to prevent inhibition of the downstream steps, either restriction digestion or PCR amplification.

3. Perform your restriction digests carefully

The reaction volume should be compatible with the downstream step (e.g., smaller than the volume of the well of an agarose gel used to resolve the fragments). For a typical cloning reaction, this is often between 25–50 µl. The volume of restriction enzyme(s) added should be no more than 10% of the total reaction volume, to ensure that the glycerol concentration stays below 5%; this is an important consideration to minimize star activity (unwanted cleavage).

4. Mind your ends

DNA ends prepared for cloning by restriction digest are ready for ligation without further modification, assuming the ends to be joined are compatible. If the ends are non-compatible, they can be modified using blunting reagents, phosphatases, etc.

DNA ends prepared by PCR for cloning may have a 3´ addition of a single adenine (A) residue following amplification using *Taq* DNA Polymerase (NEB #M0273). High-fidelity DNA polymerases, such as Q5 (NEB #M0491), leave blunt ends. PCR using standard commercial primers produces non-phosphorylated fragments, unless the primers were 5´ phosphorylated. The PCR product may need to be kinase treated to add a 5´ phosphate prior to ligation with a dephosphorylated vector.

5. Clean up your DNA prior to vector:insert joining

This can be done with gel electrophoresis or column purification (e.g., Monarch Spin PCR & DNA Cleanup Kit, NEB #T1130). Isolating the desired DNA from unwanted parent vectors and/or other DNA fragments can dramatically improve your cloning results.

Confirm digested DNA on an agarose gel prior to ligation. For a single product, run a small amount of the digest, and then column purify to isolate the remainder (e.g., Monarch Spin PCR & DNA Cleanup Kit, NEB #T1130). When multiple fragments are produced and only one is to be used, resolve the fragments on a gel and excise the desired fragment under UV light. Using longwave (365 nm) UV light will minimize any radiation-induced DNA damage to the fragment. Recover the DNA fragment from the agarose slice using a gel extraction kit (e.g., Monarch Spin DNA Gel Extraction Kit, NEB #T1120) or β-Agarase I (NEB #M0392).

6. Quantitate your isolated material

Simple quantitation methods, such as gel electrophoresis with mass standards or spectroscopic quantitation on low-input spectrophotometers (such as a NanoSpec®), ensure that the proper amount of material is used for the downstream reactions.

7. Follow the manufacturer's guidelines for the joining reaction

For traditional cloning, follow the guidelines specified by the ligase supplier. If a 3:1 molar ratio of insert to vector is recommended, try this first for best results. Using a 3:1 mass ratio is not the same thing (unless the insert and vector have the same mass). Ligation usually proceeds quickly and, unless your cloning project requires the generation of a high-complexity library that benefits from the absolute capture of every possible ligation product, long incubation times are not necessary.

Follow the manufacturer's guidelines for the joining reactions in PCR cloning and seamless cloning. If you are performing a cloning protocol for the first time, adhere to the recommended protocol for optimal results. NEB recommends using NEBioCalculator to calculate ligation ratios.

8. Use competent cells that are suited to your needs

While some labs prepare their own competent cells "from scratch" for transformations, the levels of competence achieved rarely matches the high levels attained with commercially-available competent cells. Commercially-available competent cells save time and resources, and make cloning more reproducible.

Find an overview of traditional cloning.

Optimization Tips for Golden Gate Assembly

Looking to assemble multiple DNA fragments in a single reaction? Here are some tips to keep in mind when planning your Golden Gate Assembly experiments using one of our NEBridge Golden Gate Assembly kits for BsaI-HFv2 (NEB #E1601) or BsmBI-v2 (NEB #E1602), NEBridge Ligase Master Mix (NEB #M1100), or PaqCI (NEB #R0745), our newest Type IIS restriction enzyme optimized for use in assembly, featuring a 7 base recognition site which minimizes the need for domestication of internal sites in your sequences.

Check your sequences

- Always check your assembly sequences for internal sites before choosing which Type IIS restriction endonuclease to use for your assembly. While single insert Golden Gate assembly has such high efficiencies of assembly that the desired product is obtainable regardless of the presence of an internal site, this is not true for assemblies with multiple inserts. Options include choosing a different Type IIS restriction enzyme to direct your assembly or eliminating internal sites through domestication. Our tutorial video on Golden Gate Assembly Domestication provides a full description of the many options available for internal site issues. Note the use of a Type IIS restriction enzyme with a 7 base recognition site, such as PaqCI, is less likely to have internal sites present in any given sequence.

Orient your primers

- When designing PCR primers to introduce Type IIS restriction enzyme sites, either for amplicon insert assembly or as an intermediate for pre-cloning the insert, remember that the recognition sites should always face inwards towards your DNA to be assembled. Consult the NEBridge Golden Gate Assembly Kit manuals or assembly videos for further information regarding the placement and orientation of the sites.

Choose the right plasmid

- Consider switching to the pGGAselect Destination Plasmid for your Golden Gate assembly. This versatile destination construct is included in all NEBridge Golden Gate Assembly kits and can be used for BsaI-HFv2, BsmBI-v2 or BbsI directed assemblies. It also features T7 and SP6 promoter sequences flanking the assembly site, and has no internal BsaI, BsmBI or BbsI sites. The pGGAselect plasmid can also be transformed into any *E. coli* strain compatible with pUC19 for producing your own plasmid preparation if so desired.

Choose the right buffer

- T4 DNA Ligase Buffer works best for Golden Gate Assembly with BsaI-HFv2, BsmBI-v2 and PaqCI. However, alternate buffers would be NEBuffer r1.1 for BsaI-HFv2, NEBuffer r2.1 for BsmBI-v2 & rCutSmart for PaqCI, if these buffers are supplemented with 1 mM ATP and 5-10 mM DTT. NEB also offers NEBridge® Ligase Master Mix that has been optimized for Golden Gate Assembly with our Type IIS restriction enzymes for Golden Gate.

Increase your complex assembly efficiency by increasing the Golden Gate cycling levels

- T4 DNA Ligase, BsaI-HFv2, BsmBI-v2 and PaqCI are very stable and continue to be active during extended cycling protocols; an easy way to increase assembly efficiencies without sacrificing fidelity is to increase the total cycles from 30 to 45-65, even when using long (5-minute) segments for the temperature steps.

Make sure your plasmid prep is RNA-free

- For pre-cloned inserts/modules, make sure your plasmid prep is free of RNA to avoid an overestimation of your plasmid concentrations.

Avoid primer dimers

- For amplicon inserts/modules, make sure your PCR amplicon is a specific product and contains no primer dimers. Primer dimers, with Type IIS restriction endonuclease sites (introduced in the primers used for the PCR reactions), would be active in the assembly reaction and result in mis-assemblies.

Avoid PCR-induced errors

- Do not over-cycle and use a proofreading high fidelity DNA polymerase, such as Q5 DNA High-Fidelity Polymerase.

Decrease insert amount for complex assemblies

- For complex assemblies involving >10 fragments, pre-cloned insert/modules levels can be decreased from 75 to 50 ng each without significantly decreasing the efficiencies of assembly.

Carefully design EVERY insert's overhang

- An assembly is only as good as its weakest junction. Research at NEB has led to an increased understanding of ligase fidelity, including the development of a comprehensive method for profiling end-joining ligation fidelity in order to predict which overhangs will result in improved accuracy. This ligase fidelity information can be paired with the appropriate Type IIS restriction enzyme chosen to direct your assembly to achieve high efficiencies and accurate complex assemblies. Please use the free NEBridge Golden Gate Assembly Tool to design primers for your Golden Gate Assembly reactions. Predict overhang fidelity or find optimal Golden Gate junctions for long sequences using our NEBridge Ligase Fidelity Tools.

Check for a sequence error if your assembly becomes non-functional

- Be aware that occasionally a pre-cloned insert/module can become corrupted by an error during propagation in *E. coli*, usually a frameshift due to slippage in a run of a single base (e.g., AAAA) by the *E. coli* DNA Polymerase. This should be suspected if previously functional assembly components suddenly become nonfunctional.

For more information on Golden Gate, visit **www.neb.com/GoldenGate**

How does Golden Gate Assembly work?

NEBridge® Ligase Master Mix Protocol Guidelines

This table provides guidance on using NEBridge Ligase Master Mix (MM) with NEB Type IIS restriction enzymes for Golden Gate Assembly. Recommendations differ based on the number of fragments to be assembled as well as the choice of Type IIS restriction enzyme. All incubations should be followed by an end soak for 5 min at 60°C prior to transformation. Store reactions at -20°C if not immediately being used for transformations.

	2 fragments*	3-6 fragments	7+ fragments**
	5 µl NEBridge Ligase MM 15 µl rxn volume 15-60 min 37°C *** -or- 15-30 cycles of 42°C X 1 min, 16°C X 1 min**** End soak 60°C X 5 min	5 µl NEBridge Ligase MM 15 µl rxn volume 30 cycles of 37°C X 1 min, 16°C X 1 min*** -or- 30 cycles of 42°C X 1 min, 16°C X 1 min**** End soak 60°C X 5 min	10 µl NEBridge Ligase MM 30 µl rxn volume 30-60 cycles of 37°C X 5 min, 16°C X 5 min*** -or- 30-60 cycles of 42°C X 5 min, 16°C X 5 min**** End soak 60°C X 5 min
BbsI-HF®	1 µl (20U)	1 µl (20U)	1 µl (50U)[a]
BsaI-HFv2	1 µl (20U)	1 µl (20U)	1 µl (20U)
BsmBI-v2	3 µl[b] (30U)	3 µl[b] (30U)	6 µl[b] (60U)
BspQI/BspQI-HF	1 µl (10U)	1 µl (10U)	2 µl (20U)
Esp3I	2 µl (20U)	3 µl (30U)	4 µl (40U)
PaqCI®	1 µl[c] (10U)	1 µl[c] (10U)	2.5 µl[c] (25U)
SapI	1 µl (10U)	1 µl (10U)	2 µl (20U)

* For 2 fragment assembly, 15 min or 15 cycles for single insert cloning; 60 min or 30 cycles for library construction

** For 7+ fragment assembly, 30 cycles for 7-13 fragment assembly; 60 cycles for 14+ fragment assembly

*** Reaction protocol for BbsI-HF, BsaI-HFv2, BspQI-HF, Esp3I, PaqCI and SapI

**** Reaction protocol for BsmBI-v2 and BspQI. Optimum reaction temperature is 42°C rather than 37°C.

a = Requires use of NEB #R3539M (50U/µl)

b = Use of less enzyme will reduce performance

c = Recommended PaqCI Activator : PaqCI ratio is 1:1 (pmol:U). Use 0.5 µl of PaqCI Activator (20 µM) for 2 and 3-6 fragments; 1.25 µl of PaqCI Activator (20 µM) for 7+ fragments

Joel and Marilyn are members of our Production Team. Joel (left) is an Automation Specialist and has been with NEB for 5 years. He helps organize NEB's annual Halloween Party and is involved with many clubs on campus. Marilyn is the Automation Supervisor and has been with NEB for 3 years. She is also a member of our Cat Owners Club.

Type IIS Restriction Enzymes

Type IIS restriction enzymes recognize asymmetric DNA sequences and cleave outside of their recognition sequence. They are useful for many applications, including Golden Gate Assembly. NEB currently offers over 50 Type IIS restriction enzymes.

Enzyme	Heat Inact.	NEBuffer	Reaction Temp.	Activity at 37°C	Storage Temp.	Recognition Sequence	Recognition Sequence Length	Overhang Length	Isoschizomers from NEB	Methylation Sensitivity**	Enzyme Sub-type
AcuI	Y	rCutSmart	37°C		-20°C	CTGAAG(16/14)	6	2			IIC
AlwI	N	rCutSmart	37°C		-20°C	GGATC(4/5)	5	1		dam	
BaeI SAM	Y	rCutSmart	25°C	100%	-20°C	(10/15)ACNNNNGTAYC(12/7)	7	5 & 5			IIC
BbsI *	Y	NEBuffer r2.1	37°C		-80°C	GAAGAC(2/6)	6	4			IIT
BbsI-HF *	Y	rCutSmart	37°C		-20°C	GAAGAC(2/6)	6	4			IIT
BbvI 2*site	Y	rCutSmart	37°C		-20°C	GCAGC(8/12)	5	4			
BccI	Y	rCutSmart	37°C		-20°C	CCATC(4/5)	5	1			
BceAI	Y	NEBuffer r3.1	37°C		-20°C	ACGGC(12/14)	5	2		CpG	
BcgI 2*site	Y	NEBuffer r3.1	37°C		-20°C	(10/12)CGANNNNNNTGC(12/10)	6	2 & 2		dam; CpG	IIC
BciVI	Y	rCutSmart	37°C		-20°C	GTATCC(6/5)	6	1			
BcoDI	N	rCutSmart	37°C		-20°C	GTCTC(1/5)	5	4	BsmAI	CpG	IIT
BfuAI 2*site	Y	NEBuffer r3.1	50°C	25%	-20°C	ACCTGC(4/8)	6	4	BspMI	CpG	
BmrI	Y	NEBuffer r2.1	37°C		-20°C	ACTGGG(5/4)	6	1			
BpmI 2*site	Y	NEBuffer r3.1	37°C		-20°C	CTGGAG(16/14)	6	2			IIC
BpuEI	Y	rCutSmart	37°C		-20°C	CTTGAG(16/14)	6	2			IIC
BsaI-HF®v2 *	Y	rCutSmart	37°C		-20°C	GGTCTC(1/5)	6	4		dcm; CpG	IIT
BsaXI	N	rCutSmart	37°C		-20°C	(9/12)ACNNNNNCTCC(10/7)	6	3 & 3			IIC
BseRI	Y	rCutSmart	37°C		-20°C	GAGGAG(10/8)	6	2			IIC
BsgI 2*site	Y	rCutSmart	37°C		-20°C	GTGCAG(16/14)	6	2			IIC
BsmAI	N	rCutSmart	55°C	50%	-20°C	GTCTC(1/5)	5	4	BcoDI	CpG	
BsmBI-v2 *	Y	NEBuffer r3.1	55°C	10%	-20°C	CGTCTC(1/5)	6	4	Esp3I	CpG	IIT
BsmFI	Y	rCutSmart	37°C		-20°C	GGGAC(10/14)	5	4		CpG; dcm	IIC
BsmI	Y	rCutSmart	65°C	10%	-20°C	GAATGC(1/-1)	6	2			IIT
BspCNI	Y	rCutSmart	37°C		-20°C	CTCAG(9/7)	5	2			IIC
BspMI 2*site	Y	NEBuffer r3.1	37°C		-20°C	ACCTGC(4/8)	6	4	BfuAI		
BspQI *	Y	NEBuffer r3.1	50°C	50%	-20°C	GCTCTTC(1/4)	7	3	SapI		IIT
BspQI-HF	Y	rCutSmart	37°C		-20°C	GCTCTTC(1/4)	7	3	SapI		IIT
BsrDI	Y	NEBuffer r2.1	37°C		-20°C	GCAATG(2/0)	6	2			IIT
BsrI	Y	NEBuffer r3.1	65°C	10%	-20°C	ACTGG(1/-1)	5	2			IIT
BtgZI *	Y	rCutSmart	60°C	50%	-20°C	GCGATG(10/14)	6	4		CpG	IIC
BtsCI	Y	rCutSmart	50°C	25%	-20°C	GGATG(2/0)	5	2			
BtsI-v2	Y	rCutSmart	37°C		-20°C	GCAGTG(2/0)	6	2			IIT
BtsIMutI	Y	rCutSmart	55°C	50%	-20°C	CAGTG(2/0)	5	2			IIT
CspCI 2*site	Y	rCutSmart	37°C		-20°C	(11/13)CAANNNNNGTGG(12/10)	7	2 & 2			IIC
EarI	Y	rCutSmart	37°C		-20°C	CTCTTC(1/4)	6	3		CpG	IIT
EciI	Y	rCutSmart	37°C		-20°C	GGCGGA(11/9)	6	2		CpG	IIC
Esp3I *	Y	rCutSmart	37°C		-20°C	CGTCTC(1/5)	6	4	BsmBI-v2	CpG	IIT
FauI	Y	rCutSmart	55°C	50%	-20°C	CCCGC(4/6)	5	2		CpG	
FokI 2*site	Y	rCutSmart	37°C		-20°C	GGATG(9/13)	5	4		dcm; CpG	
HgaI	Y	NEBuffer r1.1	37°C		-20°C	GACGC(5/10)	5	5		CpG	
HphI	Y	rCutSmart	37°C		-20°C	GGTGA(8/7)	5	1		dam; dcm	
HpyAV	Y	rCutSmart	37°C		-20°C	CCTTC(6/5)	5	1		CpG	
MboII 2*site	Y	rCutSmart	37°C		-20°C	GAAGA(8/7)	5	1		dam	
MlyI	Y	rCutSmart	37°C		-20°C	GAGTC(5/5)	5	0			
MmeI 2*site	Y	rCutSmart	37°C		-20°C	TCCRAC(20/18)	6	2		CpG	IIC
MnlI	Y	rCutSmart	37°C		-20°C	CCTC(7/6)	4	1			
NmeAIII 2*site	Y	rCutSmart	37°C		-20°C	GCCGAG(21/19)	6	2			IIC
PaqCI 2*site	Y	rCutSmart	37°C		-20°C	CACCTGC(4/8)	7	4		CpG	
PleI 2*site	Y	rCutSmart	37°C		-20°C	GAGTC(4/5)	5	1		CpG	
SapI *	Y	rCutSmart	37°C		-20°C	GCTCTTC(1/4)	7	3	BspQI		IIT
SfaNI	Y	NEBuffer r3.1	37°C		-20°C	GCATC(5/9)	5	4		CpG	

* Cited for use in Golden Gate Assembly according to current literature

** Methylation sensitivity applies to the recognition motif only

2*site Through suppression experiments and published reports, NEB has identified that these enzymes require more than one recognition site on the substrate to cleave optimally. For more information, see Restriction Enzyme Cleavage: 'single-site' enzymes and 'multi-site' enzymes.

Optimization Tips for NEBuilder® HiFi DNA Assembly and NEB Gibson Assembly®

Looking to assemble multiple DNA fragments in a single reaction? Here are some tips to keep in mind when planning your NEBuilder HiFi DNA Assembly or Gibson Assembly experiments.

Decide How You Want to Generate the Linearized Vector. You can Choose from Two Methods:

1) Restriction enzyme digestion: good for large plasmids you don't want to amplify; background may be higher if undigested vector is present.

2) PCR: achieves lower background versus restriction enzyme digestion, but is limited by the size of the vector. Typically, vectors up to 10 kb can be amplified; for amplicons greater than 10 kb, divide into 2 fragments.

Design the Primers

- Use the NEBuilder Assembly Tool (**NEBuilder.neb.com**) to design the primers and check the sequence of the final assembly. Primers will contain the overlap sequence. We recommend watching the tutorials before using the tool for the first time. There is one for restriction enzyme digestion and another for PCR. The videos can be found at **NEBuilderHiFi.com**
- Make sure the overlap is the correct length for the number of fragments in the assembly: Refer to the section titled "Use the Correct Amount of DNA" for more details.

Column Purify the PCR Products

- If you do not purify the PCR products, limit the unpurified PCR products to 20% of the reaction volume (4 µl for a standard 20 µl reaction).
- If PCR produces a single band of the correct size and the yield is good, DNA purification is not necessary.
- If PCR produces multiple products or a smear, it is best to optimize the PCR. If it is not possible to optimize, purify the products using gel extraction. Be careful, however, as gel extraction can introduce guanadine thiocynate (from the gel dissolving buffer) and can reduce the efficiency of the assembly reaction. To minimize this contamination, trim the gel slice so that a smaller amount of gel dissolving buffer is required. Due to the potential for residual guanidine salt being present in fragments isolated by gel-extraction, PCR or DNA column purification (NEB #T1130) is preferable to gel extraction (NEB #T1120).

Use the Correct Amount of DNA

- Make sure you calculate the optimum ratio of insert(s):vector. If the ratio is not ideal, we recommend using NEBioCalculator (**NEBioCalculator.neb.com**) to determine molar amounts.

  For NEBuilder HiFi DNA Assembly:

  2-3 fragments: 15-20 nt overlaps, total DNA = 0.03-0.2 pmol, 2 fold molar excess of each insert:vector

  4-6 fragments: 20-30 nt overlaps, total DNA = 0.2-0.5 pmol, 1:1 molar ratio of each insert:vector

  We recommend using NEBuilder Protocol Calculator (NEBuilderCalculator.neb.com) to generate your customized protocol.

  For NEB Gibson Assembly:

  2-3 fragments: 15-25 nt overlaps, total DNA = 0.02-0.5 pmol, 2-3 fold molar excess of each insert:vector

  4-6 fragments: 20-80 nt overlaps, total DNA = 0.2-1.0 pmol, 1:1 molar ratio of each insert:vector

Perform a PCR Assay to Determine if the Assembly is Successful

- Determine if the assembly works *in vitro* by amplifying the assembled product directly from the assembly reaction. Dilute 1 µl of the assembly reaction with 3 µl water then use 1 µl as a template in a 50 µl PCR. Use primers that anneal to the vector and amplify across the insert. Do not use primers that anneal across the assembly junction because this can lead to false positive results. If you can amplify the assembled product but cannot recover clones by transformation, then the problem is either with the transformation step, or the inability of the cells to maintain the transformed construct due to toxicity.

  Check the reaction conditions, DNA amounts, overlap sequences and perform the assembly control.

Always use High Competency Cells with a Transformation Efficiency of 10^8 – 10^9 cfu/µg

- We recommend NEB 5-alpha High Efficiency Competent *E. coli* (NEB #C2987) or NEB 10-beta High Efficiency Competent *E. coli* (NEB #C3019).

  More information can be found on NEBuilderHiFi.com

How does NEBuilder HiFi DNA Assembly work?

Preparation of Insert and Vectors

Insert From a Plasmid Source

- Digest plasmid with the appropriate restriction enzymes to produce a DNA fragment that can be cloned directly into a vector. Unidirectional cloning is achieved with restriction enzymes that produce non-compatible ends.

Insert From a PCR Product

- Design primers with appropriate restriction sites to clone unidirectionally into a vector
- Addition of 6 bases upstream of the restriction site is sufficient for digestion with most enzymes
- If fidelity is a concern, choose a proofreading polymerase such as Q5 High-Fidelity DNA Polymerase (NEB #M0491)
- Visit **www.NEBPCRPolymerases.com** for additional guidelines for PCR optimization
- Purify PCR product by running the DNA on an agarose gel and excising the band or by using a spin column (e.g., Monarch® Spin DNA Gel Extraction Kit, NEB #T1120, Monarch Spin PCR & DNA Cleanup Kit, NEB #T1130)
- Digest with the appropriate restriction enzyme

Standard Restriction Enzyme Protocol

DNA	1 µg
10X NEBuffer	5 µl (1X)
Restriction Enzyme	10 units is sufficient, generally 1 µl is used
Nuclease-free Water	To 50 µl
Incubation Time	1 hour*
Incubation Temperature	Enzyme dependent

** Can be decreased by using a Time-Saver qualified enzyme*

Time-Saver Restriction Enzyme Protocol

DNA	1 µg
10X NEBuffer	5 µl (1X)
Restriction Enzyme	1 µl
Nuclease-free Water	To 50 µl
Incubation Time	5–15 minutes*
Incubation Temperature	Enzyme dependent

** Time-Saver qualified enzymes can also be incubated overnight with no star activity*

Insert from Annealed Oligos

- Annealed oligos can be used to introduce a fragment (e.g., promoter, polylinker, etc.)
- Anneal two complementary oligos that leave protruding 5´ or 3´ overhangs for ligation into a vector cut with appropriate enzymes
- Non-phosphorylated oligos can be phosphorylated using T4 Polynucleotide Kinase (NEB #M0201)

Typical Annealing Reaction

Oligo 1	20 µM Final concentration
Oligo 2	20 µM Final concentration
NEBuffer r2.1	5 µl
Nuclease-free Water	To 50 µl
Incubation	95°C for 5 minutes, cool slowly to room temp.

Vector

- Digest vector with appropriate restriction enzymes. Enzymes that leave non-compatible ends are ideal as they prevent vector self-ligation

Dephosphorylation

- Dephosphorylation is sometimes necessary to prevent self-ligation. NEB offers four products for dephosphorylation of DNA:
- Quick CIP (NEB #M0525), Shrimp Alkaline Phosphatase (rSAP) (NEB #M0371) and Antarctic Phosphatase (AP) (NEB #M0289) are heat-inactivatable phosphatases. They work in all NEBuffers, but AP requires supplementation with Zn^{2+}.

Dephosphorylation of 5´ ends of DNA using Quick CIP

DNA	1 pmol of DNA ends
10X rCutSmart Buffer	2 µl
Quick CIP	1 µl
Nuclease-free Water	To 20 µl
Incubation	37°C for 10 minutes
Heat Inactivation	80°C for 2 minutes

Note: Scale larger reaction volumes proportionally.

Blunting

- In some instances, the ends of the insert or vector require blunting
- PCR with a proofreading polymerase will leave a predominantly blunt end
- T4 DNA Polymerase (NEB #M0203) or Klenow (NEB #M0210) will fill in a 5´ overhang and chew back a 3´ overhang
- The Quick Blunting Kit (NEB #E1201) is optimized to blunt and phosphorylate DNA ends for cloning in less than 30 minutes
- Analyze agarose gels with longwave UV (365 nM) to minimize UV exposure that may cause DNA damage

Blunting with the Quick Blunting Kit

DNA	Up to 5 µg
10X Blunting Buffer	2.5 µl
dNTP Mix (1 mM)	2.5 µl
Blunt Enzyme Mix	1 µl
Nuclease-free Water	To 25 µl
Incubation	Room temperature; 15 minutes for RE-digested DNA sheared or 30 minutes for nebulized DNA or PCR products*
Heat Inactivation	70°C for 10 minutes

** PCR-generated DNA must be purified before blunting using a purification kit (NEB #T1130), phenol extraction/ethanol precipitation, or gel extraction (NEB #T1120).*

Phosphorylation

- For ligation to occur, at least one of the DNA ends (insert or vector) should contain a 5´ phosphate
- Primers are usually supplied non-phosphorylated; therefore, the PCR product will not contain a 5´ phosphate
- Digestion of DNA with a restriction enzyme will always produce a 5´ phosphate
- A DNA fragment can be phosphorylated by incubation with T4 Polynucleotide Kinase (T4 PNK, NEB #M0201). T4 PNK can be inactivated at 65°C for 20 minutes.

Phosphorylation with T4 PNK

DNA (20 mer)	up to 300 pmol of 5´ termini
10X T4 PNK Buffer	5 µl
10 mM ATP	5 µl (1 mM final conc.)
T4 PNK	1 µl (10 units)
Nuclease-free Water	To 50 µl
Incubation	37°C for 30 minutes

Purification of Vector and Insert

- Purify the vector and insert by either running the DNA on an agarose gel and excising the appropriate bands or by using a spin column, such as Monarch Spin DNA Gel Extraction Kit or Spin PCR & DNA Cleanup Kit (5 µg) (NEB #T1120 or T1130)
- DNA can also be purified using β-Agarase I (NEB #M0392) with low melt agarose, or an appropriate spin column or resin
- Analyze agarose gels with longwave UV (360 nM) to minimize UV exposure that may cause DNA damage

Ligation of Vector and Insert

- Use a molar ratio between 1:1 and 1:10 of vector to insert (1:3 is typical). Use NEBioCalculator to calculate molar ratios.
- If using T4 DNA Ligase (NEB #M0202) or the Quick Ligation Kit (NEB #M2200), thaw and resuspend the Ligase Buffer at room temp. If using Ligase Master Mixes, no thawing is necessary.
- The Quick Ligation Kit (NEB #M2200) is optimized for ligation of both sticky and blunt ends
- Instant Sticky-end Ligase Master Mix (NEB #M0370) is optimized for instant ligation of sticky/cohesive ends
- Blunt/TA Ligase Master Mix (NEB #M0367) is optimized for ligation of blunt or single base overhangs, which are the more challenging type of ends for T4 DNA Ligase
- Following ligation, chill on ice and transform
- DO NOT heat inactivate when using the Quick Ligation Buffer or Ligase Master Mixes, as this will inhibit transformation
- Electroligase (NEB #M0369) is optimized for ligation of both sticky and blunt ends and is compatible with electroporation (i.e., no cleanup step required)
- Improved Golden Gate Assembly can be achieved by selecting high fidelity overhangs [Potapov, V. et al. (2018) *ACS Synth. Biol.* 7(11), 2665–2674.
- Use NEBridge Ligase Fidelity Tools (ligasefidelity.neb.com) for help designing your high-fidelity reactions

1:3 Ligation with the Quick Ligation Kit

Vector DNA (4 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	37.5 ng (0.060 pmol)
2X Quick Ligation Buffer	10 µl
Quick T4 DNA Ligase	1 µl
Nuclease-free Water	20 µl (mix well)
Incubation	Room temperature for 5 minutes

1:3 Ligation with Instant Sticky-end Ligase Master Mix

Vector DNA (4 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	37.5 ng (0.060 pmol)
Master Mix	5 µl
Nuclease-free Water	To 10 µl
Incubation	None

1:3 Ligation with Blunt/TA Ligase Master Mix

Vector DNA (4 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	37.5 ng (0.060 pmol)
Master Mix	5 µl
Nuclease-free Water	To 10 µl
Incubation	Room temperature for 15 minutes

Transformation

- If recombination is a concern, then use the *recA*⁻ strains NEB 5-alpha Competent *E. coli* (NEB #C2987), NEB-10 beta Competent *E. coli* (NEB #C3019) or NEB Stable Competent *E. coli* (NEB #C3040)
- NEB 10-beta Competent *E. coli* works well for constructs larger than 5 kb
- NEB Stable Competent *E. coli* (NEB #C3040) can be used for constructs with repetitive sequences such as lentiviral constructs
- If electroporation is required, use NEB 10-beta (NEB #C3020) Electrocompetent *E. coli*
- Use pre-warmed selection plates
- Perform several 10-fold serial dilutions in SOC or NEB 10-beta/Stable Outgrowth Medium for plating

Transformation with NEB 5-alpha Competent *E. coli*

DNA	1–5 µl containing 1 pg – 100 ng of plasmid DNA
Competent *E. coli*	50 µl
Incubation	On ice for 30 minutes
Heat Shock	Exactly 42°C for exactly 30 seconds
Incubation	On ice for 5 minutes Add 950 µl room temperature SOC 37°C for 60 minutes, with shaking

Troubleshooting Guide for Cloning

We strongly recommend running the following controls during transformations. These controls may assist in identifying which step(s) in the cloning workflow has failed.

- Transform 100 pg – 1 ng of uncut vector to check cell viability, calculate transformation efficiency and verify the antibiotic resistance of the plasmid.
- Transform the cut vector to determine the amount of background due to undigested plasmid. The number of colonies in this control should be < 1% of the number of colonies in the uncut plasmid control transformation (from control #1).
- Transform a vector only ligation reaction. The ends of the vector should not be able to re-ligate because either they are incompatible (e.g., digested with two restriction enzymes that do not generate compatible ends) or the 5´ phosphate group has been removed in a dephosphorylation reaction (e.g., blunt ends treated with rSAP). This control transformation should yield the same number of colonies as control #2.
- Digest vector DNA with a single restriction enzyme, re-ligate and transform. The ends of the vector DNA should be compatible and easily joined during the ligation reaction, resulting in approximately the same number of colonies as control #1.

The cloning workflow often benefits from an accurate quantitation of the amount of DNAs that are being worked with. We recommend quantification of DNAs whenever possible.

Problem	Cause	Solution
Few or no transformants	Cells are not viable	• Transform an uncut plasmid (e.g., pUC19) and calculate the transformation efficiency of the competent cells. • If the transformation efficiency is low (< 10^4) re-make the competent cells or consider using commercially available high efficiency competent cells.
	Incorrect antibiotic or antibiotic concentration	• Confirm antibiotic and antibiotic concentration
	DNA fragment of interest is toxic to the cells	• Incubate plates at lower temperature (25–30°C). • Transformation may need to be carried out using a strain that exerts tighter transcriptional control over the DNA fragment of interest (e.g., NEB 5-alpha F´ I^q Competent *E. coli* (NEB #C2992))
	If using chemically competent cells, the wrong heat-shock protocol was used	• Follow the manufacturer's specific transformation protocol (Note: going above the recommended temperature during the heat shock can result in competent cell death)
	If using electrocompetent cells, PEG is present in the ligation mix	• Clean up DNA prior to transformation with the Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130) • Try NEB's ElectroLigase (NEB #M0369)
	If using electrocompetent cells, arcing was observed or no voltage was registered	• Clean up DNA prior to ligation with the Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130) • Tap the cuvette to get rid of any trapped air bubbles • Be sure to follow the manufacturer's specified electroporation parameters
	Construct is too large	• Select a competent cell strain that can be transformed efficiently with large DNA constructs [≥ 10 kb, we recommend trying NEB 10-beta Competent *E. coli* (NEB #C3019)] or NEB Stable Competent *E. coli* (NEB #C3040) • For very large constructs (> 10 kb), consider using electroporation
	Construct may be susceptible to recombination	• Select a *recA*⁻ strain such as NEB 5-alpha (NEB #C2987), NEB 10-beta (NEB #C3019) or NEB Stable (NEB #C3040) Competent *E. coli*
	The insert comes directly from mammalian or plant DNA and contains methylated cytosines, which are degraded by many *E. coli* strains	• Use a strain that is deficient in McrA, McrBC and Mrr, such as NEB 10-beta Competent *E. coli* (NEB #C3019)
	Too much ligation mixture was used	• Use < 5 µl of the ligation reaction for the transformation
	Inefficient ligation	• Make sure that at least one fragment being ligated contains a 5´ phosphate moiety • Vary the molar ratio of vector to insert from 1:1 to 1:10. Use NEBioCalculator to calculate molar ratios • Purify the DNA to remove contaminants such as salt and EDTA with Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130) • ATP will degrade after multiple freeze-thaws; repeat the ligation with fresh buffer • Heat inactivate or remove the phosphatase prior to ligation • Ligation of single base-pair overhangs (most difficult) may benefit from being carried out with Blunt/TA Master Mix (NEB #M0367), Quick Ligation Kit (NEB #M2200) or concentrated T4 DNA Ligase (NEB #M0202) • Test the activity of the ligase by carrying out a ligation control with Lambda-HindIII digested DNA (NEB #N3012)
	Inefficient phosphorylation	• Purify the DNA prior to phosphorylation with Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130). Excess salt, phosphate or ammonium ions may inhibit the kinase. • If the ends are blunt or 5´ recessed, heat the substrate/buffer mixture for 10 minutes at 70°C. Rapidly chill on ice before adding the ATP and enzyme, then incubate at 37°C. • ATP was not added. Supplement the reaction with 1 mM ATP, as it is required by T4 Polynucleotide Kinase (NEB #M0201) • Alternatively, use 1X T4 DNA Ligase Buffer (NEB #B0202) (contains 1 mM ATP) instead of the 1X T4 PNK Buffer

Troubleshooting Guide for Cloning (continued)

Problem	Cause	Solution
Few or no transformants	Inefficient blunting	• Heat inactivate or remove the restriction enzymes prior to blunting • Clean up the PCR fragment prior to blunting with Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130) • Sonicated gDNA should be blunted for at least 30 minutes • Do not use > 1 unit of enzyme/µg of DNA • Do not incubate for > 15 minutes • Do not incubate at temperatures > 12°C (for T4 DNA Polymerase, NEB #M0203) or > 24°C (for Klenow, NEB #M0210) • Make sure to add a sufficient amount of dNTPs to the reaction (33 µM each dNTP for DNA Polymerase I, Large (Klenow) Fragment, NEB #M0210 and 100 µM each dNTP for T4 DNA Polymerase, NEB #M0203). • When using Mung Bean Nuclease (NEB #M0250), incubate the reaction at room temperature. Do not use > 1 unit of enzyme/µg DNA or incubate the reaction > 30 minutes.
	Inefficient A-Tailing	• Clean up the PCR prior to A-tailing. NEB recommends the Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130). High-fidelity polymerases will remove any non-templated nucleotides.
	Restriction enzyme(s) didn't cleave completely	• Check the methylation sensitivity of the enzyme(s) to determine if the enzyme is blocked by methylation of the recognition sequence • Use the recommended buffer supplied with the restriction enzyme • Clean up the DNA to remove any contaminants that may inhibit the enzyme. NEB recommends the Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130). • When digesting a PCR fragment, make sure to have at least 6 nucleotides between the recognition site and the end of the DNA molecule
Colonies don't contain a plasmid	Antibiotic level used was too low	• Increase the antibiotic level on plates to the recommended amount • Use fresh plates with fresh antibiotics
	Satellite colonies were selected	• Choose large, well-established colonies for analysis
Colonies contain the wrong construct	Recombination of the plasmid has occurred	• Use a *recA*⁻ strain such NEB 5-alpha (NEB #C2987), NEB 10-beta (NEB #C3019) or NEB Stable (NEB #C3040) Competent *E. coli*
	Incorrect PCR amplicon was used during cloning	• Optimize the PCR conditions • Gel purify the correct PCR fragment. NEB recommends the Monarch Spin DNA Gel Extraction Kit (NEB #T1120).
	Internal recognition site was present	• Use NEBcutter to analyze insert sequence for presence of an internal recognition site
	DNA fragment of interest is toxic to the cells	• Incubate plates at lower temperature (25–30°C) • Transformation may need to be carried out using a strain that exerts tighter transcriptional control of the DNA fragment of interest (e.g., NEB 5-alpha F´ I^q Competent *E. coli*) (NEB #C2992)
	Mutations are present in the sequence	• Use a high-fidelity polymerase (e.g., Q5 High-Fidelity DNA Polymerase, NEB #M0491) • Re-run sequencing reactions
Too much background	Inefficient dephosphorylation	• Heat inactivate or remove the restriction enzymes prior to dephosphorylation
	Kinase is present/active	• Heat inactivate the kinase after the phosphorylation step. Active kinase will re-phosphorylate the dephosphorylated vector.
	Restriction enzyme(s) didn't cleave completely	• Check the methylation sensitivity of the restriction enzyme(s) to be sure it is not inhibited by methylation of the recognition sequence • Use the recommended buffer supplied with the restriction enzyme • Clean up the DNA to remove contaminants. (e.g., too much salt). NEB recommends the Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130).
	Antibiotic level is too low	• Confirm the correct antibiotic concentration
Ran the ligation on a gel and saw no ligated product	Inefficient ligation	• Make sure at least one DNA fragment being ligated contains a 5´ phosphate • Vary the molar ratios of vector to insert from 1:1 to 1:10. Use NEBioCalculator to calculate molar ratios. • Purify the DNA to remove contaminants such as salt and EDTA. NEB recommends the Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130). • ATP will degrade after multiple freeze-thaws; repeat the ligation with fresh buffer • Heat inactivate or remove the phosphatase prior to ligation • Ligation of single base-pair overhangs (most difficult) may benefit from being carried out with Blunt/TA Master Mix (NEB #M0367), Quick Ligation Kit (NEB #M2200) or concentrated T4 DNA Ligase (NEB #M0202) • Test the activity of the ligase by carrying out a ligation control with Lambda-HindIII digested DNA (NEB #N3012)
The ligated DNA ran as a smear on an agarose gel	The ligase is bound to the substrate DNA	• Treat the ligation reaction with Proteinase K (NEB #P8107) prior to running on a gel
The digested DNA ran as a smear on an agarose gel	The restriction enzyme(s) is bound to the substrate DNA	• Lower the number of units • Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the DNA or use Gel Loading Dye, Purple (6X) (NEB #B7024)
	Nuclease contamination	• Use fresh, clean running buffer • Use a fresh agarose gel • Clean up the DNA. NEB recommends Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130).

Troubleshooting Guide for Cloning (continued)

Problem	Cause	Solution
Incomplete restriction enzyme digestion	Cleavage is blocked by methylation	• DNA isolated from a bacterial source may be blocked by Dam and Dcm methylation • DNA isolated from eukaryotic source may be blocked by CpG methylation • If the enzyme is inhibited by Dam or Dcm methylation, grow the plasmid in a *dam-/dcm-* strain (NEB #C2925) or use PCR DNA
	Salt inhibition	• Enzymes that have low activity in salt-containing buffers (NEBuffer r3.1) may be salt sensitive, so clean up the DNA prior to digestion. NEB recommends the Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130). • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. Monarch Spin kits (NEB #T1110, #T1120, #T1130) use columns that have been designed to minimize salt carry over into the eluted DNA, so using them can minimize this issue. To prevent this, DNA solution should be no more than 25% of total reaction volume
	Inhibition by PCR components	• Clean up the PCR fragment prior to restriction digest. NEB recommends Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130).
	Using the wrong buffer	• Use the recommended buffer supplied with the restriction enzyme
	Too few units of enzyme used	• Use at least 5–10 units of enzyme per µg of DNA
	Incubation time was too short	• Increase the incubation time
	Digesting supercoiled DNA	• Some enzymes have a lower activity on supercoiled DNA. Increase the number of enzyme units in the reaction.
	Presence of slow sites	• Some enzymes can exhibit slower cleavage towards specific sites. Increase the incubation time, 1–2 hours is typically sufficient.
	Two sites required	• Some enzymes require the presence of two recognition sites to cut efficiently. For more information, visit the table "Restriction Enzymes Requiring Multi-sites" on neb.com.
	DNA is contaminated with an inhibitor	• Assay substrate DNA in the presence of a control DNA. Control DNA will not cleave if there is an inhibitor present. Miniprep DNA is particularly susceptible to contaminants. NEB recommends the Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130). • Clean DNA with a spin column, we recommend Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130), or increase volume to dilute contaminant
Extra bands in the gel	If larger bands than expected are seen in the gel, this may indicate binding of the enzyme(s) to the substrate	• Lower the number of units in the reaction • Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the substrate or add Gel Loading Dye, Purple (6X) (NEB #B7024)
	Star activity	• Use the recommended buffer supplied with the restriction enzyme • Decrease the number of enzyme units in the reaction • Make sure the amount of enzyme added does not exceed 10% of the total reaction volume. This ensures that the total glycerol concentration does not exceed 5% v/v • Decrease the incubation time. Using the minimum reaction time required for complete digestion will help prevent star activity. • Try using a High-Fidelity (HF) restriction enzyme. HF enzymes have been engineered for reduced star activity.
	Partial restriction enzyme digest	• Enzymes that have low activity in salt-containing buffers (e.g., NEBuffer r3.1) may be salt sensitive. Make sure to clean up the DNA prior to digestion. NEB recommends Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130). • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. Monarch Spin kits (NEB #T1110, #T1120, #T1130) use columns that have been designed to minimize salt carry over into the eluted DNA, so using them can minimize this issue. To prevent this, DNA solution should be no more than 25% of total reaction volume • Clean-up the PCR fragment prior to restriction digest. NEB recommends Monarch Spin PCR & DNA Cleanup Kit (NEB #T1130). • Use the recommended buffer supplied with the restriction enzyme • Use at least 3–5 units of enzyme per g of DNA • Digest the DNA for 1–2 hours
No PCR fragment amplified	Used the wrong primer sequence	• Double check the primer sequence
	Incorrect annealing temperature	• Use the NEB Tm calculator to determine the correct annealing temperature (www.neb.com/TmCalculator)
	Incorrect extension temperature	• Each polymerase type has a different extension temperature requirement. Follow the manufacturer's recommendations.
	Too few units of polymerase	• Use the recommended number of polymerase units based on the reaction volume
	Incorrect primer concentration	• Each polymerase has a different primer concentration requirement. Make sure to follow the manufacturer's recommendations.
	Mg^{2+} levels in the reaction are not optimal	• Titrate the Mg^{2+} levels to optimize the amplification reaction. Follow the manufacturer's recommendations.
	Difficult template	• With difficult templates, try different polymerases and/or buffer combinations
The PCR reaction is a smear on a gel	If bands are larger than expected it may indicate binding of the enzyme(s) to the DNA	• Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the DNA
Extra bands in PCR reaction	Annealing temperature is too low	• Use the NEB Tm calculator to determine the annealing temperature of the primers
	Mg^{2+} levels in the reaction are not optimal	• Titrate the Mg^{2+} levels to optimize the amplification reaction. Make sure to follow the manufacturer's recommendations.
	Additional priming sites are present	• Double check the primer sequence and confirm it does not bind elsewhere in the DNA template
	Formation of primer dimers	• Primer sequence may not be optimal. Additional primers may need to be tested in the reaction.
	Incorrect polymerase choice	• Try different polymerases and/or buffer combinations

Optimization Tips for Your Cloning Reactions

New England Biolabs offers a wide selection of reagents for your cloning experiments. For more information, visit **ClonewithNEB.com**. The following tips can be used to help optimize each step in your cloning workflow. Tips for restriction enzyme digestion and amplification can be found earlier in the technical reference section, or at www.neb.com.

cDNA Synthesis

Starting Material

- Intact RNA of high purity is essential for generating cDNA for cloning applications
- Total RNA or mRNA can be used in the reverse transcription reaction. Total RNA is generally sufficient for cDNA synthesis reactions. However, if desired, mRNA can be easily obtained using a PolyA Spin mRNA Isolation Kit (NEB #S1560) or Magnetic mRNA Isolation Kit (NEB #S1550).
- The amount of RNA required for cDNA cloning depends on the abundance of the transcript-of-interest. In general, 1 ng to 1 µg total RNA or 0.1–100 ng mRNA are recommended.

Product Selection

- Streamline your reaction setup by using the ProtoScript II First Strand cDNA Synthesis Kit (NEB #E6560). This kit combines ProtoScript II Reverse Transcriptase (NEB #M0360), a thermostable M-MuLV (RNase H^-) Reverse Transcriptase, and recombinant RNase Inhibitor in an enzyme Master Mix, along with a separate Reaction Mix containing dNTPs. Additionally, the kit contains two optimized reverse transcription primer mixes.

Yield

- ProtoScript II Reverse Transcriptase is capable of generating cDNA of more than 10 kb up to 48°C. We recommend 42°C for routine reverse transcription.
- You can increase the yield of a long cDNA product by doubling the amount of enzyme and dNTPs

Additives

- For most RT-PCR reactions, RNase H treatment is not required. But for some difficult amplicons or sensitive assays, add 2 units of *E. coli* RNase H to the reaction and incubate at 37°C for 20 minutes

Phosphorylation

Enzyme

- T4 Polynucleotide Kinase (NEB #M0201) and T4 DNA Ligase (NEB #M0202) can be used together in the T4 DNA Ligase Buffer
- T4 Polynucleotide Kinase is inhibited by high levels of salt (50% inhibition by 150 mM NaCl), phosphate (50% inhibition by 7 mM phosphate) and ammonium ions (75% inhibited by 7 mM $(NH_4)_2SO_4$)
- If using T4 Polynucleotide Kinase and working with 5´-recessed ends, heat the reaction mixture for 10 min at 70°C, chill rapidly on ice before adding the ATP (or Ligase Buffer containing ATP) and enzyme, then incubate at 37°C

Additives

- The addition of PEG 8000 (up to 5%) can improve results

Dephosphorylation

Enzyme

- When dephosphorylating a fragment following a restriction enzyme digest, a DNA clean up step is required if the restriction enzyme(s) used is NOT heat inactivatable. We recommend the Monarch Spin PCR & DNA Cleanup Kit (5 µg) (NEB #T1130).
- When working with Quick CIP (NEB #M0525), rSAP (NEB #M0371) or AP (NEB #M0289), which are heat-inactivatable enzymes, a DNA clean-up step after dephosphorylation is not necessary prior to the ligation step.

Additives

- AP requires the presence of Zn^{2+} in the reaction, so don't forget to supplement the reaction with 1X Antarctic Phosphatase Reaction Buffer when using other NEBuffers

Blunting/End Repair

Enzyme

- Make sure that you choose the correct enzyme to blunt your fragment. The Quick Blunting Kit (NEB #E1201), T4 DNA Polymerase (NEB #M0203) and DNA Polymerase I, Large (Klenow) Fragment (NEB #M0210) will fill 5´ overhangs and degrade 3´ overhangs. Mung Bean Nuclease (NEB #M0250) degrades 5´ overhangs.
- T4 DNA Polymerase and DNA Polymerase I, Large (Klenow) Fragment are active in all NEBuffers. Please remember to add dNTPs.

Clean-up

- When trying to blunt a fragment after a restriction enzyme digestion, if the restriction enzyme(s) used are heat inactivatable, then a clean-up step prior to blunting is not needed. Alternatively, if the restriction enzyme(s) used are not heat inactivatable, a DNA clean-up step is recommended prior to blunting.
- When trying to blunt a fragment amplified by PCR, a DNA clean-up step is necessary prior to the blunting step to remove the nucleotides and polymerase
- When trying to dephosphorylate a fragment after the blunting step, you will need to add a DNA clean-up step (e.g., Monarch Spin PCR & DNA Cleanup Kit, NEB #T1130) after the blunting and before the addition of the phosphatase

Temperature

- When trying to blunt a fragment with Mung Bean Nuclease, the recommended temperature of incubation is room temperature, since higher temperatures may cause sufficient breathing of the dsDNA ends that the enzyme may degrade some of the dsDNA sequence. The number of units to be used and time of incubation may be determined empirically to obtain best results.

Heat Inactivation

- Mung Bean Nuclease reactions should not be heat inactivated. Although Mung Bean Nuclease can be inactivated by heat, this is not recommended because the DNA begins to "breathe" before the Mung Bean Nuclease is inactivated and undesirable degradation occurs at breathing sections. Purify DNA by phenol/chloroform extraction and ethanol precipitation or spin column purification [e.g., Monarch Spin PCR & DNA Cleanup Kit (5 µg) (NEB #T1130).

Optimization Tips for Your Cloning Reactions (continued)

A-tailing

- If the fragment to be tailed has been amplified with a high-fidelity polymerase, the DNA needs to be purified prior to the tailing reaction. For this we recommend the Monarch Spin PCR & DNA Cleanup Kit, NEB #T1130). Otherwise, any high-fidelity polymerase present in the reaction will be able to remove any non-templated nucleotides added to the end of the fragments.

DNA Ligation

Reaction Buffers

- T4 DNA Ligase Buffer (NEB #B0202) should be thawed on the bench or in the palm of your hand, and not at 37°C (to prevent breakdown of ATP)
- Once thawed, T4 DNA Ligase Buffer should be placed on ice
- Ligations can be performed in any of the four standard restriction endonuclease NEBuffers or in T4 Polynucleotide Kinase Buffer (NEB #B0201) supplemented with 1 mM ATP
- When supplementing with ATP, use ribo-ATP (NEB #P0756). Deoxyribo-ATP will inhibit ligation.
- Before ligation, completely inactivate restriction enzyme by heat inactivation, spin column (e.g., Monarch Spin PCR & DNA Cleanup Kit, NEB #T1130) or Phenol/EtOH purification

DNA

- Heat inactivate (AP, rSAP, Quick CIP) before ligation
- Keep total DNA concentration between 5–10 µg/ml
- Vector:Insert molar ratios between 1:1 and 1:10 are optimal for single insertions. Use NEBioCalculator at **NEBioCalculator.neb.com** to calculate molar ratios.
- For cloning more than one insert, we recommend the NEBuilder® HiFi DNA Assembly Master Mix (NEB #E2621) or Cloning Kit (NEB #E5520)
- If you are unsure of your DNA concentration, perform multiple ligations with varying ratios

Ligase

- For cohesive-end ligations, standard T4 DNA Ligase. Instant Sticky-end Ligase Master Mix or the Quick Ligation Kit are recommended.
- For blunt and single-base overhangs the Blunt/TA Ligase Master Mix is recommended
- For ligations that are compatible with electroporation, ElectroLigase is recommended
- Standard T4 DNA Ligase can be heat inactivated at 65°C for 20 minutes
- Do not heat inactivate the Quick Ligation Kit or the ligase master mixes

Transformation

- Add between 1–5 µl of ligation mixture to competent cells for transformation
- Extended ligation with PEG causes a drop off in transformation efficiency
- Electroporation is recommended for larger constructs (> 10,000 bp). Dialyze samples or use a spin column first if you have used the Quick Ligation Kit or ligase master mixes.
- ElectroLigase is recommended for ligations that will be electroporated

Transformation

Thawing

- Cells are best thawed on ice
- DNA should be added as soon as the last trace of ice in the tube disappears
- Cells can be thawed by hand, but warming decreases efficiency

DNA

- For best results, the volume of transformed DNA should not exceed 10% of the total volume of cells

Incubation & Heat Shock

- Incubate on ice for 30 minutes. Expect a 2-fold loss in transformation efficiency for every 10 minutes this step is shortened.
- Both temperature and time are specific to the transformation volume and vessel. Typically, 30 seconds at 42°C is recommended, except when using BL21 (NEB #C2530) which requires exactly 10 seconds.

Outgrowth

- Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in transformation efficiency for every 15 minutes this step is shortened.
- SOC and NEB 10-beta/Stable Outgrowth Medium give 2-fold higher transformation efficiency than LB medium
- Incubation with shaking or rotation results in 2-fold higher transformation efficiency

Plating

- Selection plates can be used warm or cold, wet or dry with no significant effects on transformation efficiency
- Warm, dry plates are easier to spread and allow for the most rapid colony formation

DNA Contaminants to Avoid

Contaminant	Removal Method
Detergents	Ethanol precipitate
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspending
PEG	Column purify (e.g., Monarch Spin PCR & DNA Cleanup Kit (5 µg)) or phenol/chloroform extract and ethanol precipitate

Tips for Plasmid DNA Purification

The following tips can be used to help optimize your plasmid purification when using our Monarch Spin Plasmid Miniprep Kit (NEB #T1110).

1 Sample Preparation

Grow your bacterial culture overnight to obtain a sufficient quantity of cells containing the plasmid DNA.

DO

- Use a fresh growth media plate and antibiotic.
- Inoculate growth media from a single colony.
- Ensure you use the proper antibiotic at the correct concentration.
- Harvest the culture during the transition from logarithmic growth to stationary phase (typically 12–16 hours for growth in LB medium).

DON'T

- ProtoScript II Reverse Transcriptase is capable of generating cDNA of more than 10 kb up to 48°C. We recommend 42°C for routine reverse transcription.
- You can increase the yield of a long cDNA product by doubling the amount of enzyme and dNTPs

2 Resuspend

Resuspend the bacterial pellet thoroughly in a buffer solution to prepare the cells for the lysis step.

DO

- If you are working with a low-copy plasmid, increase the number of cells processed and scale up the buffers accordingly.
- Ensure the cell pellet is completely resuspended before adding the Lysis Buffer.
- If using the Monarch Spin Plasmid Miniprep Kit, be sure to add RNase A to Buffer B1 to avoid RNA contamination.

DON'T

- Don't use more cells than recommended (up to 5 ml, equivalent to 15 OD units), as this can result in inefficient cell lysis and clogging of the matrix.

3 Lyse

Lyse the bacterial cells by adding a lysis buffer, which releases the plasmid DNA into the solution.

DO

- Confirm the color change from light pink to dark pink and transparent during the lysis process
- Promptly move on to the neutralization step after the lysis step to prevent plasmid denaturation.
- Mix carefully by inversion after cell lysis to avoid host chromosomal DNA contamination.

DON'T

- Don't extend the incubation time (ideally, no more than 2 minutes) in the presence of sodium hydroxide (in the Monarch Buffer B2 Lysis Buffer) during the lysis step, as this can separate the DNA strands and irreversibly denature the plasmid.
- Don't mix vigorously or vortex after cell lysis and before pelleting cell debris, as this can shear the host chromosomal DNA and contaminate the plasmid. Note: if your prep contains contaminating genomic DNA that was sheared during lysis, it may co-purify with the plasmid and will likely appear as a high-molecular-weight band on an agarose gel.

4 Neutralize

Neutralize the lysate with a neutralization buffer, allowing gDNA, proteins and other cellular debris to precipitate out, while the plasmid remains in the solution.

DO

- If RNase A was added to the buffer in the Resuspension step, ensure the sample is well mixed and incubate for the recommended time to allow for RNA degradation. For the Monarch Spin Plasmid Miniprep Kit, incubate for 2 minutes.
- Gently invert the sample tube enough times to ensure a complete and uniform color change to yellow.
- Make sure cell debris appears in abundance and is fully compacted into the pellet after centrifugation.

5 Bind

Bind the plasmid DNA to a silica column by passing the lysate through it, allowing the plasmid DNA to adhere to the column.

DO

- Only transfer the supernatant to the column. Make sure the lysate is free of cellular debris before applying it to the column to avoid clogging.
- Use the correct volume of lysate recommended by the kit to ensure optimal binding of plasmid DNA.

DON'T

- Do not overload the column. For the Monarch Spin Plasmid Miniprep kit, the maximum column loading volume is 800 µl. If the supernatant volume is > 800 µl, load the first 800 µl, spin through, discard flow-through and reload the remaining volume.
- Don't hurry the binding step; insufficient contact time can lead to poor DNA recovery.

6 Wash

Wash the bound DNA on the column with a wash buffer to remove any remaining contaminants and impurities.

DO

- Ensure the final wash spin time is 1 minute to enable the complete removal of the wash buffer.
- Be aware that strains like HB101 and the JM series have high amounts of endogenous carbohydrates that can interfere with downstream enzymatic manipulations of plasmid DNA. To remove excessive carbohydrates, be sure to include the first wash step with the Monarch Buffer BZ to help reduce plasmid degradation, and keep the samples on ice during preparation.

DON'T

- For the Monarch Spin Plasmid Miniprep Kit, don't skip any wash steps in the protocol.

7 Elute

Elute the purified plasmid DNA from the column by adding an elution buffer and collecting the eluate.

DO

- Use the recommended elution volumes and incubation times for typical plasmids in the < 15 kb range. Larger elution volumes and longer incubation times can increase the yield, but the sample will be more dilute.
- To improve the yield for larger plasmids, incubate the column at room temperature for 5 minutes or heat the elution buffer to 50°C before adding.
- Add the elution buffer to the center of the matrix to ensure the matrix becomes evenly wet.
- If eluting in water instead of elution buffer, be sure the water is nuclease-free and the pH is between 7-8.5. Milli-Q™ water is often slightly acidic, requiring pH adjustment. If you are storing the DNA long-term, we recommend using the supplied DNA elution buffer, which contains 0.1 mM EDTA and can help inhibit metal-dependent nucleases.
- Store the DNA at -20°C to ensure its stability, if it will not be used immediately. Consider enzymatically removing contaminating genomic DNA using Exonuclease V (RecBCD) (NEB #M0345).

DON'T

- Don't use smaller elution volumes or shorter incubation times than recommended. This can yield highly concentrated DNA, but can result in incomplete elution and low DNA yield.
- Don't store DNA in a solution containing magnesium, as this can degrade the DNA.
- Don't let the column tip contact the flow-through during the transfer to a new tube. If in doubt about ethanol carryover, re-spin the column for an additional 1 minute.

Tips for Successful DNA Gel Extraction

The following tips can be used to help optimize your DNA gel extraction when using our Monarch Spin DNA Gel Extraction Kit (NEB #T1120).

1 Dissolve Your Gel Slice Completely

Dissolving the gel completely is essential for high DNA recovery, as incomplete dissolution can lead to clogging of the column. Agarose gels using standard laboratory grade or low-melt agarose are compatible up to concentrations of 4%. Tris-Acetate-EDTA (TAE) buffer is a commonly used running buffer, especially if you're planning to use the DNA for downstream experiments; however, Tris-Borate-EDTA (TBE) buffer can also be used.

DO

- Use a clean, sharp blade to make precise cuts in the gel.
- Trim the excess gel as much as possible! Minimizing the gel surrounding your band will reduce the volume of binding buffer required and increase your DNA yield.
- Minimize the duration of DNA exposure to UV light, as prolonged exposure can damage the DNA.
- Ensure the gel is fully submerged in the buffer and incubate at 50°C for 5–10 minutes. Vortexing the tube occasionally can help accelerate the melting process.
- Extend the incubation time if a lower temperature and/or higher concentration of agarose gel is used (> 2%) to ensure complete dissolving of the gel.
- Extend the incubation time for particularly large or thick slices to ensure complete dissolution.

DON'T

- Don't store the gel slice for long periods before extraction. The gel slice can be stored in a closed microfuge tube at 4°C for up to 3 days; however, the best results will be achieved if you extract your band immediately following excision.

2 Bind Your DNA Efficiently

To improve the binding step, closely monitor your column capacity, centrifugation time, and temperature.

DO

- Let the solution cool to room temperature before proceeding.
- After loading the dissolved gel and buffer mixture on the column, spin it at 16,000 x g for a full minute to ensure the DNA binds effectively to the column matrix.

DON'T

- Don't overload the column by adding more than 800 µl or > 5 µg. If using the Monarch Spin DNA Gel Extraction Kit (#T1120), the maximum binding capacity of the column is 5 µg of purified DNA. The size recovery of DNA ranges from 50 bp to 25 kb.

3 Wash Your DNA Thoroughly

Thorough washing is key to achieving high-purity DNA.

DO

- Use the provided wash buffer to rinse the column twice to ensure efficient removal of any residual salt carry-over.
- Follow each wash with a full-speed spin to remove any residual contaminants.
- After the final wash, spin the column for an additional minute to remove all traces of ethanol. Additional centrifugation is unnecessary if using Monarch Spin DNA Gel Extraction Kit (#T1120). Note: The wash buffer, Monarch Buffer WZ, is an ethanol-based buffer designed for optimal DNA purity.

DON'T

- Don't let the tip of the column touch the flow-through. If in doubt, spin again to remove all residual wash buffer.

4 Elute Carefully

Elution is a critical step in the recovery of your DNA. The optimal range for elution volume is 5-20 µl of elution buffer.

DO

- Use the elution buffer in the kit and pre-warm it to 50°C to increase yield.
- Ensure the column is free of residual wash buffers before adding the elution buffer.
- Apply the buffer directly to the center of the column matrix.
- For long-term storage of the DNA, we recommend using the supplied elution buffer. Note: The Monarch Spin DNA Gel Extraction Kit contains the Monarch Buffer EY (10 mM Tris, 0.1 mM EDTA, pH 8.5).
- Use modified elution methods to increase the recovery of longer DNA, such as a heated elution buffer (50°C) or incubating at room temperature for 5 minutes after adding elution buffer. Longer DNA fragments bind tighter to the matrix, which may result in inefficient elution without these modifications.

DON'T

- Don't shorten or skip the incubation - incubate for a full minute.
- Don't store the sample for an extended amount of time if you have eluted it in water rather than an elution buffer. Note: If eluting in water, for maximum elution efficiency, ensure the water is nuclease-free and has a pH between 7 to 8.5. Milli-Q™ water is often slightly acidic, requiring pH adjustment before it can be used for elution.

5 Monitor Your Yield and Purity

Thorough washing is key to achieving high-purity DNA.

After elution, you can use a spectrophotometer to measure the concentration and purity of your DNA. This ensures that your sample is ready for downstream applications without any contaminants that could interfere with your experiments.

Carried-over salts will be indicated by a low $A_{260/230}$ ratio, which is why the column tip must not touch the flow through.

If you observe a faint additional band running below the expected size on a gel, it may be due to DNA denaturation. Chaotropic agents used in silica-based DNA purification can induce DNA denaturation, causing single-stranded forms of DNA to have faster mobility in a gel. To renature your sample, add NaCl to 10 mM and heat the sample to 95°C for one minute, then slowly cool to room temperature.

Troubleshooting Guide for DNA Cleanup & Plasmid Purification Using Monarch® Spin Kits

Problem	Product	Possible Cause	Solution
No DNA purified	Monarch Spin Plasmid Miniprep Kit (NEB #T1110)	Buffers added incorrectly	• Add buffers in the correct order so that the sample is bound, washed and eluted in the correct sequence • Ensure ethanol was added to wash buffer
		Plasmid loss during culture growth	• Ensure proper antibiotic and concentration was used to maintain selection during culture growth
	Monarch Spin DNA Gel Extraction Kit (NEB #T1120)	Ethanol not added to wash buffer	• Ensure the proper amount of ethanol was added to wash buffer
	Monarch Spin PCR & DNA Cleanup Kit (5 µg) (NEB #T1130)		
Low DNA yield	Monarch Spin Plasmid Miniprep Kit (NEB #T1110)	Incomplete lysis	• Pellet must be completely resuspended before addition of lysis buffer (B2) – color should change from light to dark pink • Avoid using too many cells; this can overload the column. If culture volume is larger than recommended, scale up buffers B1-B3.
		Plasmid loss during culture growth	• Ensure proper antibiotic and concentration was used to maintain selection during culture growth
		Low-copy plasmid selected	• Increase amount of cells processed and scale buffers accordingly • Review our guidance for working with low copy plasmids
		Lysis of cells during growth	• Harvest culture during transition from logarithmic growth to stationary phase (~12-16 hours)
		Incomplete neutralization	• Invert tube several times until color changes to a uniform yellow color
		Incomplete elution	• Deliver elution buffer directly to center of column • Larger elution volumes and longer incubation times can increase yield • For elution of plasmids > 10 kb, heat the DNA elution buffer to 50°C and extend incubation time to 5 minutes
	Monarch Spin DNA Gel Extraction Kit (NEB #T1120)	Reagents added incorrectly	• Be sure that buffers have been reconstituted correctly and that reagents have been added in the correct order
		Gel slice not fully dissolved	• Undissolved agarose may clog the column and interfere with binding. Incubate in gel dissolving buffer for proper time and temperature.
		Gel dissolved above 60°C	• Dissolve gel slice in specified range (37-55°C). Higher temperatures can denature DNA
		Incomplete elution during preparation	• Deliver elution buffer directly to center of column • Larger elution volumes and longer incubation times can increase yield • For elution of DNA > 10 kb, heat the elution buffer to 50°C and extend incubation time to 5 minutes • Multiple rounds of elution can also be performed
	Monarch Spin PCR & DNA Cleanup Kit (5 µg) (NEB #T1130)	Reagents added incorrectly	• Be sure that buffers have been reconstituted correctly and that reagents have been added in the correct order
		Incomplete elution during preparation	• Deliver elution buffer directly to center of column • Larger elution volumes and longer incubation times can increase yield • For elution of DNA > 10 kb, heat the elution buffer to 50°C and extend incubation time to 5 minutes • Multiple rounds of elution can also be performed
Low DNA quality	Monarch Spin Plasmid Miniprep Kit (NEB #T1110)	Plasmid degradation	• Be cautious of strains with high levels of endogenous endonuclease (e.g., HB101 and JM 100 series)
		Plasmid is denatured	• Limit incubation with plasmid lysis buffer (B2) to two minutes, as NaOH in the buffer can denature the plasmid
		gDNA contamination	• Use careful inversion mixing after cell lysis to avoid shearing of host cell chromosomal DNA. Do not vortex.
		RNA contamination	• Incubate sample in neutralization buffer for the full 2 minutes. For cell culture volumes > 3 ml, increase the spin after neutralization to 5 minutes.
		Improper storage	• Elute DNA in DNA elution buffer or nuclease-free water, and store at -20°C. Do not store in solutions containing magnesium.
Low DNA purity	Monarch Spin Plasmid Miniprep Kit (NEB #T1110)	Ethanol has been carried over	• Centrifuge final wash for 1 minute to ensure complete removal • Ensure column tip does not come in contact with flow through
		Excessive salt in sample	• Use both plasmid wash buffers and do not skip wash steps
		Excessive carbohydrate has been carried over	• Avoid strains with high amounts of endogenous carbohydrate (e.g., HB101 and JM 100 series). Be sure to follow protocol and include plasmid wash buffer.
	Monarch Spin DNA Gel Extraction Kit (NEB #T1120)	Gel stain not fully dissolved	• Undissolved agarose may leach salts into the eluted DNA
		Ethanol has been carried over	• Centrifuge final wash for 1 minute to ensure complete removal • Ensure column tip does not come in contact with flow through
		Trace amounts of salts have been carried over	• Ensure column tip does not come in contact with new tube for elution
	Monarch Spin PCR & DNA Cleanup Kit (5 µg) (NEB #T1130)	Ethanol has been carried over	• Centrifuge final wash for 1 minute to ensure complete removal • Ensure column tip does not come in contact with flow through
		Trace amounts of salts have been carried over	• Ensure column tip does not come in contact with new tube

Guidelines for Choosing Sample Input Amounts When Using the Monarch® Spin gDNA Extraction Kit

Genomic DNA yield, purity and integrity vary immensely based on sample type, input amount and sample condition. Below, we have provided some empirical yield and DIN data from a wide variety of sample types, as well as guidance on the maximal input amounts for each of those samples when using the Monarch Spin gDNA Extraction Kit (NEB #T3010). It is very important not to overload the column and the buffer system when extracting and purifying gDNA, as DNA yields, purity, integrity, and length may suffer.

Sample Type	Recommended Input Amount	Typical Yield (µg)	DIN	Maximum Input Amount
Tissue*				
Tail (mouse)	10 mg	12–20	8.5–9.5	25 mg
Ear (mouse)	10 mg	18–21	8.5–9.5	10 mg
Liver (mouse and rat)	10 mg	15–30	8.5–9.5	15 mg
Kidney (mouse)	10 mg	10–25	8.5–9.5	10 mg
Spleen (mouse)	10 mg	30–70	8.5–9.5	10 mg
Heart (mouse)	10 mg	9–10	8.5–9.5	25 mg
Lung (mouse)	10 mg	14–20	8.5–9.5	15 mg
Brain (mouse and rat)	10 mg	4–10	8.5–9.5	12 mg
Muscle (mouse and rat)	10 mg	4–7	8.5–9.5	25 mg
Muscle (deer)	10 mg	5	8.5–9.5	25 mg
Blood**				
Human (whole)	100 µl	2.5–4	8.5–9.5	100 µl
Mouse	100 µl	1–3	8.5–9.5	100 µl
Rabbit	100 µl	3–4	8.5–9.5	100 µl
Pig	100 µl	3.5–5	8.5–9.5	100 µl
Guinea pig	100 µl	3–8	8.5–9.5	100 µl
Cow	100 µl	2–3	8.5–9.5	100 µl
Horse	100 µl	4–7	8.5–9.5	100 µl
Dog	100 µl	2–4	8.5–9.5	100 µl
Chicken (nucleated)	10 µl	30–45	8.5–9.5	10 µl
Cells				
HeLa	1 x 10^6 cells	7–9	9.0–9.5	5 x 10^6 cells
HEK293	1 x 10^6 cells	7–9	9.0–9.5	5 x 10^6 cells
NIH3T3	1 x 10^6 cells	6–7.5	9.0–9.5	5 x 10^6 cells
Bacteria				
E. coli (gram-negative)	2 x 10^9 cells	6–10	8.5–9.0	2 x 10^9 cells
Rhodobacter sp. (gram-negative)	2 x 10^9 cells	6–10	8.5–9.0	2 x 10^9 cells
B. cereus (gram-positive)	2 x 10^9 cells	6–9	8.5–9.0	2 x 10^9 cells
Archaea				
T. kodakarensis	2 x 10^9 cells	3–5	8.5–9.0	2 x 10^9 cells
Yeast				
S. cerevisiae	5 x 10^7 cells	0.5–0.6	8.5–9.0	5 x 10^7 cells
Saliva/buccal cells***				
Saliva (human)	200 µl	2–3	7.0–8.0	500 µl
Buccal swab (human)	1 swab	5–7	6.0–7.0	1 swab

* Tissue gDNA yields are shown for frozen tissue powder, frozen tissue pieces and RNAlater-stabilized tissue pieces. Though frozen tissue powder results in highly-intact gDNA, lower yields can be expected than when using frozen or RNAlater-stabilized tissue pieces. Residual nuclease activity in tissue pieces will cut the gDNA, resulting in a slightly smaller overall size; however, this gDNA is optimal for silica-based purification.

** Human whole blood samples stabilized with various anticoagulants (e.g., EDTA, citrate and heparin) and various counter-ions were evaluated and results were comparable in all cases. Additionally, all indicated blood samples were tested both as fresh and frozen samples, yielding comparable results. Human samples were donated by healthy individuals; yields from unhealthy donors may differ.

***Buccal swabs and saliva samples partially consist of dead cell material with degraded gDNA. Therefore, the purified gDNA from those samples will naturally have lower DIN values.

Guidelines for Handling Tissue Samples When Using the Monarch® Spin gDNA Extraction Kit

In general, tissue samples should be processed immediately. If processing of the tissue samples is delayed for several hours, the quality of the isolated gDNA will be lower, particularly for metabolically active organ tissues. In many cases, tissue samples need to be stabilized before genomic DNA purification can be performed. Adequate sample storage can be carried out in one of the following ways:

- Flash frozen tissue samples are stored as whole pieces at -80°C.
- Flash frozen tissue samples are pulverized under liquid nitrogen and subsequently stored at -80°C as tissue powder.
- Tissue samples are incubated with stabilizing agents like RNAlater (Thermo Fisher Scientific) to enable transport at room temperature or on ice, or to enable safe mid-term storage at 4°C or -20°C. Additionally, cutting and preparing aliquots of stabilized samples is significantly more convenient than using fresh or frozen samples.

Below is a list of recommendations for preparing tissue samples from each of the 3 options mentioned above.

Fresh & Frozen Tissue Pieces

- Keep fresh samples on ice and frozen samples frozen (e.g. by storing on dry ice). Label and pre-cool reaction tubes on ice or a cooling block.
- Do not use more tissue than recommended (See "Choosing Input Amounts").

Fresh Tissue

- Cut appropriately-sized tissue fragment into small pieces and weigh out the exact amount by transferring small tissue pieces into reaction tube positioned on a micro balance.
- Keep tubes cold and start lysis as soon as possible.

Frozen Tissue

- Use a clean, frozen cooling block or the bottom side of a frozen metal reaction tube stand for cutting frozen tissue into smallest possible pieces. Samples are most easily cut when they are processed shortly before thawing.
- Weigh the desired amount by transferring small tissue pieces into a pre-chilled reaction tube positioned on a micro balance.
- Keep tubes frozen or on ice, and start lysis as soon as possible. In samples that have been frozen, ice crystals have destroyed cell structures and nucleases have free access to the genomic DNA. Work with the smallest possible tissue pieces to allow for a rapid inactivation of nucleases by Proteinase K. Make sure all tissue pieces are able to move freely in the lysis buffer before immediately starting lysis at 56°C.

Frozen Tissue Powder

- Label and pre-cool reaction tubes on dry ice. Keep tubes containing tissue powder on dry-ice and use small pre-chilled scoops that allow for the transfer of 5 or 10 mg frozen tissue powder at a time. Tare pre-chilled tube on the micro-balance and transfer appropriate amount of frozen tissue powder to tube for weighing. Work quickly to prevent the tube from warming up on the balance. Keep the aliquoted samples on dry ice to ensure the powder stays frozen.
- When adding Proteinase K and Tissue Lysis Buffer, mix immediately so that the tissue powder is released from the tube wall and dispersed evenly over the lysis buffer. It is important to start lysis at 56°C immediately; add the reaction components to one tube, mix and place at 56°C immediately, then proceed with the next tube. Do not dispense Proteinase K and Tissue Lysis Buffer to all tubes at once.

Stabilized Tissue Samples

If stabilized sample was frozen, thaw first. Remove stabilizing solution from the outside of the tissue sample by blotting on a paper towel or other absorbent paper. Cut the tissue sample into small pieces and weigh the desired amount in a reaction tube (see "Choosing Input Amounts"). Keep tubes cold. Although rapid processing of the samples is recommended, it is not as critical as for fresh or frozen samples because of the presence of the stabilizing agent. Stabilized tissues contain proteins that have an altered fiber structure. These proteins are more difficult for Proteinase K to digest and a fraction of insoluble fiber will remain even if lysis is complete and the lysate looks mostly clear. Since these fibers will block the membrane binding sites when the lysate is spun through, centrifugation of the lysate before loading on the column is recommended for best yield and purity. This is particularly important for brain and fibrous tissue samples (e.g. muscle).

Troubleshooting Guide for Genomic DNA Purification When Using the Monarch® Spin gDNA Extraction Kit

Problem	Cause	Solution
Low Yield		
Cells	Frozen cell pellet was thawed and/or resuspended too abruptly	• Thaw cell pellets slowly on ice and flick tube several times to release the pellet from bottom of tube. Use cold PBS, and resuspend gently by pipetting up and down 5–10 times until pellet is dissolved
	Cell Lysis Buffer was added concurrently with enzymes	• Add Proteinase K and RNase A to sample and mix well before adding the Cell Lysis Buffer
Blood	Blood was thawed, allowing for DNase activity	• Keep blood samples frozen and add Proteinase K, RNase A and Blood Lysis Buffer directly to the frozen samples. Start lysis right away & thaw upon incubation.
	Blood sample is too old	• Fresh (unfrozen) whole blood should not be older than 1 week. Older samples will show progressive DNA degradation and loss of yield.
	Formation of hemoglobin precipitates	• Species with high hemoglobin content (e.g., guinea pig) may accumulate insoluble hemoglobin complexes that clog the membrane. Reduce Proteinase K lysis time from 5 to 3 minutes.
Tissue	Tissue pieces are too large	• Cut starting material to the smallest possible pieces or grind with liquid nitrogen. In large tissue pieces, nucleases will destroy the DNA before the Proteinase K can lyse the tissue.
	Membrane is clogged with tissue fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge lysate at maximum speed for 3 minutes, as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
	Sample was not stored properly	• Samples stored for long periods of time at room temperature, 4°C or -20°C, will show degradation and loss of gDNA. Flash freeze tissue samples with liquid nitrogen or dry ice and store at -80°C. Alternatively, use stabilizing reagents to protect the gDNA.
	Genomic DNA was degraded (common in DNase-rich tissues)	• Organ tissues (e.g., pancreas, intestine, kidney, liver) contain significant amounts of nucleases. Store properly to prevent DNA degradation. Keep on ice during sample preparation. Refer to the protocol for the recommended amount of starting material and Proteinase K to use.
	Column is overloaded with DNA	• Some organ tissues (e.g., spleen, kidney, liver) are extremely rich in genomic DNA. Using inputs larger than recommended will result in the formation of tangled, long-fragment gDNA that cannot be eluted from the silica membrane. Reduce the amount of input material.
	Incorrect amount of Proteinase K added	• Most samples are digested with 10 µl Proteinase K, but for brain, kidney and ear clips, use 3 µl.
DNA Degradation		
Tissue	Tissue samples were not stored properly	• Samples stored for long periods of time at room temperature, 4°C or -20°C, will show degradation and loss of gDNA. Flash freeze tissue samples with liquid nitrogen or dry ice and store at -80°C. Alternatively, use stabilizing reagents to protect the gDNA.
	Tissue pieces are too large	• Cut starting material to the smallest possible pieces or grind with liquid nitrogen. In large tissue pieces, nucleases will degrade the DNA before Proteinase K can lyse the tissue.
	High DNase content of soft organ tissue	• Organ tissues (e.g., pancreas, intestine, kidney, liver) contain significant amounts of nucleases. Store properly to prevent DNA degradation. Keep on ice during sample preparation. Refer to the protocol for the recommended amount of starting material and Proteinase K to use.
Blood	Blood sample is too old	• Fresh (unfrozen) whole blood should not be older than 1 week. Older samples will show progressive DNA degradation and loss of yield.
	Blood was thawed, allowing for DNase activity	• Keep frozen blood samples frozen and add enzymes and lysis buffer directly to the frozen samples
Salt Contamination		
	Guanidine salt was carried over into the eluate: • The binding buffer contains guanidine thiocyanate (GTC) which shows very strong absorbance at 220-230 nm. • The most common way that salt is introduced into the eluate is by allowing the buffer/lysate mixture to contact the upper column area.	• When transferring the lysate/binding buffer mix, avoid touching the upper column area with the pipet tip and always pipet carefully onto the silica membrane. • Avoid transferring any foam that may have been present in the lysate; foam can enter into the cap area of the spin column. • Close the caps gently to avoid splashing the mixture into the upper column area and move the samples with care in and out of the centrifuge. • If salt contamination is a concern, invert the columns a few times (or vortex briefly) with gDNA Wash Buffer as indicated in the protocol.

Troubleshooting Guide for Genomic DNA Purification When Using the Monarch® Spin gDNA Extraction Kit (continued)

Problem	Cause	Solution
Protein Contamination		
Tissue	Incomplete digestion	• Cut samples to the smallest possible pieces. Incubate sample in the lysis buffer for an extra 30 minutes to 3 hours to degrade any remaining protein complexes.
	Membrane is clogged with tissue fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small, indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge the lysate at maximum speed for 3 minutes as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
Blood	High hemoglobin content	• Some blood samples (e.g., horse) are rich in hemoglobin, evidenced by their dark red color. Extend lysis time by 3–5 minutes for best purity results.
	Formation of hemoglobin precipitates	• Species with high hemoglobin content (e.g., guinea pig) may accumulate insoluble hemoglobin complexes that clog the membrane. Reduce Proteinase K lysis from 5 to 3 minutes.
RNA Contamination		
Tissue	Too much input material	• DNA-rich tissues (e.g., spleen, liver and kidney) will become very viscous during lysis and may inhibit RNase A activity. Do not use more than the recommended input amount.
	Lysis time was insufficient	• Extend lysis time by 30 minutes to 3 hours after the tissue piece has completely dissolved
Tissue Digestion Takes Too Long		
	Tissue pieces too large	• Cut tissue pieces to the smallest possible size or grind with liquid nitrogen before starting lysis
	Tissue pieces are stuck to bottom of tube	• Vortex to release pieces from the tube bottom, and immediately after adding Proteinase K and Tissue Lysis Buffer
	Too much starting material	• Use recommended input amount
Tissue Lysate Appears Turbid		
	Formation of indigestible fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge lysate at maximum speed for 3 minutes, as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
Ratio $A_{260}/A_{230} > 2.5$		
	Slight variations in EDTA concentration in eluates	• EDTA in elution buffer may complex with cations like Mg^{2+} and Ca^{2+} samples present in genomic DNA, which may lead to higher than usual A_{260}/A_{230} ratio. In some cases, this ratio exceeds a value of 3.0 and is consistent with highly pure samples. In these cases, the elevated value does not have any negative effect on downstream applications.

Guidelines for Choosing Sample Input Amounts When Using the Monarch® Spin RNA Isolation Kit (Mini)

RNA yield, purity, and integrity vary immensely based on sample type, input amount and sample condition. Below, we have provided some empirical yield and RIN data from a wide variety of sample types, as well as guidance on the maximal input amounts for each of those samples when using the Monarch Spin RNA Isolation Kit (Mini) (NEB #T2110). It is very important not to overload the column when extracting and purifying RNA, as yields, purity and integrity may suffer.

Sample Type		Recommended Input	Average Yield (µg)	Observed RIN	Maximum Starting Material
Cultured Cells					
HEK 293		1 x 10^6 cells	12–14	9–10	5 x 10^6 cells
NIH3T3		1 x 10^6 cells	8–12	9–10	1 x 10^7 cells
Blood or Plasma [1]					
Human	Fresh	200 µl	0.5–1.0	7–8	200 µl
	Frozen	200 µl	0.5–1.0	7–8	200 µl
Rat	Frozen	200 µl	5–6	9	200 µl
Blood Cells					
PBMC (isolated from 5 ml whole blood)		5 ml	1–3	7	5 x 10^6 cells
Tissue					
Rat liver (Frozen stabilized)		10 mg	40–60	8–9	20 mg
Rat spleen (Frozen stabilized)		10 mg	40–50	9	20 mg
Rat kidney (Frozen stabilized)		10 mg	7–10	9	50 mg
Rat brain (Frozen stabilized)		10 mg	5–8	8–9	50 mg
Rat muscle (Frozen stabilized)		10 mg	2–3	8–9	50 mg
Mouse heart (Frozen stabilized)		10 mg	5–6	8–9	50 mg
Yeast					
S. cerevisiae	Frozen with bead homogenizer	1 x 10^7 cells	20–40	9–10**	5 x 10^7 cells
	Fresh with Zymolyase®	1 x 10^7 cells	20–40	9**	5 x 10^7 cells
Bacteria					
E. coli	Frozen with bead homogenizer	1 x 10^9 cells	10	10	1 x 10^9 cells
	Frozen with lysozyme	1 x 10^9 cells	70	10	1 x 10^9 cells
B. cereus	Frozen with bead homogenizer	1 x 10^9 cells	15–20	9	1 x 10^9 cells
	Frozen with lysozyme	1 x 10^9 cells	20–30	9–10	1 x 10^9 cells
Plant					
Corn leaf (Frozen pulverized with bead homogenizer)		100 mg	40–60	8*	100 mg
Tomato leaf (Frozen pulverized with bead homogenizer)		100 mg	40–60	8*	100 mg
Onion leaf (Fresh with bead homogenizer)		50 mg	4–6	8*	50 mg
Root		50 mg	8–10	8*	50 mg
Insects					
Mosquito (Preserved in ethanol dry ice bath)		10 mg	20–30	9*	20 mg
House fly (Preserved in ethanol dry ice bath)		10 mg	10–20	9*	10 mg

[1] A protocol for nucleated blood (e.g., birds, reptiles) is also available.

* Contain RNA with an atypical ribosomal profile that is not applicable for standard RIN measurement. Users are encouraged to set their own threshold or assess RNA integrity by usually inspecting the bands generated by the automated gel electrophoresis program.

** *S. cerevisiae* total RNA was run on an Agilent® Nano 6000 Chip using plant assay.

Troubleshooting Guide for Monarch® Spin RNA Isolation Kit (Mini)

Problem	Cause	Solution
Clogged column	Sample input higher than recommended	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. See Guidelines for Choosing Sample Input Amounts.
	Insufficient lysis	• Increase time of digestion or homogenization. • Centrifuge sample to pellet debris and use only supernatant for next steps. • Use larger volume of buffer for lysis and homogenization.
Low RNA yield	Insufficient lysis	• Increase time of digestion or homogenization. • Centrifuge sample to pellet debris and use only supernatant for next steps. • Use larger volume of buffer for lysis and homogenization.
	Sample is degraded	• Use RNA preservation reagents to maintain RNA integrity during storage. • Use best practices when working with RNA, harvest samples quickly, flash-freeze samples or preserve in stabilization reagent as soon as possible. • To minimize risk of contaminating RNases, clean surfaces and tools with RNase-removal reagents, always handle samples with appropriate PPE. • See Important Notes Before Starting in product manual.
	Sample input higher than recommended	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. Overloading leads to inefficient purification, insufficient elution and reduced yield. See Guidelines for Choosing Sample Input Amounts.
Low RNA quality	Sample is degraded	• Use RNA preservation reagents to maintain RNA integrity during storage. • Use best practices when working with RNA, harvest samples quickly, flash-freeze samples or preserve in stabilization reagent as soon as possible. • To minimize risk of contaminating RNases, clean surfaces and tools with RNase-removal reagents, always handle samples with appropriate PPE.
	Salt/ethanol carryover	• Low $A_{260/230}$ values indicate residual guanidine salts have been carried over during elution. Ensure wash steps are carried out prior to eluting sample. Do not skip any washes with Buffer BX and Buffer WZ. • Use care to ensure the column tip does not contact the flow-through. If unsure, please repeat centrifugation. When reusing collection tubes, blot rim of tube on a soft paper towel prior to reattachment to the column to remove any residual wash buffer. • Add additional wash step and/or extend spin time for final wash.
	Residual protein carryover	• Low $A_{260/280}$ values indicate residual protein in the purified sample. Ensure the Proteinase K step was utilized for the recommended time. Ensure samples have no debris prior to addition of ethanol and loading onto RNA purification column. Do not skip any washes with Buffer BX and Buffer WZ.
DNA contamination	DNA carryover	• Perform optional on-column DNase I treatment to remove unwanted gDNA from lysed sample. • Perform in-tube/off-column DNase I treatment to remove gDNA. See Appendix in product manual.
	Sample input higher than recommended	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded.
Low performance of RNA in downstream steps	Salt and/or ethanol carryover has occurred	• Use care to ensure the column tip does not contact the flow-through after the final wash. If unsure, please repeat centrifugation. • Be sure to spin the column for 2 minutes following the final wash with Monarch Buffer WZ. • When reusing collection tubes, blot rim of tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer. • Add additional wash step and/or extend spin time for final wash.
Unusual spectrophotometric readings	RNA concentration is too low for spectrophotometric analysis	• For more concentrated RNA, elute with 10 µl of nuclease-free water. • Increase amount of starting material (within kit specifications). See Guidelines for Choosing Input Amounts or product manual.
	Silica fines in eluate	• Re-spin eluted samples and pipet aliquot from the top of the liquid to ensure the $A_{260/230}$ is unaffected by possible elution of silica particles.

Troubleshooting Guide for RNA Cleanup

Our troubleshooting guide below outlines some of the most common pain points that scientists encounter during RNA cleanup with Monarch kits.

Problem	Cause	Solution
Low RNA yield	Reagents added incorrectly	• Check protocol to ensure correct buffer reconstitution, order of addition of buffers and ethanol, and proper handling of column flow-through and eluents.
	Insufficient mixing of reagents	• Ensure the ethanol is thoroughly mixed with RNA sample and RNA cleanup binding buffer before applying the sample to the RNA cleanup column.
	Incomplete elution during prep	• Ensure the nuclease-free water used for elution is delivered directly to the center of the column so that the matrix is completely saturated. Larger elution volumes, multiple elutions, and longer incubation times can increase yield of RNA, but will dilute the sample and may increase processing times. For typical RNA samples, the recommended elution volumes and incubation times should be sufficient.
	High degree of RNA secondary structure	• Binding and elution of smaller RNAs (< 45 nt) can be affected by secondary structure of the RNA molecules. If poor yield of a small RNA is observed, we recommend diluting your sample with 2 volumes of ethanol instead of one volume in Step 2 of the protocol.
Purified RNA is degraded	RNase contamination	• In order to avoid RNase contamination during RNA cleanup, make sure to work on a clean lab bench, wear gloves and use disposable RNase-free pipet tips and microfuge tubes (not provided). Keep all kit components tightly sealed when not in use.
	Improper storage of RNA	• Purified RNA should be used immediately in downstream applications or stored at -70°C.
Low $A_{260/230}$ ratios	Residual guanidine salt carry-over	• Ensure wash steps are carried out prior to eluting sample. Use care to ensure the tip of the column does not contact the flow-through. If unsure, repeat centrifugation. When reusing collection tubes, blot the rim of the tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer.
Low performance of RNA in downstream steps	Salt and/or ethanol carry-over	• Ethanol and salt remaining after the washes may inhibit downstream applications. Use care to ensure that the tip of the column does not come into contact with the flow-through. If in doubt, re-centrifuge for 1 minute to ensure traces of salt and ethanol are not carried over in the eluted RNA.
	DNA contamination	• DNA removal may be necessary for certain applications. Incubate RNA sample with DNase I (NEB #M0303) and cleanup RNA using the Monarch Spin RNA Cleanup Protocol.

Guidelines for Choosing Sample Input Amounts When Using the Monarch® HMW DNA Extraction Kit for Cells & Blood

The table below provides data on minimum, maximum, and recommended input amounts for various cell lines and blood samples using the Monarch HMW DNA Extraction Kit for Cells & Blood (NEB #T3050). Data on yield, purity and RNA content is also provided. Samples that were successfully tested in standard ligation-based Oxford Nanopore Technologies® sequencing runs are indicated. RNA content was determined by HPLC analysis of nucleoside content after digestion of 1 µg of eluted nucleic acid with the Nucleoside Digestion Mix (NEB #M0649). Yields from blood samples vary by donor due to different leukocyte content; yield can vary up to 3-fold by donor. Similar yield and purity results were obtained with different anticoagulants (e.g., EDTA, citrate, heparin and PAXgene Blood DNA tubes were tested).

Using input amounts below the recommended minimum will reduce yields drastically. Exceeding maximum input amounts will result in DNA eluates that are highly viscous and difficult to dissolve and will reduce purity of the isolated DNA. Results are shown for samples that were lysed with agitation at 2,000 rpm.

Cells

	Minimum Input (Cells)	Maximum Input (Cells)*	Recommended Input Amount (Cells)	Yield (µg) FROM 1 x 10^6 cells	Purity Ratios		RNA content	Validated for ONT sequencing?
					$A_{260/280}$	$A_{260/230}$		
HEK293	1 x 10^5	1 x 10^7	1 x 10^6	11.5–13	1.86	2.4	≤ 1%	Yes
HeLa	1 x 10^5	1 x 10^7	1 x 10^6	12.9	1.86	2.4	≤ 1%	Yes
NIH3T3	1 x 10^5	1 x 10^7	1 x 10^6	9.4	1.86	2.4	≤ 1%	Yes
Jurkat	1 x 10^5	1 x 10^7	1 x 10^6	13.7	1.86	2.5	≤ 1%	Yes
K562 (suspension cells)	1 x 10^5	1 x 10^7	1 x 10^6	13.7	1.86	2.4	≤ 1%	Yes
HCT116	1 x 10^5	1 x 10^7	1 x 10^6	16.9	1.86	2.5	≤ 1%	Yes
A549	1 x 10^5	1 x 10^7	1 x 10^6	12.7	1.86	2.3	≤ 1%	Yes
U5Os	1 x 10^5	1 x 10^7	1 x 10^6	10.6	1.86	2.4	≤ 1%	Yes
HepG2	1 x 10^5	1 x 10^7	1 x 10^6	13.4	1.81	2.2	≤ 1%	Yes
NCI-460	1 x 10^5	1 x 10^7	1 x 10^6	9.5	1.86	2.4	≤ 1%	Yes
SK-N-SH	1 x 10^5	1 x 10^7	1 x 10^6	9.5	1.86	2.4	≤ 1%	Yes
Aa23	1 x 10^5	1 x 10^7	1 x 10^6	8.7	1.81	2.3	≤ 1%	Yes

Mammalian Blood

		Minimum Input (µl)	Maximum Input (µl)	Recommended Input (µl)	Yield (µg) for 500 µl**	Purity Ratios		RNA content	Validated for ONT sequencing?
						$A_{260/280}$	$A_{260/230}$		
Human***	Fresh	100	2,000	500	12–32	1.86	2.4	≤ 1%	Yes
	Frozen	100	2,000	500	9–30	1.86	2.4	≤ 1%	Yes
Mouse	Fresh	100	2,000	500	7–11	1.88	2.4	≤ 1%	Yes
	Frozen	100	2,000	500	16–17	1.88	2.4	≤ 1%	ND
Rat (fresh only)	Fresh	100	2,000	500	29–38	1.87	2.4	≤ 1%	Yes
Rabbit	Fresh	100	500	200	12–15	1.72	1.9	≤ 1%	Yes
	Fresh	100	500	200	200 µl: 4–5	1.89	2.4	≤ 1%	Yes
	Frozen	100	500	200	200 µl: 4–5	1.89	2.4	≤ 1%	Yes
Pig	Fresh	100	2,000	500	up to 42	1.86	2.4	≤ 1%	Yes
	Frozen	100	2,000	500	up to 40	1.86	2.4	≤ 1%	Yes
Horse	Fresh	100	2,000	500	16	1.86	2.3	≤ 1%	Yes
	Frozen	100	2,000	500	22.3	1.86	2.4	ND	ND
Cow	Fresh	200	2,000	500	7	1.86	2.4	≤ 1%	Yes
	Frozen	200	2,000	500	9.1	1.86	2.4	ND	ND
Rhesus monkey	Fresh	100	2,000	500	52	1.86	2.4	≤ 1%	Yes
	Frozen	100	2,000	500	52.6	1.86	2.5	ND	ND
Goat (fresh only)	Fresh	100	2,000	500	24	1.87	2.4	≤ 1%	Yes
Sheep (fresh only)	Fresh	100	2,000	500	15.3	1.87	2.4	ND	ND

Nucleated Blood

		Minimum Input (µl)	Maximum Input (µl)	Recommended Input (µl)	Yield (µg) for 5 µl**	Purity Ratios		RNA content	Validated for ONT sequencing?
						$A_{260/280}$	$A_{260/230}$		
Chicken	Fresh	2	20	5	33	1.86	2.5	ND	Yes
	Frozen	2	20	5	30	1.86	2.5	ND	ND
Turkey	Fresh	2	20	5	37	1.87	2.4	ND	Yes
	Frozen	2	20	5	28	1.87	2.5	ND	ND

ND = Not determined

* For low agitation speeds, do not exceed 5 x 106 cells

** Unless otherwise stated

***Compatible with K2-EDTA, Na-citrate, Na-heparin, PAXgene® Blood DNA

Guidelines for Choosing Sample Input Amounts When Using the Monarch® HMW DNA Extraction Kit for Tissue, Bacteria and Other Samples

The table below provides guidance on the minimum, maximum, and recommended input amounts for various sample types when using the Monarch HMW DNA Extraction Kit for Tissue (NEB #T3060). Data on yield, purity and RNA content is also provided. Samples that were successfully tested in standard ligation-based Oxford Nanopore Technologies sequencing runs are indicated. Using input amounts that exceed the maximum will lead to challenges in solubility and viscosity, and purity may be affected. If more starting material is required, splitting the sample and performing multiple preps is recommended. RNA content was determined by HPLC analysis of nucleoside content after digestion of 1 µg of eluted nucleic acid with the Nucleoside Digestion Mix (NEB #M0649). Using input amounts below the recommended minimums will reduce yields drastically.

		Minimum Input (mg)	Maximum Input (mg)	Recommended Input (mg)	Yield (µg) for recommended input (Yield per mg)	Purity Ratios		RNA Content	Validated for ONT Sequencing?
						$A_{260/280}$	$A_{260/230}$		
Mammalian Tissue									
Mouse brain	Fresh	2**	20	15	12–21	1.87	2.39	ND	Yes
	Frozen	2**	20	15	15–21 (1–1.5)	1.86	2.48	ND	Yes
Mouse liver	Fresh (w/NaCl)	2	15	10	7	1.84	2.10	1.2%	Yes
	Frozen (w/NaCl)	2	15	10	17–19 (1.7–1.9)	1.89	2.50	ND	Yes
	Fresh*	2	15	10	20	1.84	1.52+	8.7%	Yes
	Frozen*	2	15	10	27–31 (2.7–3.1)	1.89	1.93++	ND	Yes
Mouse muscle	Fresh	2**	25	20	8–9	1.87	2.25	2.1%	Yes
	Frozen	2**	25	20	12–16 (0.6–0.8)	1.87	2.30	ND	Yes
Mouse kidney	Fresh	2	15	10	23–34	1.86	2.44	ND	Yes
	Frozen	2	15	10	32–41 (3.2–4.1)	1.86	2.53	0.8%	Yes
Mouse tail	Frozen	2**	25	20	20 (1.8–2.1)	1.86	2.43	ND	Yes+++
Mouse ear punch	Fresh	2**	15	10	15–16 (1.5–1.6)	1.86	2.29	ND	Yes
Rat kidney	Frozen	2	15	10	20–25	1.87	2.40	ND	Yes
Bacteria									
E. coli (Gram-negative)	Frozen	5 x 10^8 cells	5 x 10^9 cells	1 x 10^9 cells	8–9	1.89	2.31	1.7%	Yes
B. cereus (Gram-positive)	Frozen	2 x 10^8 cells	4 x 10^8 cells	2 x 10^8 cells	4–5	1.86	2.20	3.9%	Yes
M. luteus (Gram-positive)	Frozen	ND	ND	1 x 10^8 cells	2.0	1.89	2.09	ND	ND
Amphibian									
X. laevis	Fresh	ND	ND	3–4	5	1.86	2.51	2.3%	ND
Yeast									
S. cerevisiae	Fresh	ND	ND	20 x 10^7 cells	3–6***	1.90	2.01	ND	ND
Insect									
A. aegypti	Frozen	ND	ND	15	6	1.84	2.53++	2.7%	ND
NEMATODE									
*C. elegans***	Frozen	ND	ND	2 plates	8.2	1.91	2.5	ND	ND

ND = Not determined

* Standard protocol without recommended NaCl treatment.

** If working with input amounts < 5 mg, refer to the product manual for guidance on reducing buffer volumes.

*** Total nucleic acid yields are 4-10 µg and 6-12 µg for haploid and diploid strains, respectively. Though an RNase A step is included, RNA is co-purified. Yields may vary depending on the strain.

**** Rotor-stator homogenization is recommended.

\+ Measured with Nanodrop One; systems that differentiate turbidity in the content profiling will give higher values.

++ Measured with Unchained Labs Lunatic (formerly Trinean DropSense16); devices without content profiling that differentiates turbidity may give lower values.

+++ Size selection is recommended.

Genetic Markers

A *genotype* indicates the genetic state of the DNA in an organism. It is a theoretical construct describing a genetic situation that explains the observed properties (phenotype, see below) of a strain. *E. coli* genotypes list only genes that are defective (1). If a gene is not mentioned, then it is not known to be mutated*, **. Prophages and plasmids that were present in the original K-12 strain (F, λ, e14, rac) are normally listed only if absent. However, for simplicity, we have not listed I except when it is present, and we have listed F and its variants in all cases. Parentheses or brackets surround a prophage or plasmid when listed. Genes are given three-letter, lower-case, italicized names (e.g.,*dam*) that are intended to be mnemonics suggesting the function of the gene (here, **D**NA **a**denine **m**ethylase). If the same function is affected by several genes, the different genes are distinguished with uppercase italic letters (e.g., *recA, recB, recC, recD* all affect **rec**ombination). Proper notation omits superscript + or – in a genotype, but these are sometimes used redundantly for clarity, as with F´ *lac*$^{-}$ *proA*$^{+}$*B*$^{+}$. Deletion mutations are noted as Δ, followed by the names of deleted genes in parentheses, [e.g., Δ(*lac-pro*)]. All genes between the named genes are also deleted. Specific mutations are given allele numbers that are usually italic arabic numerals (e.g.,*hsdR17*) and may be characterized as *am*=amber (UAG) mutation or *ts*=inactive at high temperature, as appropriate. Some common alleles[e.g., Δ(*lac-pro*)X111] break the rules. If two strains' genotypes list a gene with the same allele number, they should carry exactly the same mutation.

The *phenotype* of a strain is an observable behavior, e.g., Lac^{-} fails to grow on lactose as a sole carbon source. Phenotypes are capitalized and in Roman type, and the letters are always followed by superscript + or – (or sometimes r, resistant, or s, sensitive). Although phenotypes do not, strictly speaking, belong in a genotype, they are sometimes included following the genotype designation when the former is not obvious from the latter [e.g., ^{rpsL104} (Str^{r})– gene name from **r**ibosomal **p**rotein, **s**mall subunit, S12, confers resistance to streptomycin].

Some common genes of interest are described below and on the next page; a catalogue of genetically defined genes can be found in reference 2 and on the very useful internet site maintained by the *E. coli* Genetic Stock Center (CGSC) at Yale University <http://cgsc.biology.yale.edu/>. Additional information from CGSC can be obtained from curator Mary Berlyn by e-mail <cgsc@yale.edu>.

* Most *E. coli* laboratory strains have been heavily mutagenized over forty years of study, and different lines may carry different, so far undiscovered, mutations that may or may not affect your situation. For this reason, it is sometimes useful to try more than one line or strain background in your experiments.

** *E. coli* B and its derivatives are naturally Lon^{-} and Dcm^{-}. We have listed this in brackets even though it is the wild type state for these strains.

dam	Endogenous adenine methylation at GATC sequences is abolished. *dam* strains have a high recombination frequency, express DNA repair functions constitutively, and are poorly transformed by Dam-modified plasmids. Used for making DNA susceptible to cleavage by some restriction enzymes (e.g., BclI).
dcm	Endogenous cytosine methylation at CCWGG sequences is abolished. Used for making DNA susceptible to cleavage by some restriction enzymes (e.g., AvaII).
dnaJ	One of several "chaperonins" is inactive. This defect has been shown to stabilize certain mutant proteins expressed in *E. coli*.
dut	dUTPase activity is abolished. This mutation, in combination with *ung*, allows incorporation of uracil into DNA. Used for oligonucleotide mutagenesis.
endA	Activity of nonspecific Endonuclease I is abolished. DNA preparations are thought to be of higher quality when prepared from *endA* strains.
e14	An excisable prophage-like element, present in K-12 but missing from many derivatives. e14 carries the *mcrA* gene among others, therefore e14^{-} strains are McrA^{-}.
F	A low-copy number self-transmissible plasmid. F´ factors carry portions of the *E. coli* chromosome, most notably the *lac* operon and *proAB* on F´ *lac*$^{-}$ *proA*$^{+}$*B*$^{+}$.
fhuA	An iron uptake receptor is mutated. This mutation confers resistance to phage T1 (ferric hydroxamate uptake). Former name is *tonA*.
gal	The ability to metabolize galactose is abolished.
glnV	See ***supE***.
gyrA	A point mutation in DNA gyrase, subunit A. This mutation confers resistance to the antibiotic nalidixic acid.
hflA	This mutation results in high frequency lysogenization by λ.
hsdR, hsdS	DNA that does not contain methylation of certain sequences is recognized as foreign by EcoKI or EcoBI and restricted (degraded). These enzymes recognize different sequences and are encoded by different alleles of *hsdRMS*. *hsdR* mutations abolish restriction but not protective methylation (r^{-}m^{+}), while *hsdS* mutations abolish both (r^{-}m^{-}). DNA made in the latter will be restricted when introduced into a wild-type strain. See *E. coli* K-12.
lacI^{q}	The *lac* repressor is overproduced, turning off expression from P*lac* more completely.
lacZ	β-galactosidase activity is abolished.
lacZ:: T7gene 1	The phage T7 RNA polymerase (= gene 1) is inserted into the lacZ gene.
lacY	Lactose permease activity is abolished.
	Δ(*lac*) = deletion; there are four common deletions involving *lac*:
	Δ(*lacZ*)M15 expresses a fragment that complements the *lac* α-fragment encoded by many vectors. These vectors will yield blue color on X-Gal only if the host carries ΔM15.
	ΔU169, ΔX111, and ΔX74 all delete the entire *lac* operon from the chromosome,in addition to varying amounts of flanking DNA. ΔX111 deletes *proAB* as well, so that the cell requires proline for growth on minimal medium, unless it also carries F´ *lac proA*$^{+}$*B*$^{+}$.
lon	Activity of a protease responsible for degrading aberrant proteins is abolished. Some eukaryotic proteins are stabilized in *lon* strains. *E. coli* B naturally lacks Lon.
lysY	The lysozyme gene from the T7 bacteriophage is mutated. The mutation K128Y eliminates lysozyme activity, but the mutant protein still binds to and inhibits T7 RNA polymerase.

References
(1) Demerec et al. (1966) *Genetics*, 54, 61–76.
(2) Berlyn, M.K.B. (1996). In F. C. Niedhardt et al. (Ed.), *Escherichia coli and Salmonella: cellular and molecular biology*, (2nd ed.), Vol. 2, (pp. 1715–1902). ASMPress.
(3) Raleigh, E.A. et al. (1991) *J. Bacteriol.*, 173, 2707–2709.

Genetic Markers (continued)

malB	The *malB* region encompasses the genes *malEFG* and *malK lamB malM*. Δ(*malB*) deletes most or all of this region and eliminates expression of Maltose Binding Protein (MalE).
mcrA, mcrBC	A restriction system that requires methyl mcrBC cytosine is abolished. DNA containing methylcytosine in some sequences is restricted by Mcr$^+$. *dcm*$^-$ modified DNA is not restricted by Mcr$^+$. Δ(*mcrC-mrr*) deletes six genes: *mcrC-mcrB-hsdS-hsdM-hsdR-mrr; mcrA* is lost with e14. See *E. coli* K12.
mrr	A restriction system that requires cytosine or adenine methylation is abolished; however, *dam*$^-$, *dcm*$^-$ or EcoKI-modified DNA is not restricted by Mrr$^+$. The methylcytosine-dependent activity is also known as McrF (3). See *E. coli* K12.
mtl	The ability to metabolize the sugar alcohol mannitol is abolished.
ompT	Activity of outer membrane protease (protease VII) is abolished.
phoA	Activity of alkaline phosphatase is abolished.
Prc	See ***tsp***.
recA	Homologous recombination is abolished; particularly desirable when working with sequences containing direct repeats > 50 bp.
recB, recC	Exonuclease and recombination activity of Exonuclease V is abolished. Homologous recombination is much reduced in *recB recC* strains that are not also *sbcB* or *sbcA*. Stability of inverted repeat sequences is enhanced in *recB recC* strains, especially if they are also *sbcB sbcC*. Plasmid replication may be aberrant.
recD	Exonuclease activity of ExoV is abolished, but recombination activity is elevated. Inverted repeat sequences in λ can be propagated in *recD* strains. Plasmid replication is aberrant.
recF	Plasmid-by-plasmid homologous recombination is abolished.
recJ	Plasmid-by-plasmid homologous recombination is abolished.
relA1	Lacks ppGpp synthesis during the stringent response to amino acid starvation; activity of ATP:GTP 3´-pyrophosphotransferase (EC2.7.6.5) is abolished.
rfbD	Lacks functional TDP-rhamnose synthetase, and thus does not synthesize the cell surface O-antigen.
rpoH	(also known as *htpR*) Lack of this heat-shock transcription factor abolishes expression of some stress-induced protease activities in addition to *lon*. Some cloned proteins are more stable in *rpoH*am *sup* Cts strains at high temperature.
sbcB	Exo I activity is abolished. Strains carrying *recB recC* and *sbcB* are usually also *sbcC*. These quadruple mutant strains are recombination-proficient and propagate inverted repeats in λ, but plasmid replication is aberrant.
sbcC	Usually found with *recB recC sbcB*. However, strains carrying *sbcC* alone are recombination-proficient and stably propagate inverted repeats both in λ and in plasmids.
sulA	Mutations in this gene allows cells to divide and recover from DNA damage in a lon mutant background (suppressor of Lon).
supC(ts)	A thermosensitive tyrosine-inserting ochre (UAA) and amber (UAG) suppressor tRNA. Nonsense mutations in the same strain are suppressed only at low temperatures. Now called ***tyrT***.
supE	A glutamine-inserting amber (UAG) suppressor tRNA; required for growth of some phage vectors. Now called ***glnV***.
supF	A tyrosine-inserting amber (UAG) suppressor tRNA; required for lytic growth of S7 or S100 λ phage, such as λgt11. Now called ***tyrT***.
thi-1	The ability to synthesize thiamine is abolished (vitamin B1).
traD	The self-transmissibility of the F factor is severely reduced.
tsp	A periplasmic protease that may degrade secreted or cytoplasmically overexpressed proteins after lysis is abolished. Now called ***prc***.
tsx	Confers resistance to bacteriophage T6.
tyrT	See ***supC***, ***supF***.
ung	Uracil N-glycosylase activity is abolished. Uracil incorporated into DNA is removed by Ung$^+$, leaving baseless site. See *dut*.
xyl	The ability to metabolize the sugar xylose is abolished.
(P1)	The cell carries a P1 prophage. Such strains express the P1 restriction system.
(P2)	The cell carries a P2 prophage. This allows selection against Red$^+$ Gam$^+$ λ (Spi$^-$ selection).
(ϕ80)	The cell carries the lambdoid prophage ϕ80. A defective ϕ80 prophage carrying the *lac* M15 deletion is present in some strains.
(Mu)	Mu prophage; Mud means the phage is defective.

Enhancing Transformation Efficiency

Transformation efficiency is defined as the number of colony forming units (cfu) that would be produced by transforming 1 µg of plasmid into a given volume of competent cells. However, 1 µg of plasmid is rarely transformed. Instead, efficiency is routinely calculated by transforming 100 pg–1 ng of highly purified supercoiled plasmid under ideal conditions. Transformation Efficiency (TE) is calculated as: TE = Colonies/µg/Dilution. Efficiency calculations can be used to compare cells or ligations. Our recommended protocols and tips are presented here to help you achieve maximum results.

Recommended Protocols

High Efficiency Transformation Protocol

1. Thaw cells on ice for 10 minutes
2. Add 1 pg–100 ng of plasmid DNA (1–5 µl) to cells and mix without vortexing
3. Place on ice for 30 minutes
4. Heat shock at 42°C for 10–30 seconds or according to recommendations. For BL21, use exactly 10 seconds.
5. Place on ice for 5 minutes
6. Add 950 µl of room temperature SOC or NEB 10-beta/Stable Outgrowth Medium.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Mix cells without vortexing and perform several 10-fold serial dilutions in SOC or NEB 10-beta/Stable Outgrowth Medium.
9. Spread 50–100 µl of each dilution onto pre-warmed selection plates and incubate overnight at 37°C (30°C for SHuffle® strains) or according to recommendations

5 Minute Transformation Protocol

(10% efficiency compared to above protocol)

1. Thaw cells in your hand
2. Add 1 pg–100 ng of plasmid DNA (1–5 µl) to cells and mix without vortexing
3. Place on ice for 2 minutes
4. Heat shock at 42°C for 30 seconds or according to recommendations.
5. Place on ice for 2 minutes
6. Add 950 µl of room temperature SOC or NEB 10-beta/Stable Outgrowth Medium. Immediately spread 50–100 µl onto a selection plate and incubate overnight at 37-42°C. (30°C for SHuffle strains) NOTE: Selection using antibiotics other than ampicillin may require some outgrowth prior to plating.

Transformation Tips

Thawing

- Cells are best thawed on ice
- DNA should be added as soon as the last trace of ice in the tube disappears
- Cells can be thawed by hand, but warming above 0°C decreases efficiency

Incubation of DNA with Cells on Ice

- Incubate on ice for 30 minutes. Expect a 2-fold loss in TE for every 10 minutes this step is shortened.

Heat Shock

- Both temperature and time are specific to the transformation volume and vessel. Typically, 30 seconds at 42°C is recommended.

Outgrowth

- Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in TE for every 15 minutes this step is shortened.
- SOC gives 2-fold higher TE than LB medium
- Incubation with shaking or rotation results in 2-fold higher TE

Plating

- Selection plates can be used warm or cold, wet or dry with no significant effects on TE
- Warm, dry plates are easier to spread and allow for the most rapid colony formation

DNA

- DNA should be purified and resuspended in water or TE Buffer
- For best results, the volume of the transformed DNA should not exceed 10% of the total volume of cells
- Purification by either a spin column or phenol/chloroform extraction and ethanol precipitation is ideal
- The optimal amount of DNA is lower than commonly recognized. Using clean, supercoiled pUC19, the efficiency of transformation is highest in the 100 pg–1 ng range. However, the total colonies which can be obtained from a single transformation reaction increase up to about 100 ng.

DNA Contaminants to Avoid

Contaminant	Removal Method
Detergents	Ethanol precipitate
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspending
PEG	Column purify or phenol/chloroform extract and ethanol precipitate
DNA binding proteins (e.g., ligase)	Column purify or phenol/ chloroform extract and ethanol precipitate

Electroporation Tips

NEB 10-beta (NEB #C3020) Competent *E. coli* is available as electrocompetent cells. The following tips will help maximize transformation efficiencies.

- Pre-chill electroporation cuvettes and microcentrifuge tubes on ice
- Thaw cells on ice and suspended well by carefully flicking the tubes
- Once DNA is added, electroporation can be carried out immediately. It is not necessary to incubate DNA with cells. The maximum recommended volume of a DNA solution to be added is 2.5 µl. Addition of a large volume of DNA decreases transformation efficiency.
- DNA should be purified and suspended in water or TE. Transformation efficiency is > 10-fold lower for ligation mixtures than the control pUC19 plasmid due to the presence of ligase and salts. If used directly, ligation reactions should be heat-inactivated at 65°C for 20 min and then diluted 10-fold. For optimal results, spin columns are recommended for clean up of ligation reactions.
- Electroporation conditions vary with different cuvettes and electroporators. If you are using electroporators not specified in the protocol, you may need to optimize the electroporation conditions. Cuvettes with 1 mm gap are recommended (e.g., BTX Model 610/613 and Bio-Rad #165-2089). Higher voltage is required for cuvettes with 2 mm gap.
- Arcing may occur due to high concentration of salts or air bubbles
- It is essential to add recovery medium to the cells immediately after electroporation. One minute delay can cause a 3-fold reduction in efficiency.
- Cold and dry selection plates lead to lower transformation efficiency. Pre-warm plates at 37°C for 1 hour. Using 37°C pre-warmed recovery medium increases the efficiency by about 20%.
- Refreeze unused cells in a dry ice/ethanol bath for 5 min and then store at -80°C. Do not use liquid nitrogen. Additional freeze-thaw cycles result in lower transformation efficiency.

Find tips for successful transformation.

T7 Protein Expression

1. Transform expression plasmid into a T7 expression strain. Plate out on antibiotic selection plates and incubate overnight at 37°C (24 hours at 30°C for SHuffle strains).
2. Resuspend a single colony in 10 ml liquid culture with antibiotic
3. Incubate at 37°C until OD_{600} reaches 0.4–0.6
4. Induce with 40 µl of a 100 mM stock of IPTG (final conc. = 0.4 mM) and induce for 2 hours at 37°C (4 hours at 30°C or 16°C overnight for SHuffle strains)
5. Check expression by Coomassie stained protein gel, Western Blot or activity assay. Check expression in the total cell extract (soluble + insoluble) and the soluble fraction alone.
6. For large scale, inoculate 1 L of liquid medium (with antibiotic) with a freshly grown colony or 10 ml of freshly grown culture. Incubate at 37°C (30°C for SHuffle strains) until OD_{600} reaches 0.4–0.6. Add IPTG to 0.4 mM. Induce 2 hours at 37°C or 15°C overnight (4 hours at 30°C or 16°C overnight for SHuffle strains).

Troubleshooting Tips

No Colonies or No Growth in Liquid Culture

- Even though T7 expression is tightly regulated, there may be a low level of basal expression in the T7 Express host. If toxicity of the expressed protein is likely, transformation of the expression plasmid should be carried out in a more tightly controlled expression strain:
  - In I^q strains over-expression of the *LacI*^{q} repressor reduces basal expression of the T7 RNA polymerase
  - In *lysY* strains, mutant T7 lysozyme is produced which binds to T7 RNA polymerase, reducing basal expression of the target protein. Upon induction, newly made T7 RNA polymerase titrates out the lysozyme and results in expression of the target protein.
- Incubation at 30°C or room temperature may also alleviate toxicity issues
- Check antibiotic concentration (test with control plasmid)

No Protein Visible on Gel or No Activity

- Check for toxicity - the cells may have eliminated or deleted elements in the expression plasmid. If this is the case, test I^q and/or *lysY* strains to reduce basal expression.
- Culture cells for protein induction. Just before induction, plate a sample on duplicate plates with and without antibiotic selection. If toxicity is an issue, significantly fewer colonies will be seen on plates containing antibiotic (indicating that the plasmid has been lost) compared to plates without antibiotic.

Induced Protein is Insoluble

T7 expression often leads to very high production of protein that can result in the target protein becoming insoluble. In this case:

- Induce at lower temperatures (12–15°C overnight)
- Reduce IPTG concentration to 0.01 mM – 0.1 mM
- Induce for less time (as little as 15 minutes)
- Induce earlier in growth (OD_{600} = 0.3 or 0.4)

Don (left) is the Director of Global Quality Operations and Validation and has been with NEB for 9 years. He is also the organizer of the NEB Golf League. Jim (right) is the Associate Director of GMP Operations and has been with NEB for 8 years. Jim is a participant in several clubs on campus, and also organizes a fantastic Haunted House at Halloween for NEB employees and their families to enjoy.

Troubleshooting Guide for NEBNext® DNA Library Prep Kits

Troubleshooting guides are available at www.neb.com for NEBNext products including NEBNext RNA Library Prep, NEBNext Ultra II FS DNA Library Prep, NEBNext DNA Library Prep, NEBNext rRNA Depletion Kit (Bacteria) and NEBNext Custom RNA Depletion Design Tool with NEBNext RNA Depletion Core Reagent Set

Problem	Cause	Solution
Failed Library Prep **For example:** **• You may see nothing on the Bioanalyzer, or similar instrument** **• After amplification, you may see library fragments that are still the same size as the starting input DNA rather than ~120 bp longer than the input DNA**	Input DNA contains an inhibitor	• Ensure DNA does not contain inhibitor • Consider additional cleanup step
	Failed step - Any of the enzymatic steps can fail if a critical reagent is omitted, or if the reagent has become inactive	• Confirm reagents were added for each step in the protocol • Confirm expiration dates on reagents
Low Library Yield	Input DNA is damaged	• Use NEBNext Ultra Shear FFPE DNA Library Prep Kit (NEB #E6655)
	Adaptor is denatured	• When diluting NEBNext adaptors, use 10 mM Tris HCl (pH 7.5-8.0) with 10 mM NaCl • Keep the adaptor on ice until use
	Insufficient mixing	• Mix samples well with 80-90% of the total volume in the well or tube by pipetting up and down. Keep the tip in the liquid to avoid the formation of bubbles. • For enzymatic steps, follow the manual recommendations (usually 10 mix cycles) • Try to avoid losing sample in the pipette tip or on the source tube during transfer
	SPRI beads have dried out before elution	• Add Elution Buffer and mix before the beads turn lighter brown and start cracking • For additional tips about SPRI beads, refer to neb.com/tools-and-resources/video-library for our technical tips videos
	Incomplete ethanol removal during SPRI bead wash	• Quickly spin the tube after the last ethanol wash at each SPRI bead step, keep the tube on the magnet and remove residual ethanol with a p10 tip • For additional tips about SPRI beads, refer to neb.com/tools-and-resources/video-library for our technical tips videos
	SPRI bead sample loss	• Mix slowly to avoid droplets clinging to the inside of the tip, which may not combine with the sample before the tip is ejected. Dispense the last mix slowly into the sample tube so that the liquid stays together. Wait 1 second before pushing the pipette to the second stop. • When removing the supernatant, take care not to remove any beads. Check your tip over a white piece of paper. If beads are visible, dispense everything back into the tube and allow beads to resettle. • For additional tips about SPRI beads, refer to neb.com/tools-and-resources/video-library for our technical tips videos
	Sample storage after A-tailing	• Avoid prolonged storage of a sample before moving to ligation. If sample inputs are low, avoid overnight storage and move immediately from end prep to adaptor ligation.
	Adaptor self-ligation (Adaptor-dimer formation)	• Do not add adaptor to the ligation master mix. This can cause increased adaptor-dimer formation. • For best results, add the adaptor to the sample, mix and then add ligase master mix and ligation enhancer
	Ligation incubation temperature is too warm	• If ligation incubation occurs above 20˚C, the DNA ends may breathe, which could reduce ligation
Adaptor-dimer Formation (sharp 127 bp peak on Bioanalyzer)		• To recover the samples, repeat the bead cleanup using a 0.9 x bead ratio.
	Adaptor concentration too high	• Optimize adaptor dilution based on your sample input, quality and type using an adaptor titration experiment • Adaptor titration may need to be repeated if the source of the sample input changes (e.g., extraction method, tissue type, etc.)
	Adaptor self-ligation (Adaptor Dimer formation)	• Do not add adaptor to ligation master mix. This can cause increased adaptor-dimer formation. • For best results, add adaptor to sample, mix and then add ligase master mix and ligation enhancer. Mix again.
Adaptor or primers remaining after PCR (e.g., visible on Bioanalyzer or similar instrument after PCR)	Excess adaptor or primer used or inefficient cleanup	• Perform another 0.9 x SPRI cleanup
Overamplification (Once PCR primers are depleted, library fragments will become single stranded and/or form heteroduplexes. These appear as high molecular weight fragments on a Bioanalyzer or similar instrument.)	Too many PCR Cycles	• We recommend starting with the number of PCR cycles recommended in the product manual. The ideal number of PCR cycles for your samples may vary. • Reduce the number of PCR cycles if you are seeing overamplification • Data quality may be compromised if overamplified libraries are sequenced
	Not enough PCR primer	• Check primer concentration and ensure that you are adding the primer volume recommended in the manual • Store primers at the correct temperature to prevent degradation • Data quality may be compromised overamplified libraries are sequenced
	Too much input DNA	• The higher the input of template for the PCR, the sooner the primers will be depleted • NEBNext adaptor and PCR primers require a minimum of 3 PCR cycles. If you cannot further reduce the number of PCR cycles, consider a size selection step, or using only a fraction of the ligated library as input for PCR.

DNA Sample Input Guidelines

Choice of Kits

- For pre-sheared DNA, the following inputs are recommended:
  - NEB #E7645: 500 pg–1 µg
  - NEB #E7410: 250 ng–1 µg
  - NEB #E3325: 10 ng–200 ng
- For intact DNA, the following inputs are recommended:
  - NEB #E7805: 100 pg–500 ng
  - NEB #E7430: 50 ng–500 ng
  - NEB #E3340: 10 ng–200 ng

Integrity of DNA

- The quality of the input material directly affects the quality of the library. Absorbance measurements can be used as an indication of DNA purity. Ideally, the ratio of the absorbance at 260 nm to 280 nm should be between 1.8 – 2.0. However, measurements can be affected by the presence of RNA or small nucleic acid fragments. A DNA Integrity Number can be determined using the Agilent TapeStation® or similar instrumentation, and qPCR-based methods can also provide a measurement of DNA integrity. For low-quality DNA, consider the NEBNext Ultra Shear FFPE DNA Library Prep Kit.

Quantitation of DNA

- It is important to quantify accurately the DNA sample prior to library construction. Fluorescence-based detection which utilizes dsDNA-specific dyes, such as the Qubit® from Thermo Fisher Scientific®. Qubit is more accurate than UV spectrometer-based measurements, as the presence of RNA or other contaminants can result in overestimation of the amount of the DNA sample by the latter. This can result in use of non-optimal adaptor dilutions and numbers of PCR cycles, compromising library prep efficiency.
- Consider using the NEBNext Library Quant Kit.

RNA Sample Input Guidelines

Choice of Kits

- For directional RNA, the following inputs are recommended:
  - NEB #E7765: 10 ng–1 µg
  - NEB #E3330: 25 ng–250 ng
- For non-directional RNA, the following inputs are recommended:
  - NEB #E7770: 10 ng–1 µg

Integrity of RNA

- We recommend determining the RNA sample input using the RNA Integrity Number (RIN) estimated by the Agilent TapeStation or similar instrumentation. Ideally the RNA sample will have a RIN value of 7 or higher but NEBNext RNA products are compatible for use even with samples with low RIN values.
- RNA should be completely free of DNA, and DNA digestion of the purified RNA using RNase-free DNase I (such as that provided with the Monarch Spin RNA Isolation Kit (Mini)) is recommended

Quantitation of RNA

- It is important to quantify accurately the RNA sample prior to library construction. The concentration can be estimated with the Agilent Bioanalyzer or similar instrumentation, using a pico or nano chip. Alternatively, RNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer such as a NanoDrop®. Note that free nucleotides or organic compounds used in some RNA extraction methods also absorb UV light near 260 nm and will cause an over-estimation of RNA concentration.
- Consider using the NEBNext Library Quant Kit

Bead-based Cleanups and Size Selection

- Be sure to vortex the beads well just before use. They should form a uniform suspension. If beads have settled for a long time period without being agitated a tight bead sediment can form. When beads have not been used for several weeks, plan for extra time for bead vortexing and agitation.
- Do not over-dry the beads. Beads should still be dark brown and glossy looking when eluting. Over-drying can make resuspension difficult and reduce yield.
- Take care not to remove beads after separation. If beads are accidentally aspirated, dispense everything back, allow the beads to settle and then try again.
- Remove all of the supernatant after the bind step. After removing most of the liquid with a p200 pipette, aspirate any remaining drops with a p10 pipette if necessary. Incomplete supernatant removal can cause leftover adaptor dimer or PCR primers to remain in the libraries.
- Bead ratios for cleanup and size selection after NEBNext ligation steps are appropriate just for the ligation step. Different ratios would apply if size selection is done after PCR or at any other step in the workflow.
- When adding beads to the sample, aspirate slowly to make sure the correct volume of beads is drawn into the tip. Remove any droplets of beads from the outside of the tip and make sure you dispense the full volume into the sample.
- The NEBNext Ultra Express library prep kits include a "phased cleanup." Find a video walkthrough on neb.com

Indices/Barcodes

- When using a subset of the indices supplied in a kit, or using indices from more than one kit, it is important to optimize the combination of indices used, in order to ensure balanced sequencing reads. We provide recommendations for NEBNext index combinations in the manuals for NEBNext Oligos products, and with the NEBNext Index Oligo Selector Tool at neb.com.
- For index primers provided in vials, open only one vial at a time, to minimize the risk of contamination
- Be sure to change pipette tips for each index primer
- For 96-well plate formats, NEBNext index primers are provided in single-use plates with pierceable foil lids. To avoid risk of contamination, do not pipette from a well more than one time.

Labeling with SNAP-tag® Technology-Troubleshooting Guide

Application	Problem	Possible Cause	Solution
Cellular Labeling	No labeling	Fusion protein not expressed	• Verify transfection • Check expression of fusion protein via Western blot or SDS-PAGE with an appropriate fluorescent substrate
	Weak labeling	Poor expression and/or insufficient exposure of fusion protein to substrate	• Increase substrate concentration • Increase incubation time
		Rapid turnover of fusion protein	• Analyze samples immediately or fix cells directly after labeling • Label at lower temperature (4°C or 16°C)
	High background	Non-specific binding of substrates	• Reduce substrate concentration and/or incubation time • Allow final wash step to proceed for up to 2 hours • Include fetal calf serum or BSA during labeling
	Signal strongly reduced after short time	Instability of fusion protein	• Fix cells • Switch tag from N-terminus to C-terminus or vice versa
		Photobleaching	• Add commercially available anti-fade reagent • Reduce illumination time and/or intensity
Labeling in Solution	Precipitation	Insoluble fusion	• Test from pH 5.0 to 10.0 • Optimize salt concentration [50 to 250 mM] • Add 0.05 to 0.1% Tween 20
	Weak or no labeling	Exhaustive labeling has not been achieved	• Increase incubation time to 2 hrs at 25°C or 24 hrs at 4°C • Reduce the volume of protein solution labeled • Check expression of fusion protein via SDS-PAGE with an appropriate fluorescent substrate
	Loss of activity	Instability of fusion protein	• Reduce labeling time • Decrease labeling temperature (4°C or 16°C)

Cellular Imaging & Analysis FAQs

Q. How does SNAP-tag® labeling differ from using GFP fusion proteins?

A. GFP and SNAP-tag are both valuable technologies used to visualize proteins in live cells. GFP is an intrinsically fluorescent protein derived from *Aequorea victoria* while SNAP-tag is derived from hAGT, a human DNA repair protein. In contrast to GFP, the fluorescence of SNAP-tag fusions can be readily turned on with the addition of a variety of fluorescent probes added directly to the culture media. Substituting different fluorophores or other functionalities (biotin, magnetic beads, blocking agents) requires no new cloning or expression, merely incubation of the appropriate substrate with cells, cell lysates or recombinant proteins.

Q. What is the difference between SNAP- and CLIP-tag™?

A. SNAP-tag and CLIP-tag are both derived from O6 -alkylguanine-DNA-alkyltransferase (hAGT). SNAP-tag recognizes O^6-labeled benzylguanine substrates while CLIP-tag recognizes O^2-labeled benzylcytosine substrates. Each tag transfers the label from the substrate to itself, resulting in specific covalent labeling. In creating the tags, hAGT has been engineered to no longer interact with DNA, but rather with derivatives of the free benzylguanine or benzylcytosine substrates. The tags exhibit no cross-reactivity with one another, enabling researchers to simultaneously label fusion proteins containing SNAP- and CLIP-tags with different fluorophores in live cells.

Q. Can I clone my protein as a fusion to the N- or C-terminus of the tags?

A. Yes. SNAP- and CLIP-tags can be fused to either the N- or C-terminus of a protein of interest. However, to label surface proteins on the outside of cells, the SNAP-tag or CLIP-tag must be cloned so that it is oriented to the extracellular surface of the plasma membrane. In this orientation, the tag is accessible to its fluorophore conjugated substrate.

Q. Are the substrates toxic to cells?

A. No toxicity has been noted by proliferation or viability assays when using up to 20 µM substrate for 2 hours. Most of the substrates can be incubated with cells for 24 hours up to a concentration of 20 µM without significant toxicity.

Q. How stable is the labeled protein in mammalian cells?

A. The stability of the tagged protein in the cell is dependent upon the stability of protein of interest. Labeled SNAP-tag fusion protein has been detected for up to 2 days in mammalian cells.

Q. Are SNAP-tag substrates stable to fixation?

A. Yes. SNAP-tag substrates are derived from organic fluorophores which are stable to fixation. Fluorescently-labeled SNAP-tag fusion proteins do not lose signal intensity in contrast to some GFP spectral variants. After labeling the SNAP-tag fusion proteins, the cells can be fixed with standard fixation methods such as para-formaldehyde, ethanol, methanol, methanol/acetone etc. without loss of signal.

Q. What conditions are recommended for SNAP-tag labeling *in vitro*?

A. The SNAP-tag labeling reaction is tolerant of a wide range of buffers. The requirements of the fusion partner should dictate the buffer selected. The following buffer guidelines are recommended: pH between 5.0 and 10.0, monovalent salts (e.g. sodium chloride) between 50 mM and 250 mM and at least 1 mM DTT. Non-ionic detergents can be added to 0.5% v/v if required, but SDS and other ionic detergents should be avoided entirely because they inhibit the activity of the SNAP-tag. Metal chelating reagents (e.g., EDTA and EGTA) also inhibit SNAP-tag activity and should be avoided.

Lambda Map

48,502 base pairs
GenBank Accession #: NC_001416

For ordering information, see Cloning Plasmids and DNAs in DNA Modifying Enzymes & Cloning Technologies.

There are no restriction sites for the following:

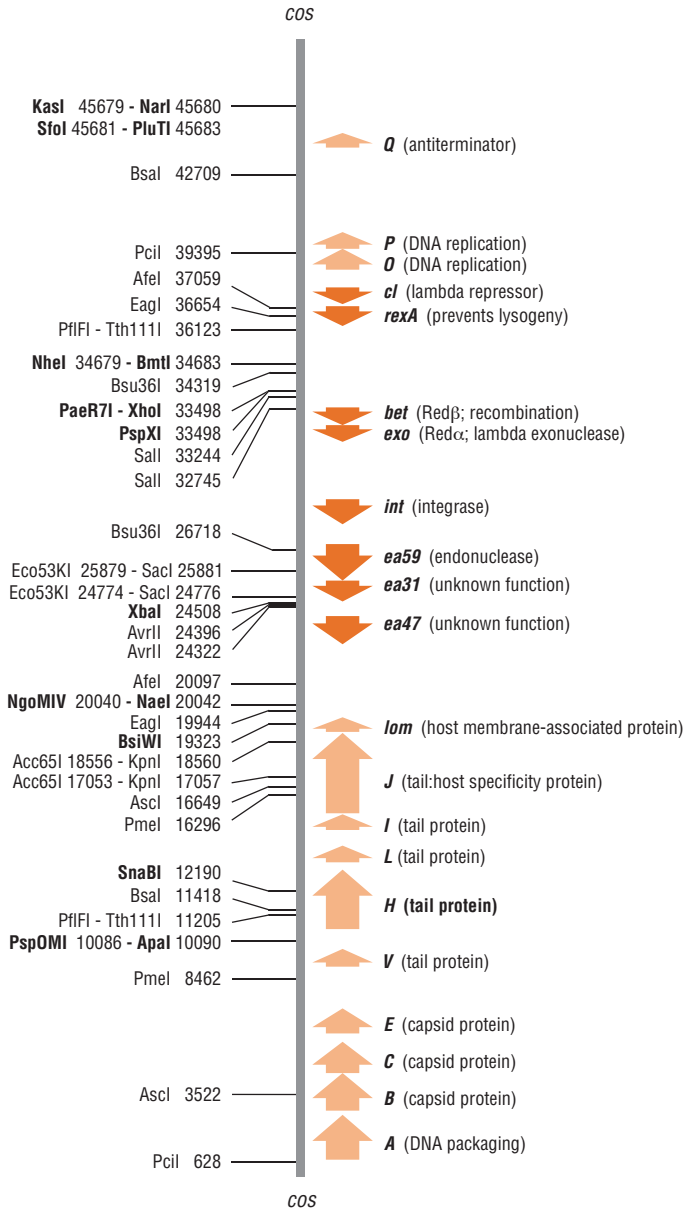
AbsI(x), AsiSI, FseI, MauBI(x), MreI(x), NotI, PacI, SfiI, SpeI, SrfI, SwaI

Lambda (λ) is a large, temperate *E. coli* bacteriophage with a linear, largely double-stranded DNA genome (1-5). At each end, the 5´ strand overhangs the 3´ strand by 12 bases. These single-stranded overhangs are complementary and anneal to form a cos site following entry into a host cell. Once annealed, the genome is a circular, completely double-stranded molecule which serves as a template for rolling-circle replication.

Many laboratory strains of lambda are derivatives of the strain λ *c*I857*ind*1 *Sam*7, which contains four point mutations relative to the wild type strain. The ind1 mutation in the cI gene creates a new HindIII site at 37584 not present in the wild type. All lambda products sold by NEB are λ *c*I857*ind*1 *Sam*7.

Numbering of the genome sequence begins at the first (5´-most) base of the left end and continues rightward from late genes nu1 and A towards the early genes. The map below shows the positions of all known ORFs larger than 200 codons.

Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand.**



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(4) Sanger, F., Coulson, A.R., Hong, G.F., Hill, D.F. and Petersen, G.B. (1982) *J. Mol. Biol.*, 162, 729–773.

(5) Daniels, D.L. et al. (1983). In R.W. Hendrix, J.W. Roberts, F.W. Stahl and R.A. Weisberg (Eds.), *Lambda II: Appendix*, New York: Cold Spring Harbor Press.

M13mp18 Map

GenBank Accession #: X02513

Revised sequence file available at www.neb.com.
For ordering information, see Cloning Plasmids and DNAs in DNA Modifying Enzymes & Cloning Technologies.

There are no restriction sites for the following:

AatII, AbsI(x), AcuI, AflII, AgeI, AhdI, AjuI(x), ApaI, ApaLI, AscI, AsiSI, AvrII, BbsI, BcgI, BciVI, BclI, BlpI, BmgBI, BmtI, BplI(x), BsaI, BsgI, BsiWI, BspEI, BspQI, BssHII, BssSI, BstAPI, BstBI, BstEII, BstXI, BstZ17I, EagI, EcoNI, EcoO109I, EcoRV, FseI, FspAI(x), HpaI, KflI(x), MauBI(x), MfeI, MluI, MreI(x), MteI(x), NcoI, NheI, NmeAIII, NotI, NruI, NsiI, PaeR7I, PaqCI, PasI(x), PflFI, PflMI, PfoI(x), PmeI, PmlI, PpuMI, PshAI, PspOMI, PspXI, PsrI(x), RsrII, SacII, SanDI, SapI, ScaI, SexAI, SfiI, SgrAI, SgrDI(x), SpeI, SrfI(x), StuI, StyI, Tth111I, XcmI, XhoI, ZraI

(x) = enzyme not available from NEB

Feature	Description	Coordinates
gene II	replication	6848-831 (cw)
gene X	replication	496-831
gene V	replication	843-1106
gene VII	minor coat protein	1108-1209
gene IX	minor coat protein	1206-1304
gene VIII	major coat protein	1301-1522
gene III	minor coat protein	1578-2852
gene VI	minor coat protein	2855-3193
gene I	phage assembly	3195-4241
gene XI (I*)	phage assembly	3915-4241
gene IV	phage assembly	4219-5499
ori	M13 origin (+) of replication	5487-5867
*lacZ*α	for α-complementation	6216-6722
MCS	multiple cloning site	6230-6286

(cw) = clockwise

M13 is a filamentous *E. coli* bacteriophage specific for male (F factor-containing) cells. Its genome is a circular, single-stranded DNA molecule 6407 bases in length, and contains 10 genes. A double-stranded form (RF) arises as an intermediate during DNA replication.

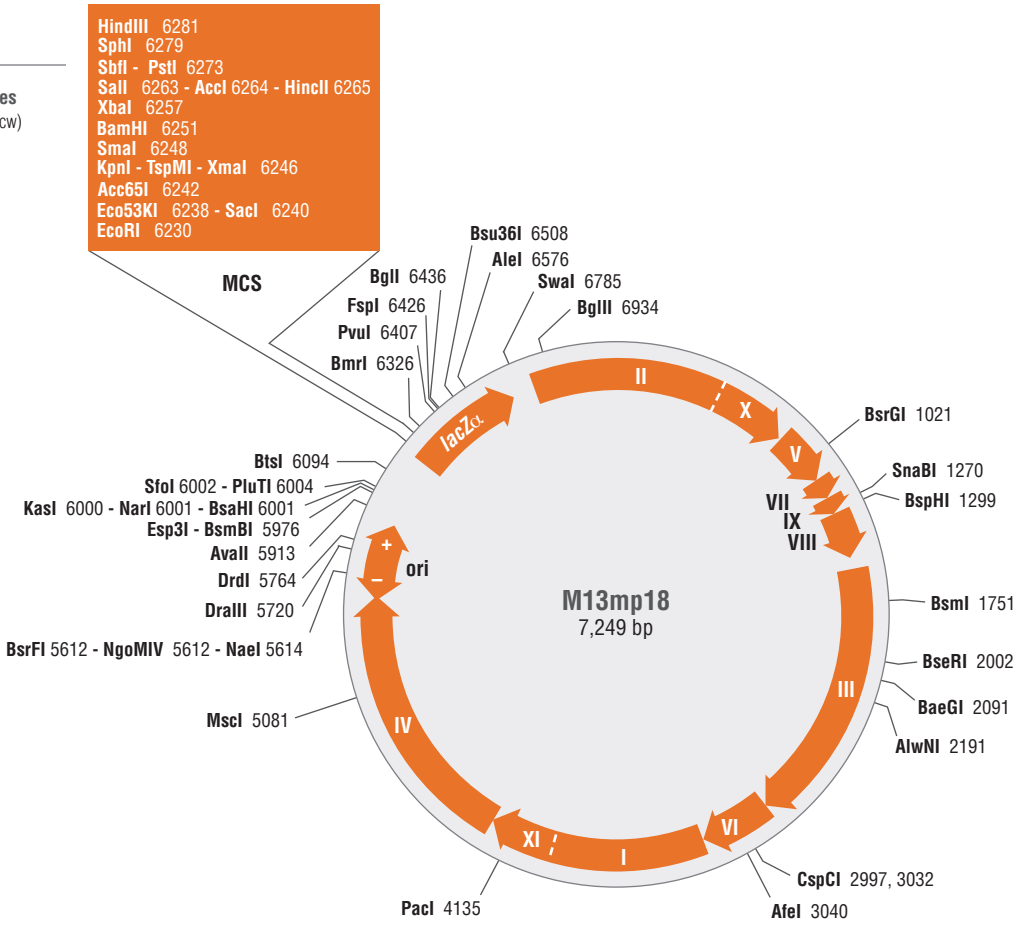
The M13mp phage vectors, derived from M13, contain the *lacZ*α gene and differ from each other by the cloning sites embedded within it. The location of cloning sites inside this gene allows screening for insertions using α-complementation. The map of M13mp18, whose multiple cloning site (MCS) was later employed to construct the plasmid pUC19, is shown below. M13mp19 is identical to M13mp18 except that the MCS region (6231-6288) is inverted.

The complete nucleotide sequences of M13mp18 and M13mp19 have been determined at New England Biolabs (1), resulting in several nucleotide changes relative to the previous sequence data (2,3).

Enzymes with unique restriction sites are shown in **bold** type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

M13 origin of replication arrows indicate the direction of synthesis of both the (+) and (–) strands.



References

(1) Stewart, F.J. (2002) unpublished observations.

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pBR322 Map

GenBank Accession #: J01749

For ordering information, see Cloning Plasmids and DNAs in DNA Modifying Enzymes & Cloning

There are no restriction sites for the following:

AbsI(x), Acc65I, AflII, AgeI, AjuI(x), AleI, AloI(x), ApaI, ArsI(x), AscI, AsiSI, AvrII, BaeI, BarI(x), BbvCI, BclI, BglII, BlpI, BmgBI, BpiI(x), BsaXI, BseRI, BsiWI, BsrGI, BssHII, BstBI, BstEII, BstXI, Bsu36I, CspCI, DraIII, Eco53KI, FalI(x), FseI, HpaI, KflI(x), KpnI, MauBI(x), MfeI, MluI, MreI(x), MteI(x), NcoI, NotI, NsiI, PacI, PaeR7I, PaqCI, PasI(x), PmeI, PmlI, PsiI, PspOMI, PspXI, PsrI(x), RsrII, SacI, SacII, SbfI, SexAI, SfiI, SgrDI(x), SmaI, SnaBI, SpeI, SrfI, StuI, SwaI, TspMI, XbaI, XcmI, XhoI, XmaI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
tet (Tc^R)	86-1276	pSC101
bla (Ap^R)	4153-3293	*Tn3*
rop	1915-2106	pMB1
origin	3122-2534	pMB1

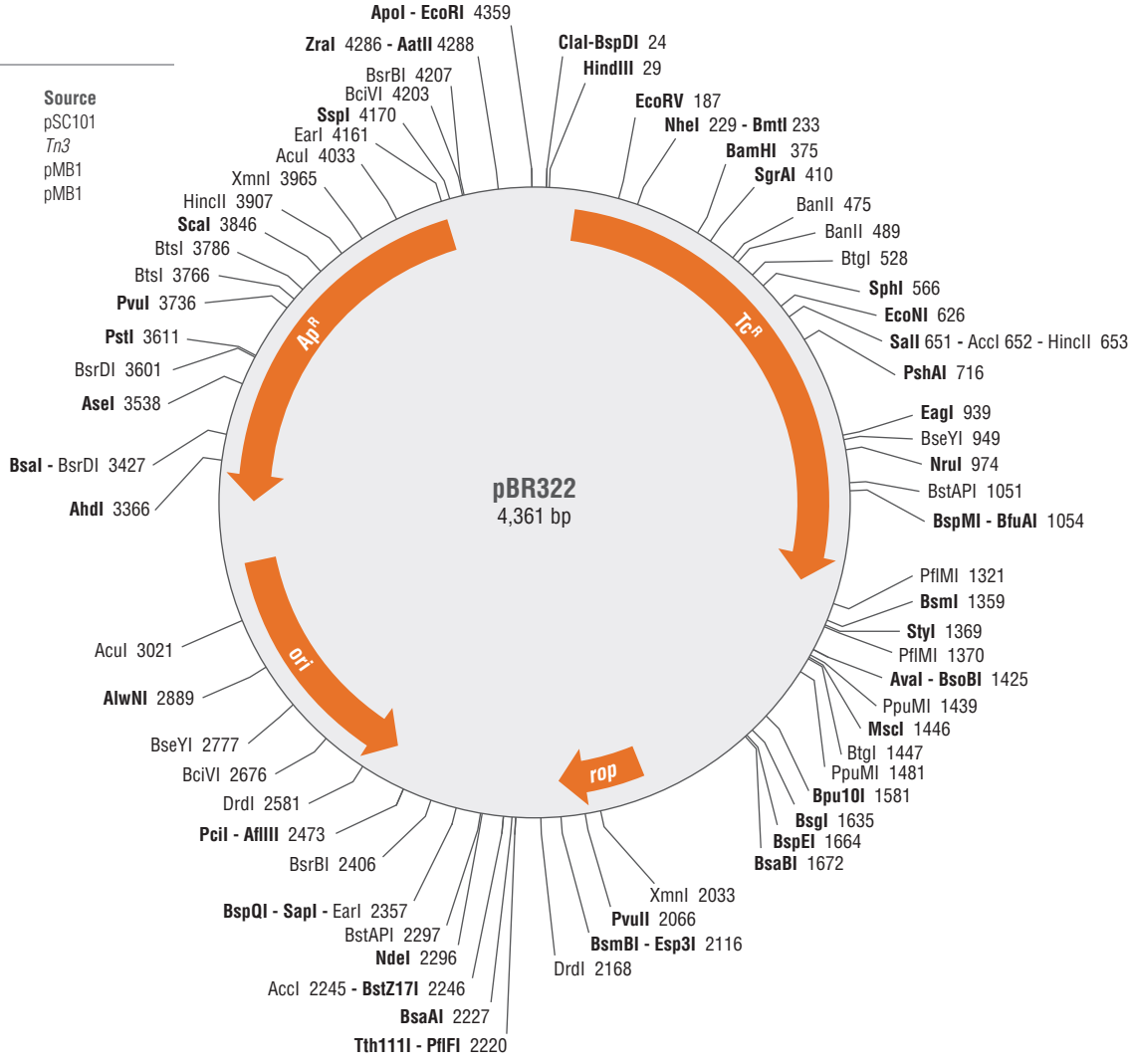
ori = origin of replication
Ap = ampicillin
Tc = tetracycline

pBR322 is an *E. coli* plasmid cloning vector containing the origin of replication from pMB1 (a plasmid in the ColE1 compatibility group; 1–3). The *rop* gene product, which regulates plasmid replication by stabilizing the interaction between RNAI and RNAII transcripts, maintains the copy number at about 20 per cell. However, pBR322 can be amplified with chloramphenicol or spectinomycin (4).

Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence.



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pKLAC2 Map

GenBank Accession #: EU196354

Sequence file available at www.neb.com.
For more information, see the *K.lactis* Protein Expression Kit (NEB #E1000).

There are no restriction sites for the following:

AatII, AbsI(x), Acc65I, AfeI, AflII, ApaI, AscI, AsiSI, AvrII, BbvCI, BlpI, Bpu10I, BsiWI, FseI, FspAI(x), KflI(x), KpnI, MauBI(x), MluI, MreI(x), MscI, MteI(x), PacI, PaqCI, PasI(x), PmeI, PmlI, PspOMI, PspXI, PsrI(x), RsrII, SfiI, SgrAI, SpeI, SrfI, SwaI, ZraI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
expression region:		
α-mating factor		
leader sequence	14-349	*K. lactis*
MCS	257-354	–
LAC4 TT region	371-953	*K. lactis*
AdH1 promoter region	1010-1712	*S. cerevisiae*
amdS	1713-3359	*A. nidulans*
LAC4 promoter region		
(5´ end)	4068-4648	*K. lactis*
origin	5102-5690	pMB1
bla (Ap^R)	6721-5861	Tn3
LAC4 promoter region		
(3´ end)	7475-9107	*K. lactis* (modified)

ori = origin of replication
Ap = ampicillin
TT = transcription terminator

pKLAC2 is an expression vector capable both of replication in *E. coli* and stable integration into the genome of the yeast *Kluyveromyces lactis* (1). It is designed for high-level expression of recombinant protein in *K. lactis* using the *K. lactis* Protein Expression Kit (NEB #E1000). pKLAC2 contains a universal multiple cloning site (MCS) that is compatible with all NEB expression systems.

In *E. coli*, it replicates using the pMB1 origin of replication from pBR322 (although the *rop* gene is missing) and carries the *bla* (Ap^R) marker for selection with ampicillin. Upon transformation of *K. lactis* GG799 competent cells (NEB #C1001), SacII- or BstXI-linearized pKLAC2 integrates into the *K. lactis* chromosome at the *LAC4* locus. Yeast transformants can be selected using the acetamidase selectable marker (*amdS*), which is expressed from the yeast *ADH1* promoter. Acetamidase expressed from pKLAC2 permits transformed cells to utilize acetamide as a sole nitrogen source on defined medium (2).

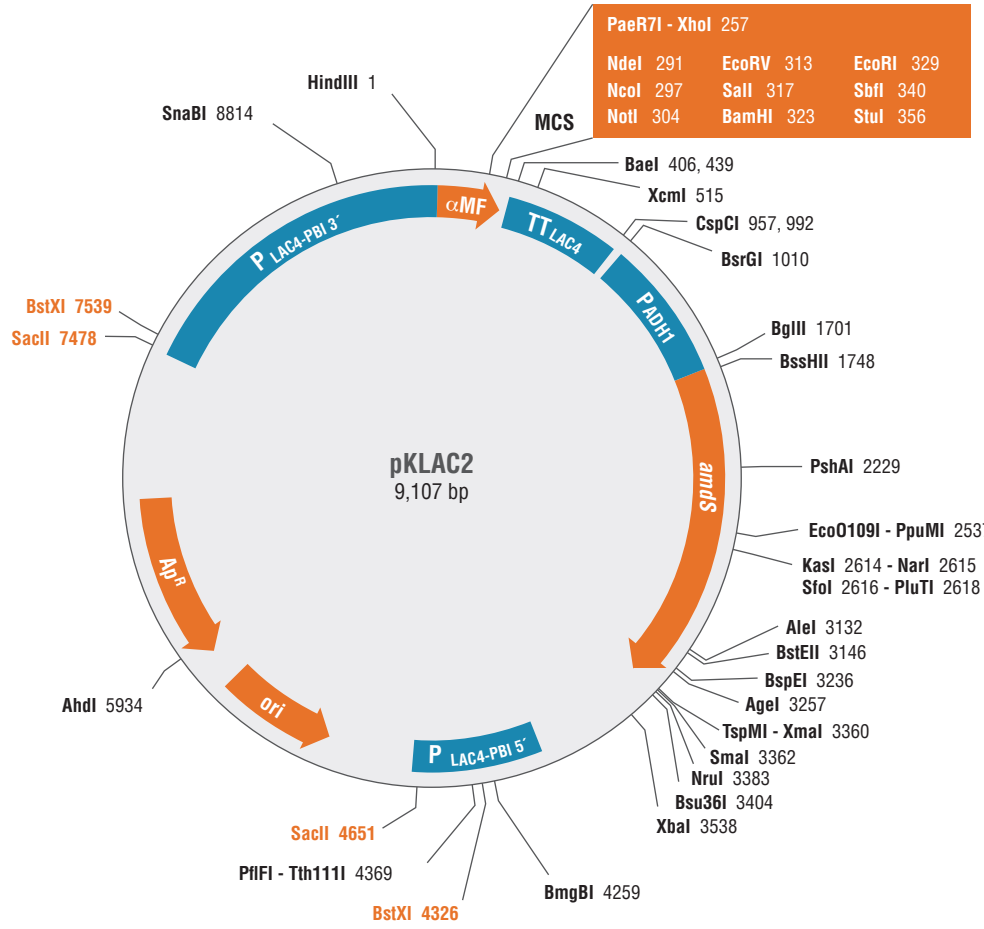
The multiple cloning site (MCS) is positioned to allow translational fusion of the K. lactis α-mating factor secretion domain (α-MF) to the N-terminus of the recombinant target protein. This directs the fusion protein to the general secretory pathway, but the α-MF domain is cleaved off in the Golgi apparatus by the Kex protease, resulting in secretion of the recombinant protein alone.

Expression of the recombinant fusion protein is driven by the *K. lactis LAC4* promoter, which has been modified to be transcriptionally silent in *E. coli* (1). This facilitates the cloning of proteins that are toxic to *E. coli*. This promoter is split such that when pKLAC2 is cleaved with SacII or BstXI, the recombinant protein and selectable marker are flanked by the two halves of the promoter. When these ends recombine with the *LAC4* promoter in the K. lactis chromosome, the result is integration of the recombinant fusion protein (driven by the *LAC4* promoter) and *amdS* upstream of the *LAC4* gene (driven by a duplicate copy of the *LAC4* promoter) (2).

Enzymes with unique restriction sites are shown in **bold** type and selected enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Components of coordinated regions are indented below the region itself.

pMB1 origin of replication coordinates include the region from the –35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. Promoter and transcription terminator coordinates represent cloned regions and not necessarily the precise functional elements.



References

(1) Colussi, P.A. and Taron, C.H. (2005) *Appl. Environ. Microbiol.*, 71, 7092–7098.

(2) van Ooyen, A.J. et al. (2006) *FEMS Yeast Res.*, 6, 381–392.

pMAL-c6T Map

Sequence file available at www.neb.com.
For ordering information, see Protein Expression & Purification.

There are no restriction sites for the following:

AatII, AbsI(x), Acc65I, AflII, AgeI, AjuI(x), AleI, ArsI(x), AscI, AsiSI, AvrII, BaeI, BarI(x), BbvCI, BmtI, BplI(x), BsaAI, BseRI, BsmFI, BspDI, BsrGI, BstBI, BstZ17I, ClaI, CspCI, DraIII, EcoNI, FalI(x), FseI, FspAI(x), KflI(x), KpnI, MauBI(x), MreI(x), MscI, MteI(x), NaeI, NcoI, NdeI, NgoMIV, NheI, NruI, NsiI, PacI, PaeR7I, PaqCI, PasI(x), PmeI, PmlI, PshAI, PspXI, PsrI(x), SacII, SexAI, SfiI, SgrAI, SmaI, SnaBI, SpeI, SphI, SrfI, StuI, StyI, SwaI, TspMI, XbaI, XhoI, XmaI, XmnI, ZraI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
lacI^q	80-1162	*E. coli*
Ptac	1405-1432	–
expression ORF	1527-2761	–
malE	1527-2721	*E. coli*
MCS	2722-2761	–
bla (ApR)	3101-3961	*Tn3*
origin	4049-4637	pMB1
rop	5007-5198	pMB1

pMAL-c6T is an *E. coli* plasmid cloning vector designed for recombinant protein expression and purification using the NEBExpress MBP Fusion and Purification System (NEB #E8201) (1–3). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322.

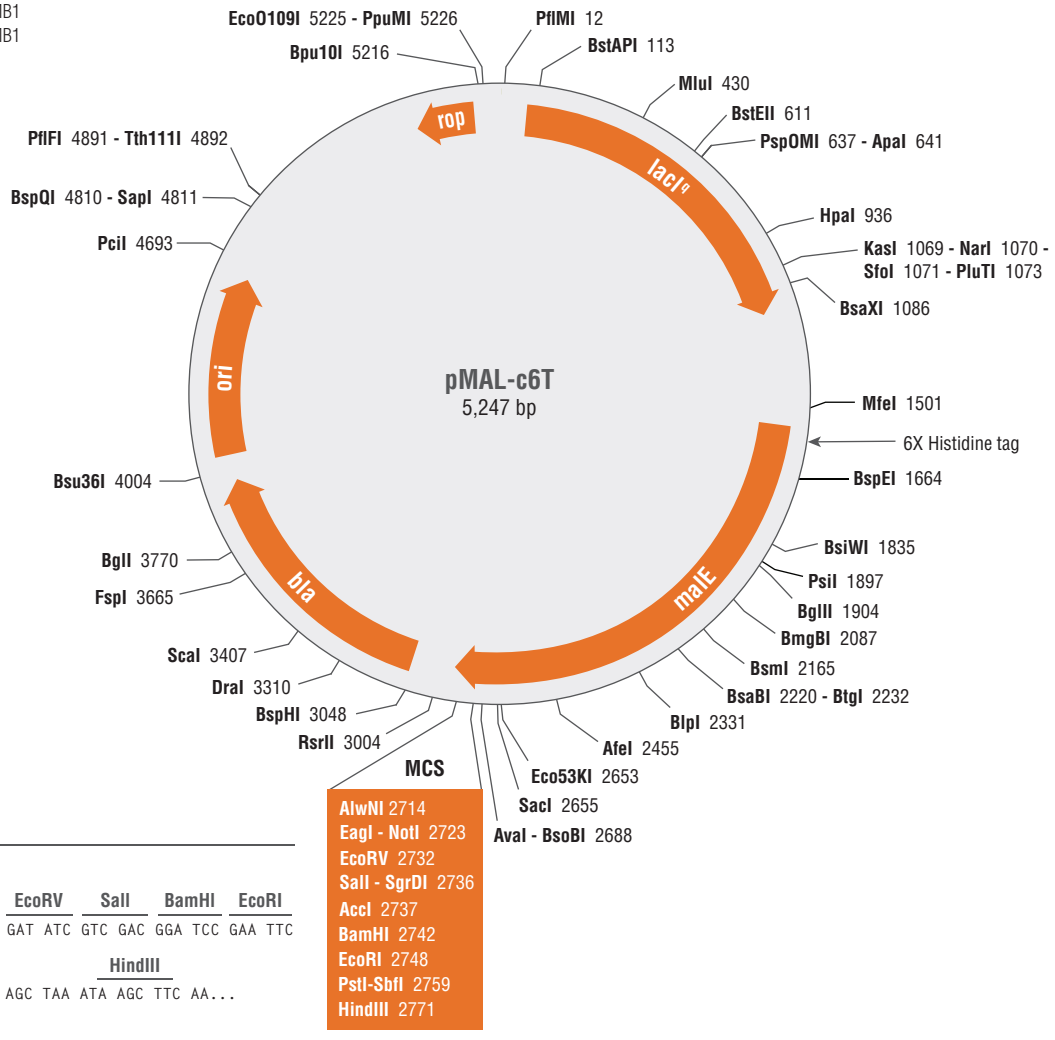
The multiple cloning site (MCS) is positioned to allow translational fusion of the *E. coli* maltose binding protein (MBP, encoded by the malE gene lacking its secretory signal sequence) to the *N*-terminus of the cloned target protein, thus resulting in an MBP-fusion protein localized in the cytoplasm. The pMAL-c6T vector contains a multiple cloning site (MCS) that is compatible with other NEB expression systems and is followed by stop codons in all three reading frames. In this vector, MBP has been engineered for tighter binding to amylose. This allows easy purification of the fusion protein, and the MBP domain can be subsequently removed using TEV Protease (NEB #P8112).

Transcription of the gene fusion is controlled by the inducible "tac" promoter (P_{tac}). Basal expression from P_{tac} is minimized by the binding of the Lac repressor, encoded by the *lacI*^{q} gene, to the *lac* operator immediately downstream of P_{tac}. A portion of the *rrnB* operon containing two terminators, derived from the vector pKK233-2, prevents transcription originating from P_{tac} from interfering with plasmid functions.

Enzymes with unique restriction sites are shown in **bold** type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

The pMB1 origin of replication includes the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point (labeled "ori") and the rop gene, which controls expression of the RNAII transcript. *bla* (Ap^R) gene coordinates include the signal sequence.



pMAL-c6T Polylinker:

	AlwNI	NotI/EagI	EcoRV	SalI	BamHI	EcoRI
5´ *malE* ...	ATG CTG ATG	GGC GGC CGC	GAT ATC	GTC GAC	GGA TCC	GAA TTC

	PstI/SbfI	HindIII
	CCT GCA GGT AATTAA ATA	AGC TAA ATA AGC TTC AA...

References

(1) Guan, C. et al. (1987) *Gene*, 67, 21–30.

(2) Maina, C.V. et al. (1988) *Gene*, 74, 365–373.

(3) Riggs, P.D. (1992). In F.M. Ausubel, et al. (Eds.), *Current Prot. in Molecular Biol.* New York: John Wiley & Sons, Inc.

pMiniT 2.0 Map

Sequence available at www.neb.com
For more information, see the NEB PCR Cloning Kit (NEB #E1202, #E1203).

There are no restriction sites for the following:

AbsI(x), Acc65I, AccI, AflII, AgeI, AjuI(x), AleI, AloI(x), ApaI, ArsI(x), AscI, AsiSI, AvrII, BaeI, BanII, BarI(x), BbsI, BbvCI, BclI, BglII, BlpI(x), BmgBI, BmtI, BpiI(x), Bpu10I, BsaI, BsaAI, BsaBI, BseRI, BsgI, BsiWI, BsmFI, BsmI, BspDI, BspEI, BsrGI, BssHII, BstAPI, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, ClaI, CspCI, DraIII, Eco53kI, EcoNI, EcoO109I, EcoRV, FalI(x), FseI, FspAI(x), HincII, HindIII, HpaI, KasI, KflI(x), KpnI, MauBI(x), MfeI, MluI, MreI(x), MscI, MteI(x), NaeI, NarI, NcoI, NgoMIV, NheI, NsiI, PasI(x), PflFI, PflMI, PfoI(x), PluTI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PsrI(x), PvuII, RsrII, SacI, SacII, SalI, SexAI, SfiI, SfoI, SgrAI, SgrDI(x), SmaI, SnaBI, SpeI, SphI, SrfI, StuI, StyI, SwaI, TspMI, Tth111I, XbaI, XcmI, XmaI

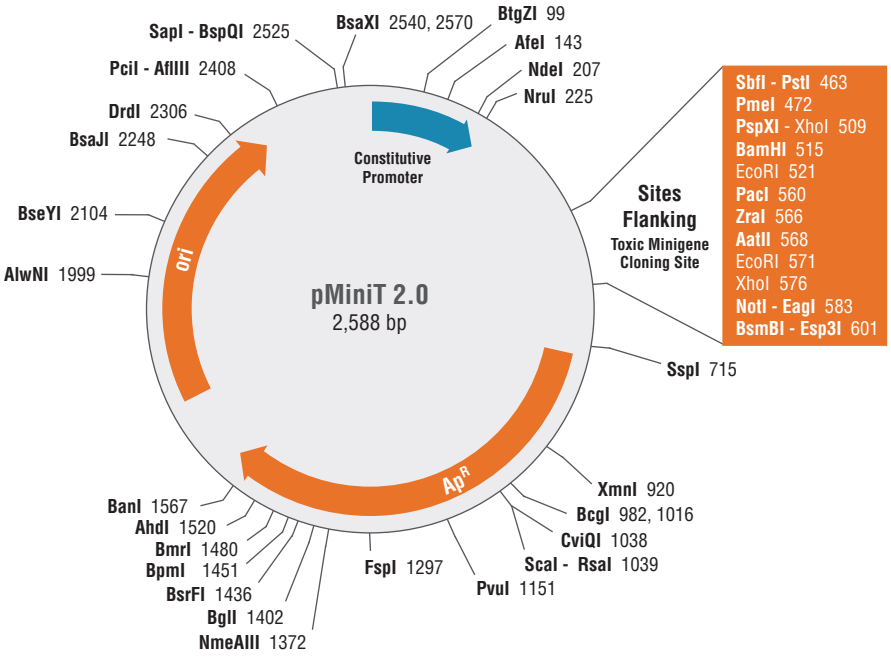
(x) = enzyme not available from NEB

Feature	Coordinates	Source
Constitutive promoter	1-214	pNK2138
SP6 promoter	479-496	SP6
Toxic minigene	541-549	–
Synthetic T7 promoter	619-602	T7
bla (Ap^R)	733-1593	*Tn3*
origin	1764-2352	pUC19

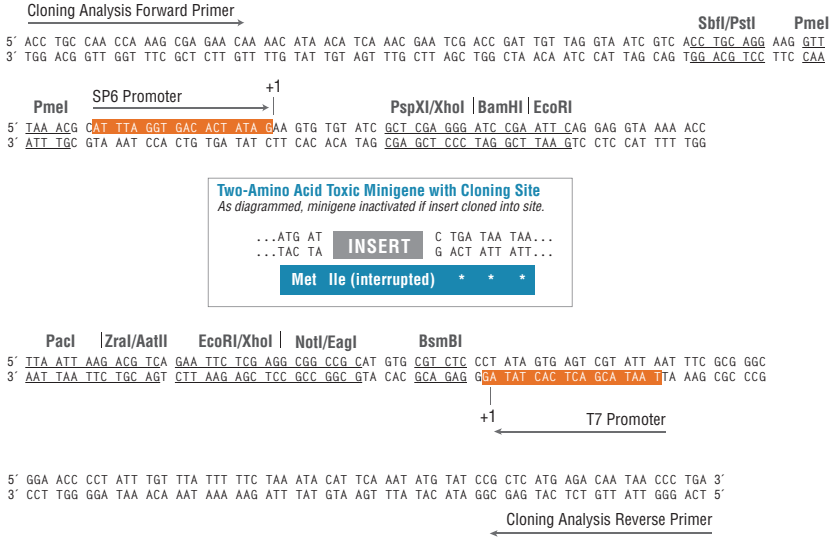
pMiniT 2.0 is an *E. coli* plasmid cloning vector designed for cloning blunt-ended or single-base overhang PCR products, or amplicons, using the NEB PCR Cloning Kit (NEB #E1202, #E1203). The pMiniT 2.0 also enables in vitro transcription using SP6 and T7 promoters. It is compatible with Golden Gate Assembly as the BsaI site has been removed from the Ampicillin resistance gene.

In *E. coli*, it replicates using the pMB1 origin of replication from pUC19 and carries the bla (Ap^R) marker for selection with ampicillin. pMiniT2.0 contains a toxic minigene that is under the control of a constitutive promoter. If the pMiniT 2.0 vector recircularizes without an insert, the toxic minigene it will cause lethal inhibition of protein synthesis and no colony will result. If the pMiniT 2.0 Vector carries an insert, a colony will grow.

The map shown below displays the construct formed if no insert is present. Unique restriction sites are shown in **bold**. Additional restriction sites that can be used for subcloning are also shown. Expanded box below shows location of sequencing primers, restriction sites for subcloning or linearization for *in vitro* transcription, RNA Polymerase promoter sequences and placement of insertion site within the toxic minigene. **Coordinates indicate position of cutsite on the top strand**.



Features within Sequence Flanking the Toxic Minigene/Cloning Site:



APPENDIX

pNEB206A Map

Sequence file available at www.neb.com.

There are no restriction sites for the following:

AbsI(x), Acc65I, AccI, AfeI, AflII, AgeI, AjuI(x), AleI, AloI(x), ApaI, ArsI(x), AsiSI, AvaI, AvrII, BaeI, BarI(x), BbsI, BclI, BfuAI, BglII, BlpI, BmgBI, BmtI, BpiI(x), BsaAI, BsaBI, BsgI, BsiWI, BsmFI, BsmI, BsoBI, BspDI, BspEI, BspMI, BsrGI, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, BtgZI, ClaI, CspCI, DraIII, EagI, EcoNI, EcoRV, FalI(x), FseI, FspAI(x), HincII, HpaI, KflI(x), KpnI, MauBI(x), MfeI, MluI, MreI(x), MscI, MteI(x), NaeI, NcoI, NgoMIV, NheI, NotI, NruI, NsiI, PaeR7I, PaqCI, PasI(x), PflFI, PflMI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PspXI, PsrI(x), RsrII, SacII, SalI, SexAI, SfiI, SgrAI, SgrDI(x), SmaI, SnaBI, SpeI, SphI, SrfI, StuI, StyI, SwaI, TspMI, Tth111I, XcmI, XhoI, XmaI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
*lacZ*α	505-146	–
cloning site	430-461	–
origin	1491-903	pUC19
bla (Ap^R)	2522-1662	Tn3

ori = origin of replication
Ap = ampicillin

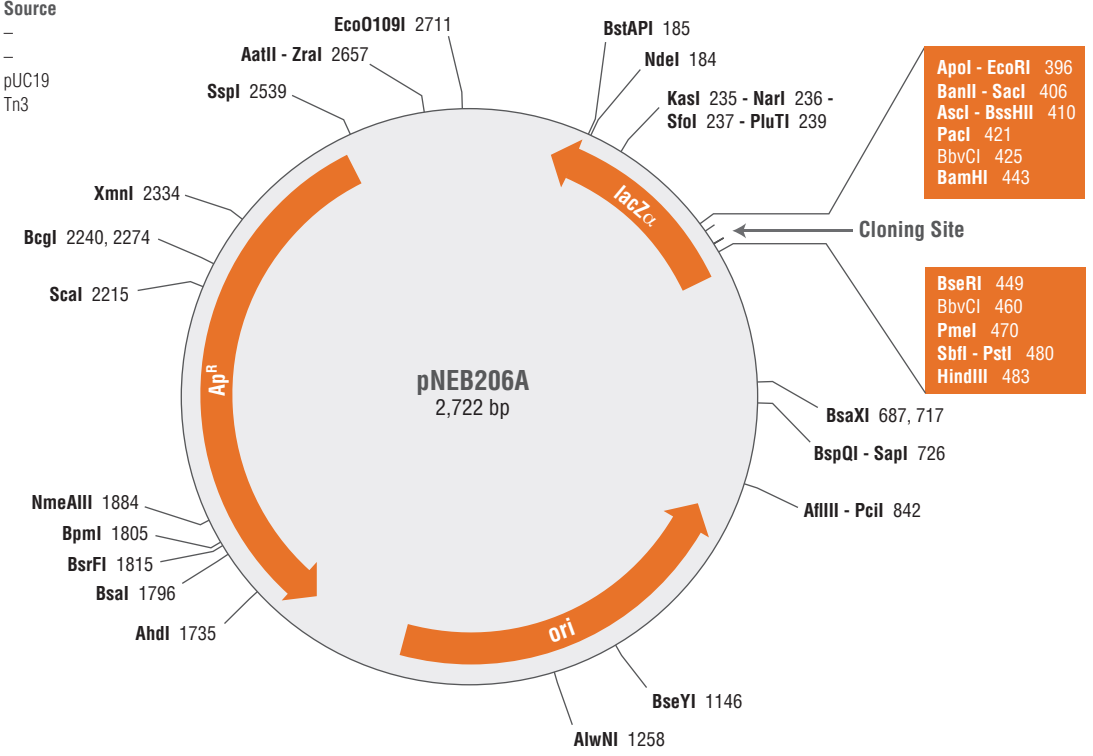
pNEB206A is an *E. coli* plasmid vector designed for fast and efficient cloning of PCR products to be used in conjunction with USER Enzyme (NEB #M5505; 1). It is derived from pNEB193 containing the high-copy pUC19 origin of replication and lacZα gene for screening of insertions at the cloning site using α-complementation (2).

The plasmid is supplied in a linearized form 2,706 bp in length (with bp 438-453 excised from the circular form), flanked by two noncomplementary 8-base 3´ overhangs at the intended cloning site. Amplification with deoxyuridine-containing primers and subsequent treatment (as defined in the protocol "Cloning with USER Enzyme" found on our website), results in PCR products with 5´ overhangs complementary to those in pNEB206A. These products can be directionally cloned into pNEB206A at high efficiency without the use of restriction enzymes or DNA ligase, forming recombinant circular molecules.

Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence. Cloning site coordinates include those bases in the circular form that are single-stranded in or missing from the supplied linear form.



pNEB206A (linearized form) cloning site:

```
          Eco53KI
            SacI        BssHII
   EcoRI            AscI       PacI       BbvCI
agtgAATTCGAGCTCAGGCGCGCCTTAATTAAGCTGAGGGAAAGT
tcacTTAAGCTCGAGTCCGCGCGGAATTAATTCGACT
      .          .          .          .
     400        410        420        430
...S  N  S  S  L  R  A  K  I  L  S  L  S  L
```

```
                                  SbfI
      BbvCI    PmeI       PstI      HindIII
        TCAGCGTTTAAACCCTGCAGGAAGCTTGGcgtaatcatggtcat
TACAGAGGAGTCGCAAATTTGGGACGTCCTTCGAACCgcattagtaccagta
      .          .          .          .          .
     460        470        480        490        500
T  E  E  A  N  L  G  Q  L  F  S  P  T  I  M  T  M
                                                 |
                                                 |
                                  lacZα translational start
```


References
(1) Bitinaite, J. and Vaiskunaite, R. (2003) unpublished observations.
(2) Yanisch-Perron, C. et al. (1985) *Gene*, 33, 103–119.

pSNAP$_f$ Map

Sequence file available at www.neb.com.
For ordering information, see Cloning Vectors in Cellular Analysis.

There are no restriction sites for the following:

AbsI(x), AfeI, AflII, AjuI(x), AlfI(x), AloI(x), AsiSI, BaeI, BarI(x), BbvCI, BlpI, BpII(x), BsiWI, BsmBI, BspDI, BspEI, BstAPI, BstBI, BstEII, ClaI, EcoNI, Esp3I, FseI, FspAI(x), KflI(x), MauBI(x), MreI(x), MteI(x), PasI(x), PfoI(x), PshAI, PsrI(x), SexAI, SgrAI, SrfI, StuI, XcmI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
CMV promoter	251-818	–
expression region	915-1564	–
MCS1	915-965	–
SNAP$_f$	969-1514	–
MCS2	1515-1564	–
IRES	1910-2500	*ECMV*
Neo^R	2536-3339	*Tn5*
origin	4094-4682	pUC19
bla (Ap^R)	4853-5713	*Tn3*

ori = origin of replication
Ap = ampicillin
Neo = neomycin
IRES = internal ribosomal entry site

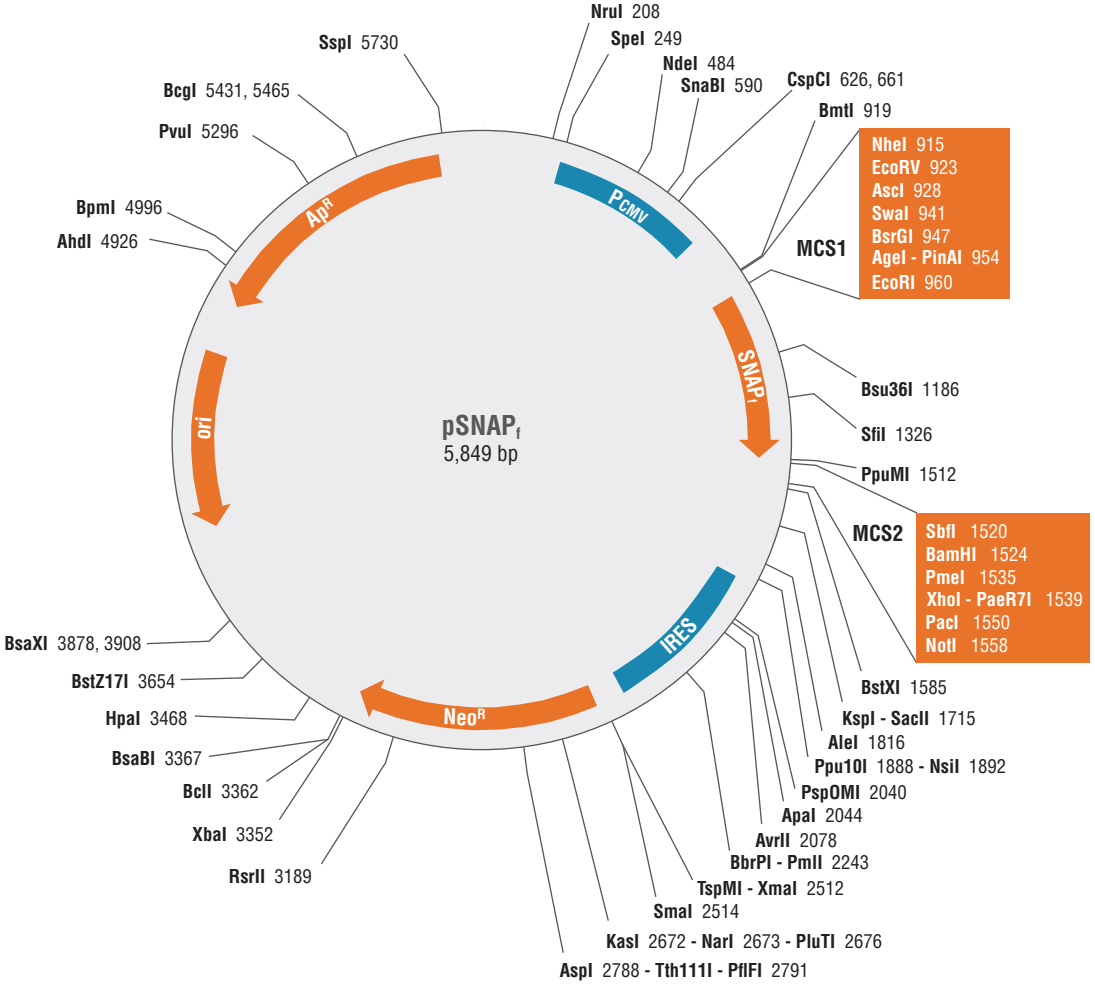
pSNAP$_f$ Vector is a mammalian expression plasmid intended for the cloning and stable or transient expression of SNAP-tag® protein fusions in mammalian cells. This plasmid encodes SNAP$_f$, a SNAP-tag protein, which is expressed under control of the CMV promoter. SNAPf is an improved version of the SNAP-tag which exhibits faster labeling kinetics. The SNAP-tag is a novel tool for protein research, allowing the specific, covalent attachment of virtually any molecule to a protein of interest. The SNAP-tag is a small protein based on human O6-alkylguanine-DNA-alkyltransferase (hAGT). SNAP-tag substrates are derivatives of benzyl purines and benzyl pyrimidines. In the labeling reaction, the substituted benzyl group of the substrate is covalently attached to the SNAP-tag. Use of this system involves two steps: sub-cloning and expression of the protein of interest as a SNAP-tag fusion, and labeling of the fusion with the SNAP-tag substrate of choice.

Codon usage of the gene is optimized for expression in mammalian cells. pSNAP$_f$ contains two multiple cloning sites to allow cloning of the fusion partner as a fusion to the N- or C-terminus of the SNAP-tag. The expression vector has an Internal Ribosome Entry Site (IRES) and a neomycin resistance gene downstream of the SNAP-tag for the efficient selection of stable transfectants.

Enzymes with unique restriction sites are shown in **bold** type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Component genes or regions of fusion ORFs are indented below the ORF itself.

pUC19 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence.



MCS1

```
     NheI          AscI          SwaI    BsrGI          EcoRI
...GCTAGC GATATCGGCG CGCCAGCATT TAAATCTGTA CAGACCGGTG AATTC
   CGATCG CTATAGCCGC GCGGTCGTAA ATTTAGACAT GTCTGGCCAC TTAAG...
```

MCS2

```
                                    XhoI
     SbfI       BamHI      PmeI                PacI        NotI
...CCTGCA GGCGGATCCG CGTTTAAACT CGAGGTTAAT TAATGAGCGG CCGC
   GGACGT CCGCCTAGGC GCAAATTTGA GCTCCAATTA ATTACTCGCC GGCG...
```

pTXB1 Map

Sequence file available at www.neb.com.
For ordering information, see the IMPACT Kit (NEB #E6901).

There are no restriction sites for the following:

Acc65I, AflII, AjuI(x), AleI, ArsI(x), AscI, AsiSI, AvrII, BaeI, BbvCI, BglII, BlpI(x), BmgBI, Bpu10I, BseRI, BspDI, BstBI, Bsu36I, ClaI, CspCI, Eco53KI, FalI(x), FseI, FspAI(x), HindIII, KflI(x), KpnI, MauBI(x), MscI, MteI(x), NcoI, NsiI, PacI, PaqCI, PasI(x), PmlI, PpuMI, PsrI(x), RsrII, SacI, SanDI(x), SbfI, SexAI, SfiI, SgrDI(x), SmaI, SnaBI, SrfI, TspMI, XmaI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
bla (Ap^R)	140-1000	*Tn3*
M13 origin	1042-1555	M13
origin	1666-2254	pMB1
rop	2814-2623	pMB1
lacI	4453-3371	*E. coli*
T7 promoter	5637-5654	T7
expression ORF	5725-6558	–
MCS	5722-5775	–
Mxe GyrA intein	5776-6369	*M. xenopi*
CBD	6400-6558	*B. circulans*

ori = origin of replication
Ap = ampicillin

pTXB1 is an *E. coli* plasmid cloning vector designed for recombinant protein expression, purification, and ligation using the IMPACT™ Kit (NEB #E6901) (1,2). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, pTXB1 also contains an M13 origin of replication.

The multiple cloning site (MCS) is positioned to allow translational fusion of the Mxe GyrA intein tag to the C-terminus of the cloned target protein (2,3). The chitin binding domain (CBD) from *B. circulans*, fused to the C-terminus of the intein, facilitates purification of the intein-target protein precursor.

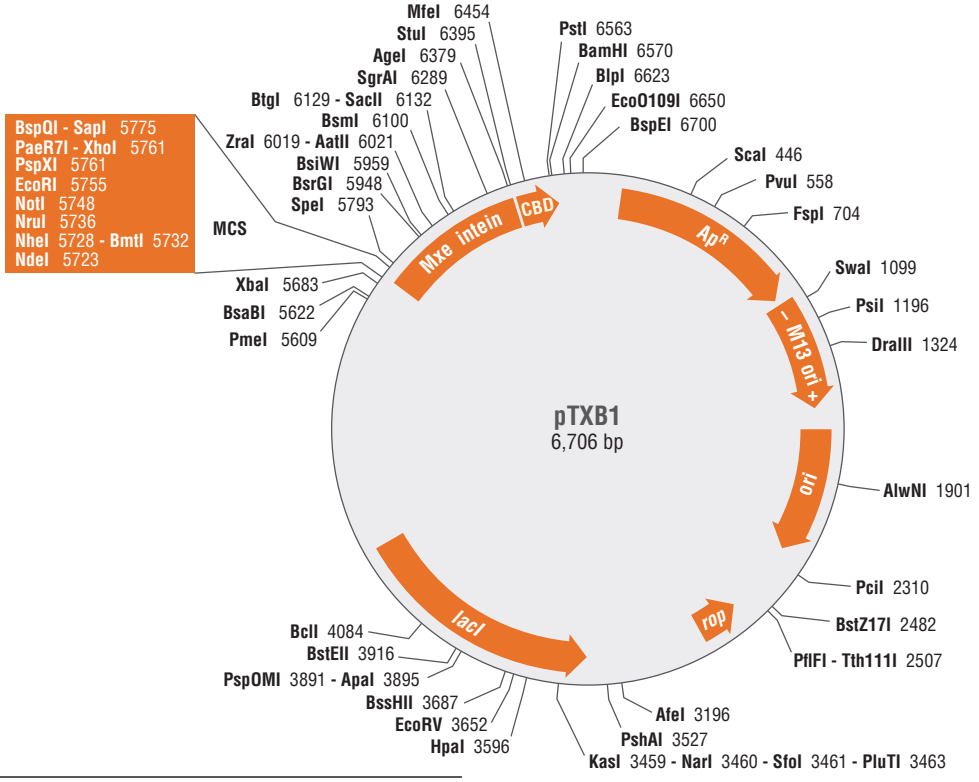
Transcription of the gene fusion is controlled by the inducible T7 promoter, requiring E. coli strains containing integrated copies of the T7 RNA polymerase gene [e.g., C2566 or BL21(DE3)] for expression. Basal expression from the T7 promoter is minimized by the binding of the Lac repressor, encoded by the lacI gene, to the lac operator immediately downstream of the T7 promoter (4). Translation of the fusion utilizes the translation initiation signal (Shine Dalgarno sequence) from the strongly expressed T7 gene 10 protein (φ10).

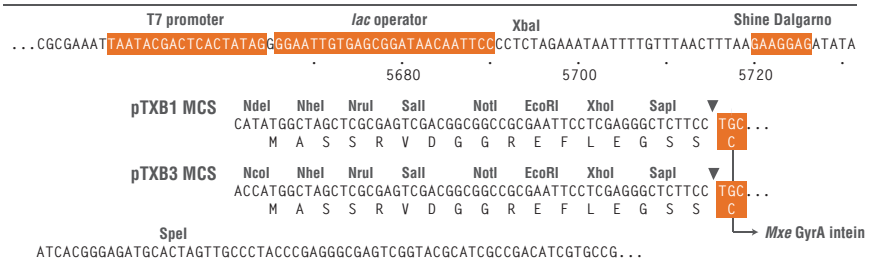
pTXB1 and pTXB3 are identical except for the MCS regions: pTXB1 contains an NdeI site, and pTXB3 an NcoI site, overlapping the initiating methionine codon of the intein fusion gene. The N-terminal cysteine residue ("Cys1") of the intein is shaded.

Enzymes with unique restriction sites are shown in **bold** type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Component genes or regions of fusion ORFs are indented below the ORF itself.

pMB1 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. For the M13 origin, the arrow shows the direction of synthesis of the (+) strand, which gets packaged into phage particles. *bla* (Ap^R) gene coordinates include the signal sequence.





References

(1) Chong, S. et al. (1997) *Gene*, 192, 271–281.
(2) Evans, T.C., Benner and Xu, M.-Q. (1998) *Protein Sci.*, 7, 2256–2264.
(3) Southworth, M.W. et al. (1999) *Biotechniques*, 27, 110–120.
(4) Dubendorff, J.W. and Studier, F.W. (1991) *J. Mol. Biol.*, 219, 45–59.

pTYB21 Map

Sequence file available at www.neb.com.
For ordering information, see the IMPACT Kit (NEB #E6901).

There are no restriction sites for the following:

AatII, AbsI(x), AflII, AgeI, AjuI(x), AscI, AsiSI, AvrII, BbvCI, BmgBI, BpiI(x), BseRI, BsiWI, BsmI, BspDI, Bsu36I, ClaI, CspCI, FalI(x), FseI, FspAI(x), KflI(x), MauBI(x), MreI(x), MteI(x), NruI, NsiI, PacI, PaeR7I, PaqCI, PasI(x), PpuMI, PspXI, PsrI(x), RsrII, SexAI, SfiI, SgrAI, SmaI, SnaBI, SrfI, TspMI, XhoI, XmaI, ZraI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
bla (Ap^R)	140-1000	*Tn3*
M13 origin	1042-1555	M13
origin	1666-2254	pMB1
rop	2814-2623	pMB1
lacI	4453-3371	*E. coli*
T7 promoter	5637-5654	T7
expression ORF	5725-7368	–
MCS	7301-7361	–
Sce VMA intein	5770-7299	*S. cerevisiae*
CBD	6595-6747	*B. circulans*

ori = origin of replication
Ap = ampicillin

pTYB21 is an *E. coli* plasmid cloning vector designed for recombinant protein expression and purification using the IMPACT™ Kit (NEB #E6901) (1,2). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, pTYB21 also contains an M13 origin of replication.

The multiple cloning site (MCS) is positioned to allow translational fusion of the *Sce* VMA intein tag to the N-terminus of the cloned target protein (2). The chitin binding domain (CBD) from *B. circulans*, facilitates purification of the intein-target protein precursor.

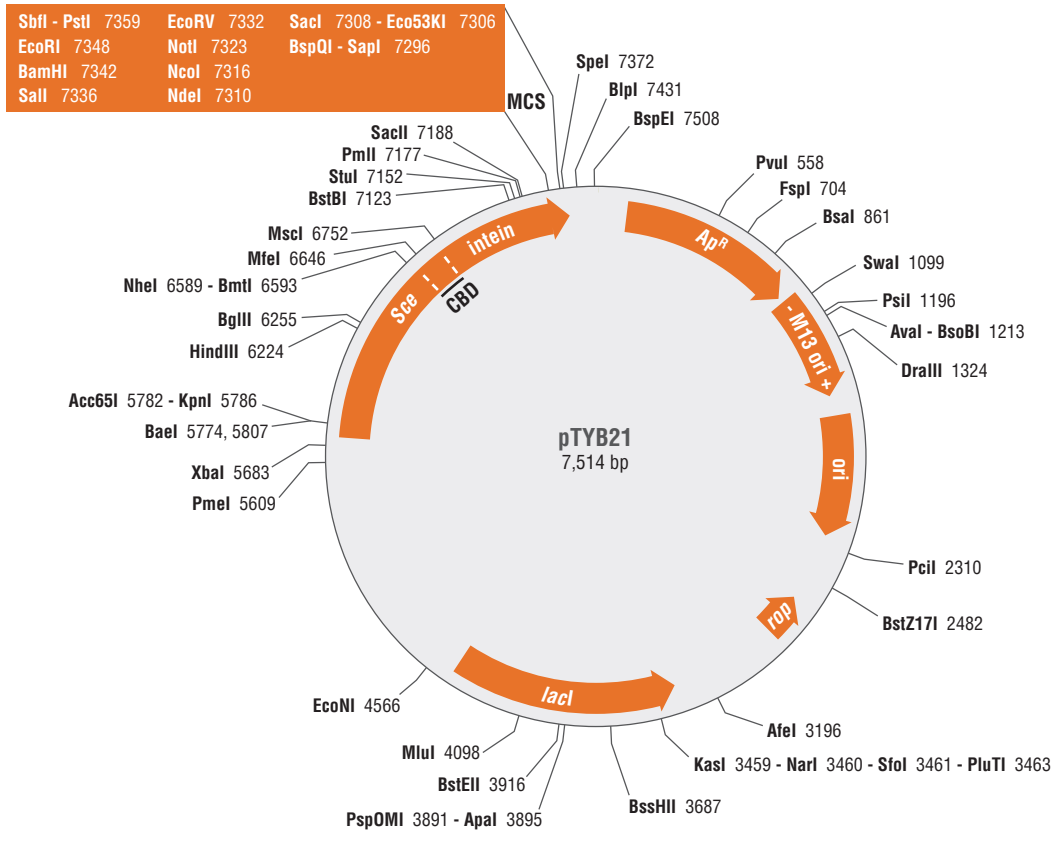
Transcription of the gene fusion is controlled by the inducible T7 promoter, requiring *E. coli* strains containing integrated copies of the T7 RNA polymerase gene [e.g., C2566 or BL21(DE3)] for expression. Basal expression from the T7 promoter is minimized by the binding of the Lac repressor, encoded by the *lacI* gene, to the *lac* operator immediately downstream of the T7 promoter (3). Translation of the fusion utilizes the translation initiation signal (Shine Dalgarno sequence) from the strongly expressed T7 gene 10 protein (φ10).

pTYB21 contains a SapI site which allows for cloning of a target gene without any extra amino acids. pTYB22 is identical to pTYB21 except for the MCS regions (see below). pTYB22 contains an NdeI site overlapping the initiating methionine codon of the intein fusion gene. pTYB21 differs from pTYB11 in that it contains a universal MCS that is compatible with all NEB expression systems.

Enzymes with unique restriction sites are shown in **bold** type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Component genes or regions of fusion ORFs are indented below the ORF itself.

pMB1 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. For the M13 origin, the arrow shows the direction of synthesis of the (+) strand, which gets packaged into phage particles. *bla* (Ap^R) gene coordinates include the signal sequence.



Sce VMA Intein ... S D H Q F L L G S Q

...TAATACGACTCACTATAGGGGAATTGTG...GAAGACGATTATTATGGGATTACTTTATCTGATGATTCTGATCATCAGTTTTTGCTTGGATCTCAG

5650 7220 7240 7260 7280

pTYB21 MCS

Sce VMA Intein → SacI SapI NdeI NcoI NotI SalI BamHI EcoRI SbfI PstI

GTTGTTGTACAGAAC GGAAGAGCTCATATGTCCATGGGCGGCCGCGATATCGTCGACGGATCCGAATTCCCTGCAGGTAATTAAATAAC...

V V V Q N G R A H M S M G G R D I V D G S E F P A G N *

pTYB22 MCS

Sce VMA Intein → BsmI NdeI NcoI NotI SalI BamHI EcoRI SbfI PstI

GTTGTTGTACAGAAT GCTGGTCATATGTCCATGGGCGGCCGCGATATCGTCGACGGATCCGAATTCCCTGCAGGTAATTAAATAAC...

V V V Q N A G H M S M G G R D I V D G S E F P A G N *

References

(1) Chong et al. (1996) *J. Biol. Chem.*, 271, 22159–22168

(2) Chong et al. (1998) *NAR*, 26, 5109–5115.

(3) Dubendorff, J.W. and Studier, F.W. (1991) *J. Mol. Biol.*, 219, 45–59.

GenBank Accession #: L09137

For ordering information, see Cloning Plasmids and DNAs in DNA Modifying Enzymes & Cloning Technologies.

There are no restriction sites for the following:

AbsI(x), AfeI, AflII, AgeI, AjuI(x), AleI, AloI(x), ApaI, ArsI(x), AscI, AsiSI, AvrII, BaeI, BarI(x), BbsI, BbvCI, BclI, BglII, BlpI, BmgBI, BmtI, BplI(x), Bpu10I, BsaAI, BsaBI, BseRI, BsgI, BsiWI, BsmFI, BsmI, BspDI, BspEI, BsrGI, BssHII, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, BtgZI, ClaI, CspCI, DraIII, EagI, EcoNI, EcoRV, FalI(x), FseI, FspAI(x), HpaI, KflI(x), MauBI(x), MfeI, MluI, MreI(x), MscI, MteI(x), NaeI, NcoI, NgoMIV, NheI, NotI, NruI, NsiI, PacI, PaeR7I, PaqCI, PasI(x), PflFI, PflMI, PmeI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PspXI, PsrI(x), RsrII, SacII, SexAI, SfiI, SgrAI, SgrDI(x), SnaBI, SpeI, SrfI, StuI, StyI, SwaI, Tth111I, XcmI, XhoI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
*lacZ*α	469-146	–
origin	1455-867	pMB1 (mutant)
bla (Ap[R])	2486-1626	*Tn3*

ori = origin of replication
Ap = ampicillin

pUC19 is a small, high-copy number E. coli plasmid cloning vector containing portions of pBR322 and M13mp19 (1). It contains the pMB1 origin of replication from pBR322, but it lacks the *rop* gene and carries a point mutation in the RNAII transcript (G 2975 in pBR322 to A 1308 in pUC19; 2). These changes together result in a temperature-dependent copy number of about 75 per cell at 37°C and > 200 per cell at 42°C (2,3). The multiple cloning site (MCS) is in frame with the *lacZ*α gene, allowing screening for insertions using α-complementation.

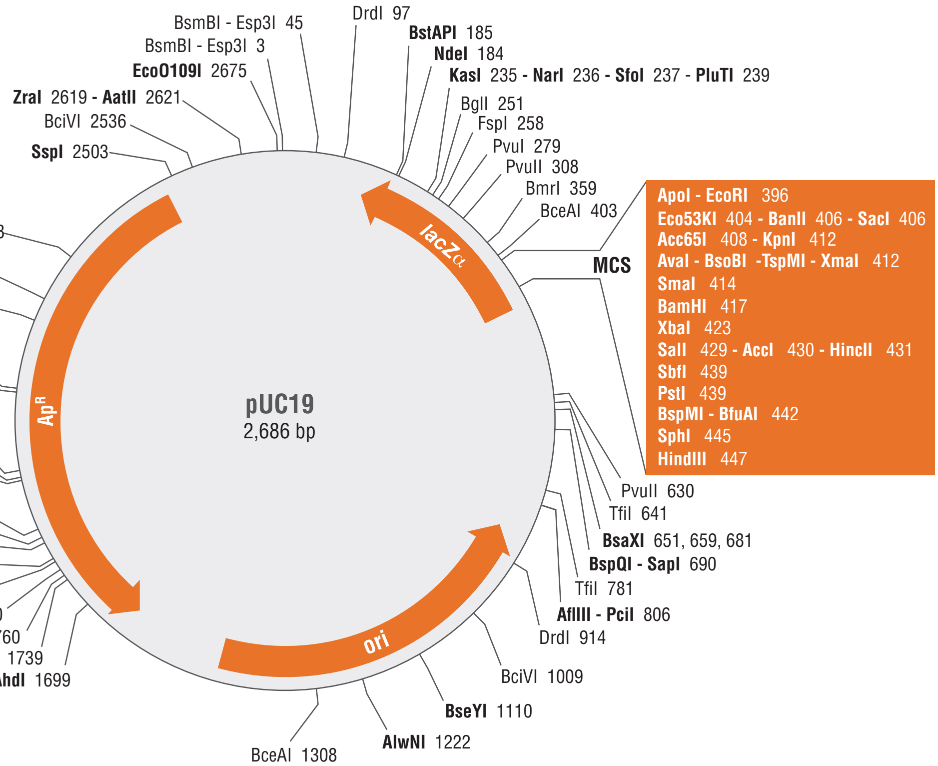
pUC18 is identical to pUC19 except that the MCS region (nt 397-454) is inverted.

pNEB193 is also identical to pUC19 except for the addition of several restriction endonuclease sites to the MCS. Its total length is 2713 bp.

Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap[R]) gene coordinates include the signal sequence.



pUC19 MCS

```
          SacI        SmaI         XbaI          SbfI
    EcoRI        KpnI       BamHI        SalI    PstI    SphI    HindIII
agtgAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGcgtaatcatggtcat
      400        410         420         430         440         450        460
...S  N  S  S  P  V  R  P  D  E  L  T  S  R  C  A  H  L  S  P  T  I  M  T  M
                                             ←——— lacZα translational start
```

pNEB193 MCS

```
          SacI        SmaI    BssHII           PacI                          SbfI
    EcoRI        KpnI    AscI       BamHI             XbaI   SalI    PmeI    PstI    SphI    HindIII
agtgAATTCGAGCTCGGTACCCGGGGGCGCGCCGGATCCTTAATTAAGTCTAGAGTCGACTGTTTAAACCTGCAGGCATGCAAGCTTGGcgtaatcatggtcat
      400        410         420         430         440         450         460         470         480         490
...S  N  S  S  P  V  R  P  R  A  P  D  K  I  L  D  L  T  S  Q  K  F  R  C  A  H  L  S  P  T  I  M  T  M
                                                                          ←——— lacZα translational start
```

References

(1) Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene*, 33, 103–119.

(2) Lin-Chao, S., Chen, W.-T. and Wong, T.-T. (1992) *Mol. Microbiol.*, 6, 3385–3393

(3) Miki, T. et al. (1987) *Protein Eng.*, 1, 327–332.

The Genetic Code

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	
	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	
5′	GCA C G U	CGA C G U OR AGA G	AAC U	GAC U	UGC U	CAA G	GAA G	GGA C G U	CAC U	AUA C U	CUA C G U OR UUA G	AAA G	AUG	UUC U	CCA C G U	UCA C G U OR AGC U	ACA C G U	UGG	UAC U	GUA C G U	3′

Second Position

First Position (5′end)	U		C		A		G		Third Position (3′ end)
U	UUU UUC UUA UUG	Phe Leu	UCU UCC UCA UCG	Ser	UAU UAC UAA UAG	Tyr Stop Stop	UGU UGC UGA UGG	Cys Stop Trp	U C A G
C	CUU CUC CUA CUG	Leu	CCU CCC CCA CCG	Pro	CAU CAC CAA CAG	His Gln	CGU CGC CGA CGG	Arg	U C A G
A	AUU AUC AUA AUG	Ile Met	ACU ACC ACA ACG	Thr	AAU AAC AAA AAG	Asn Lys	AGU AGC AGA AGG	Ser Arg	U C A G
G	GUU GUC GUA GUG	Val	GCU GCC GCA GCG	Ala	GAU GAC GAA GAG	Asp Glu	GGU GGC GGA GGG	Gly	U C A G

Termination Signals
UAA (Ochre)
UAG (Amber)
UGA (Opal)

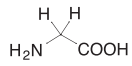
Single Letter Code

A	=	adenosine
C	=	cytidine
G	=	guanosine
T	=	thymidine
U	=	uridine
B	=	C or G or T
D	=	A or G or T
H	=	A or C or T
K	=	G or T
M	=	A or C
N	=	A or C or G or T
R	=	A or G
S	=	C or G
V	=	A or C or G
W	=	A or T
Y	=	C or T

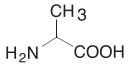
Amino Acid Structures

Each amino acid is accompanied by its three- and one-letter code, residue molecular weight (actual molecular weight minus water) and side-chain pK_a where appropriate.

Small

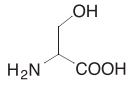


Glycine (Gly, G)
MW: 75.07

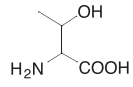


Alanine (Ala, A)
MW: 89.09

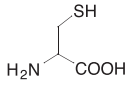
Nucleophilic



Serine (Ser, S)
MW: 105.09, pK_a ~16

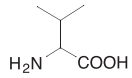


Threonine (Thr, T)
MW: 119.1, pK_a ~16

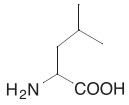


Cysteine (Cys, C)
MW: 121.2, pK_a = 8

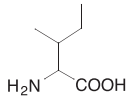
Hydrophobic



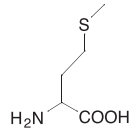
Valine (Val, V)
MW: 117.1



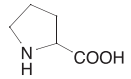
Leucine (Leu, L)
MW: 131.2



Isoleucine (Ile, I)
MW: 131.2

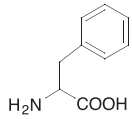


Methionine (Met, M)
MW: 149.2

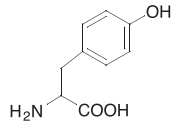


Proline (Pro, P)
MW: 115.1

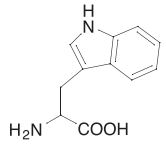
Aromatic



Phenylalanine (Phe, F)
MW: 165.2

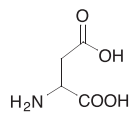


Tyrosine (Tyr, Y)
MW: 181.2, pK_a = 10.46

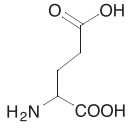


Tryptophan (Trp, W)
MW: 204.2

Acidic

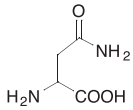


Aspartic Acid (Asp, D)
MW: 133.1, pK_a = 3.9

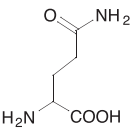


Glutamic Acid (Glu, E)
MW: 147.1, pK_a = 4.07

Amide

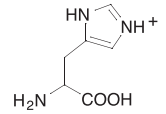


Asparagine (Asn, N)
MW: 132.1

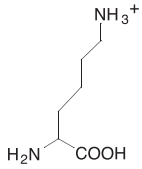


Glutamine (Gln, Q)
MW: 146.1

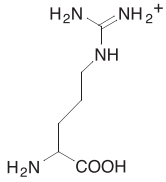
Basic



Histidine (His, H)
MW: 155.2, pK_a = 6.04



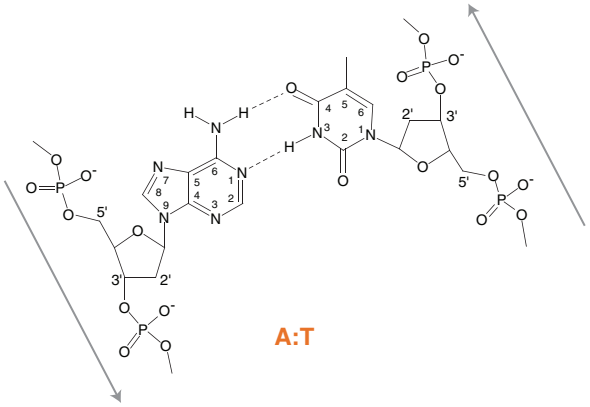
Lysine (Lys, K)
MW: 146.2, pK_a = 10.79

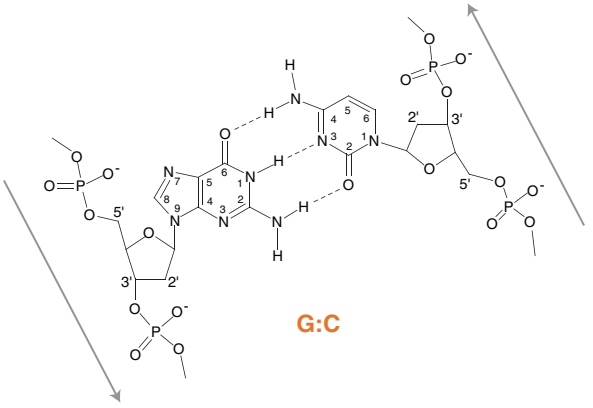


Arginine (Arg, R)
MW: 174.2, pK_a = 12.48

DNA Base Pairs

The structures of the adenosine:thymidine and guanosine:cytidine base pairs are shown in the context of the ribose phosphodiester backbones. The numbering schemes of the ribose and nucleotide moieties are indicated. Arrows indicate the polarity of each strand from 5´ to 3´.





Nucleic Acid Data

Average weight of a DNA basepair (sodium salt) = 650 daltons
1.0 A_{260} unit ds DNA = 50 µg/ml = 0.15 mM (in nucleotides)
1.0 A_{260} unit ss DNA = 33 µg/ml = 0.10 mM (in nucleotides)
1.0 A_{260} unit ss RNA = 40 µg/ml = 0.11 mM (in nucleotides)

MW of a double-stranded DNA molecule = (# of base pairs) x (650 daltons/base pair)
Moles of ends of a double-stranded DNA molecule = 2 x (grams of DNA) / (MW in daltons)
Moles of ends generated by restriction endonuclease cleavage:
a) circular DNA molecule: 2 x (moles of DNA) x (number of sites)
b) linear DNA molecule: 2 x (moles of DNA) x (number of sites) + 2 x (moles of DNA)

1 µg of 1000 bp DNA = 1.52 pmol = 9.1 x 1011 molecules
1 µg of pUC18/19 DNA (2686 bp) = 0.57 pmol = 3.4 x 1011 molecules
1 µg of pBR322 DNA (4361 bp) = 0.35 pmol = 2.1 x 1011 molecules
1 µg of M13mp18/19 DNA (7249 bp) = 0.21 pmol = 1.3 x 1011 molecules
1 µg of λ DNA (48502 bp) = 0.03 pmol = 1.8 x 1010 molecules

1 pmol of 1000 bp DNA = 0.66 µg
1 pmol of pUC18/19 DNA (2686 bp) = 1.77 µg
1 pmol of pBR322 DNA (4361 bp) = 2.88 µg
1 pmol of M13mp18/19 DNA (7249 bp) = 4.78 µg
1 pmol of λ DNA (48502 bp) = 32.01 µg

1.0 kb DNA = coding capacity for 333 amino acids ≈ 37,000 dalton protein
10,000 dalton protein ≈ 270 bp DNA
50,000 dalton protein ≈ 1.35 kb DNA

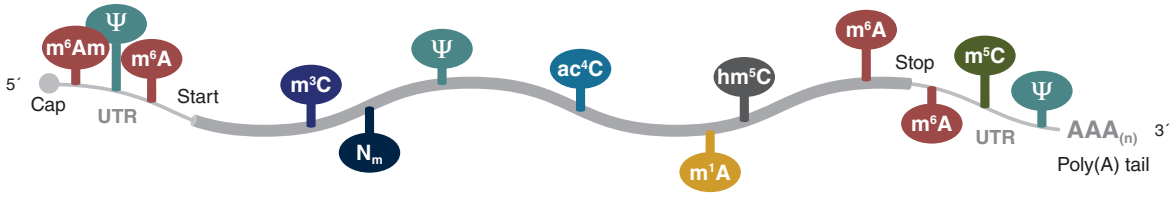
Isotope Data

Isotope	Particle Emitted	Half Life
^{14}C	β	5,730 years
^{3}H	β	12.3 years
^{125}I	γ	60 days
^{32}P	β	14.3 days
^{33}P	β	25 days
^{35}S	β	87.4 days

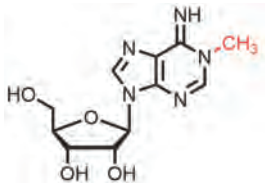
1 Ci = 1,000 mCi
1 mCi = 1,000 µCi
1 µCi = 2.2 x 10^6 disintegrations/minute
1 Becquerel = 1 disintegration/second
1 µCi = 3.7 x 10^4 Becquerels
1 Becquerel = 2.7 x 10^{-5} µCi

Messenger RNA Modifications

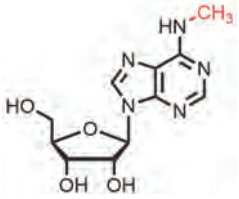
In nature, ribonucleic acid undergoes extensive chemical modification that can result in altered function or stability. The figure below shows examples of base and ribose modifications commonly found in native mRNAs



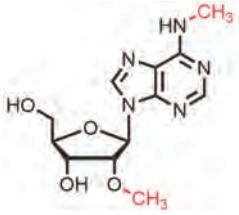
Nucleoside name
(Short name)
[New nomenclature]



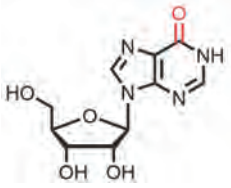
1-methyladenosine
(m^1A) [1A]



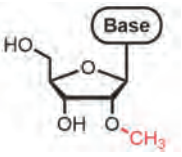
***N*^{6}-methyladenosine**
(m^6A) [6A]



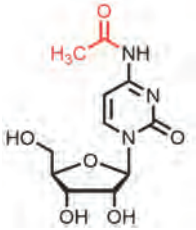
***N*^{6},2´-*O*-dimethyladenosine**
(m^6Am) [06A]



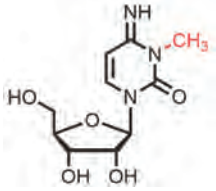
Inosine
(I) [9A]



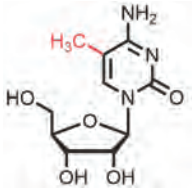
2´-*O*-methylnucleoside
(Nm) [ON]



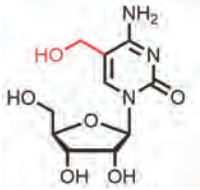
***N*^{4}-acetylcytidine**
(ac^4C) [42C]



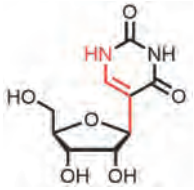
3-methylcytidine
(m^3C) [3C]



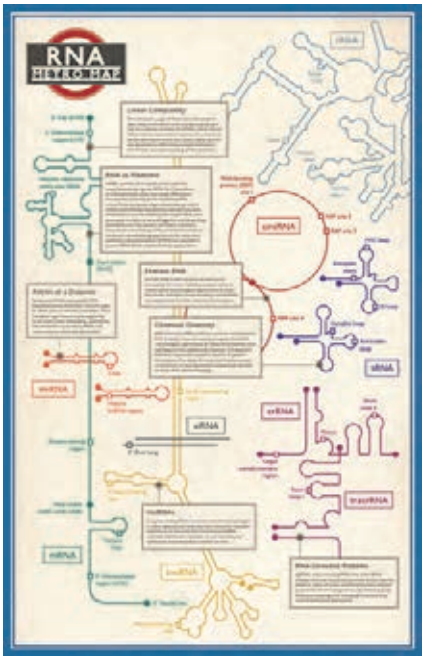
5-methylcytidine
(m^5C) [5C]



5-hydroxymethylcytidine
(hm^5C) [51C]



Pseudouridine
(Ψ) [9U]



Visit **NEBrna.com** to download our RNA Metro Map, and learn more about the various RNA structures and recent applications.

Acids and Bases

Compound	Formula	Molecular Weight	Specific Gravity	% by Weight	Conc. Reagent Molarity
Acetic acid, glacial	CH_3COOH	60.0	1.05	99.5	17.4
Formic acid	HCOOH	46.0	1.20	90	23.4
Hydrochloric acid	HCl	36.5	1.18	36	11.6
Nitric acid	HNO_3	63.0	1.42	71	16.0
Perchloric acid	$HClO_4$	100.5	1.67	70	11.6
Phosphoric acid	$H3PO_4$	98.0	1.70	85	18.1
Sulfuric acid	$H2SO_4$	98.1	1.84	96	18.0
Ammonium hydroxide	NH_4OH	35.0	0.90	28	14.8
Potassium hydroxide	KOH	56.1	1.52	50	13.5
Sodium hydroxide	NaOH	40.0	1.53	50	19.1
β-mercaptoethanol	$HSCH_2CH_2OH$	78.1	1.11	100	14.3

Protein Data

Bacterial Cells: *E. coli* or *Salmonella typhimurium*

Cell Data	per cell	per liter at 10^9 cells/ml
Wet weight	9.5×10^{-13} g	0.95 g
Dry weight	2.8×10^{-13} g	0.28 g
Total protein	1.55×10^{-13} g	0.15 g
Volume	1.15 μm^3 = 1 femtoliter	–

Protein conc. in the cell: 135 mg/ml

Theoretical maximum yield for a 1 liter culture (10^9 cells /ml) if protein of interest is:

- 0.1% of total protein: 150 µg/liter
- 2.0% of total protein: 3 mg/liter
- 50.0% of total protein: 75 mg/liter

Common Plasmid Gene Products

Gene	Gene Product # of Residues	Molecular Weight (daltons)
tet (pBR322)	401	43,267
amp (pBR322, bla)	286	31,515
kan (pACYC177, nptI)	264	29,047
cam (pACYC184, cat)	219	25,663
*lacZ*α (pUC19)	107	12,232
lacZ	1,023	116,351

Nucleotide Physical Properties

Compound	Molecular Weight	λ max (pH 7.0)	Absorbance at λ max 1 M solution (pH 7.0)
ATP	507.2	259	15,400
CTP	483.2	271	9,000
GTP	523.2	253	13,700
UTP	484.2	262	10,000
dATP	491.2	259	15,200
dCTP	467.2	271	9,300
dGTP	507.2	253	13,700
dTTP	482.2	267	9,600

Tris Buffer: pH vs Temperature

pH of Tris Buffer (0.05 M)		
5°C	25°C	37°C
7.76	7.20	6.91
7.89	7.30	7.02
7.97	7.40	7.12
8.07	7.50	7.22
8.18	7.60	7.30
8.26	7.70	7.40
8.37	7.80	7.52
8.48	7.90	7.62
8.58	8.00	7.71
8.68	8.10	7.80
8.78	8.20	7.91
8.88	8.30	8.01
8.98	8.40	8.10
9.09	8.50	8.22
9.18	8.60	8.31
9.28	8.70	8.42

Agarose Gel Resolution

% Gel	Optimum Resolution for Linear DNA (kb)
0.5	30 to 1.0
0.7	12 to 0.8
1.0	10 to 0.5
1.2	7 to 0.4
1.5	3 to 0.2

# #

A

B

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D

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Z

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- At least 70% recycled post-consumer fiber or equivalent is used
- Environmentally-friendly inks and coatings are used
- The printing facility is certified "environmental-friendly" by renowned conservation organizations

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https://projects.climatepartner.com/en/1630
https://projects.climatepartner.com/en/1610

For over 50 years, the New England Biolabs catalog has served as a resource for scientists worldwide. In line with our ongoing commitment to environmental stewardship, this issue proudly supports Osa Conservation. Their pioneering Ridge to Reef initiative is at the forefront of creating climate resilience, safeguarding biodiversity, and fostering sustainable coexistence between human communities and natural ecosystems in one of Central America's most vital landscapes.

OSA CONSERVATION IS CONNECTING HABITATS TO CREATE CLIMATE RESILIENCE FOR PEOPLE AND NATURE IN COSTA RICA

Stretching across Costa Rica's southern Pacific lies the AmistOsa region, one of Central America's richest landscapes in biodiversity. Osa Conservation is at the forefront of efforts to protect this critical ecosystem through the Ridge to Reef initiative, which focuses on building climate resilience and preserving the region's unmatched biodiversity. With habitats ranging from cloud forests and tropical lowland rainforests to the largest Pacific mangrove system and the Cocos-Galapagos swimway, the AmistOsa corridor is a biodiversity hotspot. It hosts over 6,540 species, including 400 bird species, 140 mammals, and countless endemic species that rely on this habitat for survival.

To protect this vital corridor, Osa Conservation is leading a multi-faceted conservation model. Central to their efforts is the development of the Central American Biodiversity and Climate Lab, a hub for monitoring biodiversity and climate change. The Lab consists of two key campuses: one in the lowland rainforest at the Osa Conservation Campus and a planned highland campus bordering the Amistad International Peace Park. This dual-campus strategy will create a continuous corridor from sea level to the mountaintops, providing species the connectivity they need to thrive as the climate shifts.

The organization's Ridge to Reef initiative combines scientific research, cutting-edge conservation technology, and community engagement to build a scalable model for conservation across the tropics. Through partnerships with over 370 local landowners, Osa Conservation has restored over 200 hectares of mangrove forest, planted over one million native trees, and built 28 arboreal bridges to ensure the safe movement of wildlife.

Community engagement is at the heart of Osa's work. The organization leads a Youth Nature Club that reaches over 2,000 of Costa Rica's most economically vulnerable children annually and benefits an additional 5,000 community members. Their Restoration Network empowers local farmers, youth, and volunteers to participate in citizen science and sustainable land management practices. The organization has trained volunteer rangers and expanded conservation fellowships to build the next generation of leaders in biodiversity preservation.

With a five-year plan to secure and restore new conservation land and launch the highland campus, Osa Conservation is turning the AmistOsa corridor into Central America's flagship Climate Adaptation Lifeboat. Their efforts serve as a global model for biodiversity resilience and climate adaptation, proving that people and nature can thrive together in the face of climate change.

OSA CONSERVATION

To learn more, please visit:
www.osaconservation.org

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Photo courtesy of Andrew Whitworth

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