
NEW ENGLAND
BioLabs® Inc.
be INSPIRED
drive DISCOVERY
stay GENUINE
2021•22

In loving memory

of our Founder, Donald G. Comb
(1927 – 2020)

Watch a video tribute
to New England Biolabs
Founder, Don Comb

be **INSPIRED** | *drive* **DISCOVERY** | *stay* **GENUINE**

At New England Biolabs, we are motivated by a set of core values that are still as true today as they were when the company was founded 45 years ago. These principles continue to guide us both as a company 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 30 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 1,300 publications to date, most of which are in peer-reviewed journals.

Environmental Stewardship

We continuously strive to promote ecologically sound practices and environmental sustainability in order to protect our natural resources, both locally and globally. It is our goal to continuously improve our business processes to minimize our impact on the environment. Additionally, NEB supports other organizations that are advancing environmental research and stewardship. We are excited to announce that NEB has become a Certified B Corporation™, a recognition awarded to organizations with the highest standards for social. and environmental performance, transparency and accountability. To learn more, visit **www.neb.com/environmentalphilosophy**.

Certified B Corporation

Learn more about our commitment to the environment

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. With a reliance on recombinant technologies, the majority of our products are designed and manufactured in our ISO 13485:2016 and ISO 9001:2015 certified facility in Ipswich, MA, USA. We are constantly improving the stringency and range of our quality controls to ensure that our products will perform to your expectations, every time. In addition, our manufacturing facility in Rowley, MA produces GMP-grade* materials for customers requiring an enhanced level of quality documentation and support.

nqa ISO 13485 Registered Medical Devices
nqa ISO 9001 Registered Quality Management
nqa ISO 14001 Registered Environmental Management

*See page 7 for more details.

A Unique Approach to Wastewater Treatment

Our state of the art Solar Aquatics System™ utilizes and accelerates processes found in streams and wetlands, and employs proven waste water treatment practices. This innovative method can treat up to 27,500 gallons of effluent per day through a series of filtration beds and aerated translucent tanks that host plant communities and aerobic microorganisms. The entire wastewater stream from our facility is treated, and the clean effluent is used for groundwater recharge and irrigation.

Solar Aquatics System™ is a trademark of Ecological Engineering Associates.

The NEB Facility

Our research and production facility, located in Ipswich, MA, USA, is LEED® certified, which is awarded based on environmentally-focused standards that include:

- Water efficiency
- Energy conservation
- Atmospheric protection
- Sustainable building materials and resources
- Indoor environmental quality
- Innovation and building design

LEED® is a registered trademark of the U.S. Green Building Council Corporation.

Diversity, Equity & Inclusion

We recognize that there are areas that we need to grow and are taking steps to raise our level of consciousness to injustices that have been overlooked and underestimated. Our Diversity, Equity & Inclusion Team works to address these issues, and is divided into four subgroups focusing on:

- Social Justice Philanthropy
- STEM Education & Mentorship
- Social Justice Outreach
- Diversity, Equity & Inclusion at NEB

Partnering with NEB

NEB has over 45 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 develop custom reagent solutions, and to help bring your technologies to market. Further, our global distribution network can help to ensure that your products have worldwide reach.

Customized Solutions

From development to commercialization, NEB provides the technical expertise, consistent scalable manufacturing, quality systems and a global distribution network to enable a successful long-term partnership. Our dedicated team is ready to work with you to develop novel, high performance enzymes tailored to your application, optimize these enzymes in your workflow, enable small to large scale production, generate quality controls and customize packaging. With our ISO 13485 and ISO 9001 certified manufacturing processes, as well as the ability to manufacture GMP-grade* products, you can be confident in our robust process, documentation and risk mitigation for the product you need. For more information, contact **custom@neb.com**.

Global Development

The ability of NEB to successfully operate as a both a research institute and a commercial enterprise in service of our customers is amplified by the extent of our global reach. The Global Development (GD) team at NEB operates worldwide to generate sustainable growth through an exceptional network of commercial operations that includes eight wholly owned subsidiaries located in Australia, Canada, China, France, Germany, Japan, Singapore and the United Kingdom, and over 60 distribution partners covering our involvement 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 contact **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, contact **freezers@neb.com**.

Enzymes for Innovation

The NEB catalog highlights a wide 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 the enzymology expertise at NEB, we now 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**.

What are Enzymes for Innovation?

*See page 7 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

New England Biolabs (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 safe and ethical manner. Learn more at **www.neb.com/neb-ethics**.

Supporting COVID-19 Research

NEB is supplying and supporting customers who are working diligently to develop better diagnostic tools and vaccines for the SARS-CoV-2 virus, including diagnostics for at home or point-of-care settings. Visit **www.neb.com/COVID19** to learn more, and find products and publications related to the applications below.

RNA Extraction

Extraction of RNA is often the first step in viral detection assays and has become an increasingly important laboratory technique in the midst of this global pandemic. Though NEB does not offer a kit specifically designed for viral RNA extraction, both the Monarch® Total RNA Miniprep Kit and the Monarch RNA Cleanup Kits have been used successfully to extract total RNA, including viral RNA, from clinically-relevant samples. See pages 137-138 for more information.

Virus 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. NEB has several research use only (RUO) products that can be used for detection of SARS-CoV-2 viral RNA, including our Luna® qPCR and RT-qPCR kits, which have been mentioned in several emergency use authorization (EUA) protocols. See pages 72-78 for more information.

Sequencing & Epidemiology

During the COVID-19 pandemic, sequencing has enabled scientists, often in unprecedented collaborations, to make rapid advances in epidemiology and surveillance, basic and clinical research, and diagnostics. A fast-growing number of methods are being developed to address these applications, including targeted SARS-CoV-2 sequencing protocols. Our NEBNext ARTIC kits–developed for long and short read sequencing–were based on the original work of the ARTIC Network (1) and are available for both Oxford Nanopore Technologies® and Illumina® Platforms. See pages 161-162 for more information.

Viral Biology

Proteolytic cleavage between the S1 and S2 unit of the spike protein of SARS-CoV-2 is an important part of the process to allow viral entry into the host cell after binding to the surface receptor. A subsequent cleavage on the S2 unit further triggers membrane fusion. Furin is one of the key players in a family of proteases responsible for these steps. The susceptibility of the spike protein to furin is essential to understand not only the viral biology, but also COVID-19's transmissibility.

The spike protein is also extensively glycosylated. These glycans have profound implications in modulating cell entry and membrane fusion, as well as host immune response. These glycosylations can be analyzed and profiled by PNGase F, Trypsin and Endoproteinase LysC. See pages 256-257 and 265-269 for more information.

Vaccine Development

mRNA-based vaccines are an emerging alternative to conventional vaccine approaches. Unlike protein-based vaccines, mRNA vaccines involve introduction of an mRNA sequence encoding disease-specific antigens. Once delivered into the cells, they get translated by the cellular machinery, resulting in the synthesis of protein antigens. These antigens are recognized by the immune system, and immune responses are mounted. Several mRNA vaccine candidates are either already being administered or are in development for SARS-CoV-2. mRNA vaccines can be rapidly and scalably produced *in vitro* using enzymes to transcribe and modify mRNA. See pages 199-205 for more information.

(1) Josh Quick 2020. nCoV-2019 sequencing protocol v2 (GunIt). protocols.io https://dx.doi.org/10.17504/protocols.io.bdp7i5rn.

** "GMP-grade" is a branding term NEB uses to describe reagents manufactured 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.*

GMP Capabilities

With over 45 years in enzymology expertise, NEB is a world leader in the discovery and production of reagents for the life science industry. This 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.

To better serve the needs of customers in regulated markets, NEB has opened a state-of-the-art, 43,000 sq. ft. production facility in Rowley, MA for the manufacture of GMP-grade* materials. This facility includes Quality Control and Production functions ranging from a shipping/receiving area and dedicated warehouse, to separate inoculation preparation, fermentation, purification and filling suites. Visit **www.neb.com/GMP** to learn more.

Supporting Molecular Diagnostics

The COVID-19 pandemic has highlighted many of the challenges faced by clinical labs, requiring new and innovative solutions to address them. Technology development is happening faster than ever before, exposing weaknesses in diagnostic supply chains, 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 a portfolio of products that serve as critical components for a wide array of diagnostic products and services. Extensive molecular biology and enzymology experience provide NEB with the unique ability 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.

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
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**.

Ordering Information

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Research Use Only

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For more information about commercial rights, please contact NEB's Global Business Development team at gbd@neb.com.

Notice to Buyer

The information in this Catalog is provided in good faith based on NEB's knowledge as of the publication date. The Catalog is only a guide and may not be up-to-date. For current information, please see NEB's website at www.neb.com. NEB's terms, conditions, policies and product information are subject to change at NEB's discretion.

The employee photo has been a tradition in the catalog for over 30 years. Given the current situation, we went with a more creative approach to bring the NEB family together.

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View of the moody sky over Thailand at twilight.
Credit: Boy Anupong, Getty images

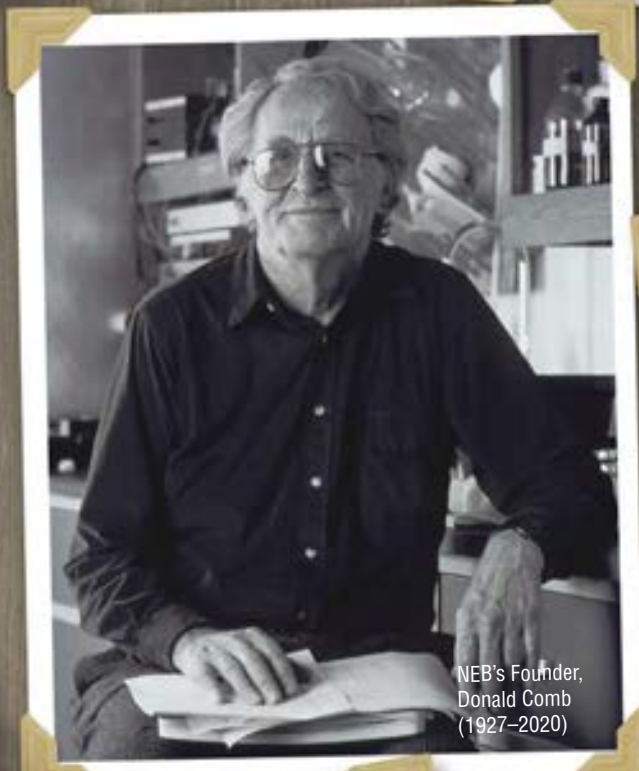
Protecting a Legacy

Each edition of the New England Biolabs Catalog contains a collection of mini-reviews that addresses various scientific, environmental and/or humanitarian topics. This year, we are dedicating the Catalog to our Founder, Donald G. Comb, and sharing some of the values that he was passionate about.

Mini-reviews were authored by Joanne Gibson, Lydia Morrison and Deana Martin. Joanne is a Marketing Communications Writer at NEB. She received her Ph.D. in Molecular Biology from the University of Sydney, Australia. Lydia is also a Marketing Communications Writer at NEB, and manages our social media program. She received her M.S. in Biochemistry and Biophysics from the University of North Carolina at Chapel Hill School of Medicine. Deana is the Associate Director of Marketing Communications. She received her Ph.D. in Biochemistry from Boston College, Chestnut Hill, MA.

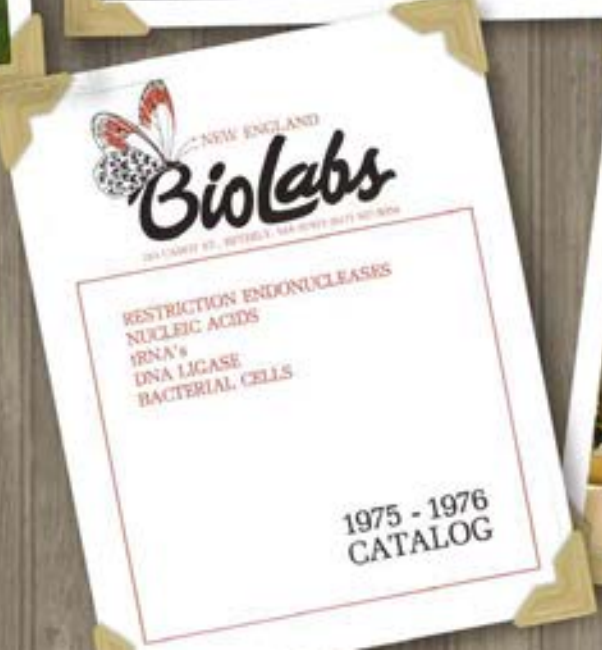
NEB's first home on Cabot St.
in Beverly, MA

NEB group photo, 2019



NEB's Founder,
Donald Comb
(1927–2020)

NEB laboratory facility
in Ipswich,MA



NEB group photo, taken at the Tozer Road Facility in Beverly, MA, 1997

First NEB group photo, 1980/81

Entrance to NEB laboratory facility in Ipswich, MA

By Scientists for Scientists

In 1974, a scientist named Don Comb left his associate professorship in the Biochemistry Department at Harvard Medical School — disenthralled with the traditional avenues of securing research funding and the rigidity of academia. He left with the goal of bringing together like-minded scientists to form a cooperative company — a company that would provide tools for molecular biologists. Further, the company would use its revenue to fund independent research projects, an idea that was unprecedented at the time. And thus, New England Biolabs®, Inc. (NEB®) was founded, with the goal of enabling life science research, both within and outside the company.

In the early days, NEB's focus was to provide high quality restriction enzymes at a fair price. Over the years, NEB researchers have discovered a wide range of enzyme specificities — as a result NEB now offers >285 restriction enzymes, the largest commercially-available selection. We have also expanded our product offerings into areas related to PCR, gene expression, sample preparation for next generation sequencing, synthetic biology, glycobiology and RNA analysis.

Don hired scientists who were passionate and curious. He encouraged them to explore their own research interests while carrying out their job responsibilities. In doing so, NEB scientists became experts in the functionality and biophysical properties of a wide range of enzyme activities, resulting in an in-depth understanding of product performance. This enabled the collegial exchange of ideas, protocols and techniques with customers — the beginnings of a more personal approach to technical support that still continues today.

This expertise also enabled NEB to produce recombinant enzymes at commercial scale, dramatically improving quality and yield — greatly advancing the field of molecular biology. As yields increased, NEB was able to lower prices and pass savings on to customers.

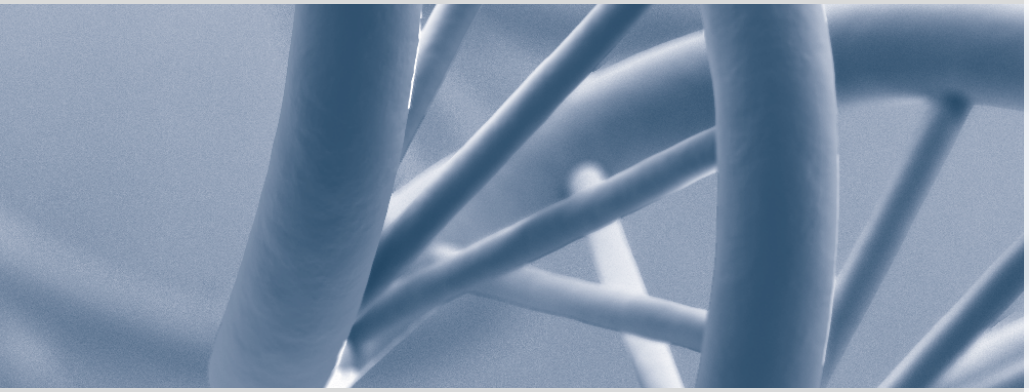
NEB remains committed to research and has maintained an active program for over 45 years. Currently, more than 30 labs participate in basic research in areas ranging from molecular biology to parasitology, and this work has resulted in over 1,300 publications to date. Basic research and publications help to ensure that NEB scientists are staying curious, driving discovery and thinking like their customers. Additionally, NEB scientists are invited to speak at renowned academic institutions and conferences around the world, yet equally value the importance of advocating for science at local high schools. This emphasis on scientific collaboration and advancement has led to the sharing of innovative products and ideas worldwide.

Don's aspiration was for NEB to remain a small, private business that focused on steady, sustainable growth. His devotion to the advancement of science, stewardship of the environment and altruistic philanthropy has been a priority since day one. NEB employees are encouraged to pursue their passions, whether it be getting involved in groundbreaking research, helping out at a local science fair, sharing ideas to improve the sustainability of our business practices, or volunteering at a local food bank — everyone feels a responsibility to each other and to the community. NEB's CEO Jim Ellard said "Don's generosity and the faith that he had in each employee resulted in a family-like culture, where everyone has a voice and all employees feel valued."

A culture built by scientists, for scientists.

Learn about NEB and the photos shown.

Restriction Endonucleases



The leader in the discovery & production of restriction enzymes.

Having supplied restriction enzymes to the research community for over 45 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. With the industry's largest research and development group dedicated to restriction enzymes, 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 a true restriction enzyme master mix. 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

309 **Performance/Activity Chart for Restriction Enzymes**

308 **Restriction Enzyme Troubleshooting Guide**

306 **Tips for Restriction Enzyme Optimization**

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

What are restriction enzymes?

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

NEB 1.1 NEB 2.1 NEB 3.1 CutSmart 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 & 1.1 - yellow, NEB r2.1 & 2.1 - blue, NEB r3.1 & 3.1 - red, rCutSmart & CutSmart - green) and supplied as 10X stocks with each enzyme. For more information, consult the Performance Chart on pages 309–314.

Epi This enzyme is EpiMark validated for epigenetics studies.

SAM This enzyme is supplied with a separate tube of S-adenosylmethionine (SAM). To obtain 100% activity, SAM should be added to the 1X reaction mix as indicated. When required, a concentrated stock of SAM is supplied with the enzyme.

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 page 321.

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. For more information see pages 309–314.

Enzymes for Innovation.

Note: Starting April 2021, NEB will begin the transition to supply all restriction enzymes with Recombinant Albumin-containing reaction buffer. For more details, see page 20 or visit **www.neb.com/BSA-free**

- AatII
- AbaSI
- AccI
- Acc65I
- AciI
- AclI
- AcuI
- AfeI
- AflII
- AflIII
- AgeI
- AgeI-HF
- AhdI
- AleI-v2
- AluI
- AlwI
- AlwNI
- ApaI
- ApaLI
- ApeKI
- ApoI
- 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
- 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
- BsrI
- BsrBI
- BsrDI
- BsrFI-v2
- BsrGI
- BsrGI-HF
- BssHII
- BssSI-v2
- BstAPI
- BstBI
- BstEII
- BstEII-HF
- BstNI
- BstUI
- BstXI
- BstYI
- BstZ17I-HF
- Bsu36I
- BtgI
- BtgZI
- BtsI-v2
- BtsIMutI
- BtsCI
- Cac8I
- ClaI
- CspCI
- CviAII
- CviKI-1
- CviQ1
- 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
- FspEI
- HaeII
- HaeIII
- HgaI
- HhaI
- HincII
- HindIII
- HindIII-HF
- HinfI
- HinP1I
- HpaI
- HpaII
- HphI
- Hpy99I
- Hpy166II
- Hpy188I
- Hpy188III
- HpyAV
- HpyCH4III
- HpyCH4IV
- HpyCH4V
- KasI
- KpnI
- KpnI-HF
- LpnPI
- MboI
- MboII
- MfeI
- MfeI-HF
- MluI
- 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
- 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
- PvuI-HF
- PvuII
- PvuII-HF
- RsaI
- RsrII
- SacI
- SacI-HF
- SacII
- SalI
- SalI-HF
- SapI
- Sau3AI
- Sau96I
- SbfI
- SbfI-HF
- ScaI-HF
- ScrFI
- SexAI
- SfaNI
- SfcI
- SfiI
- SfoI
- SgrAI
- SmaI
- SmlI
- SnaBI
- SpeI
- SpeI-HF
- SphI
- SphI-HF
- SrfI
- SspI
- SspI-HF
- StuI
- StyI-HF
- StyD4I
- SwaI
- TaqI-v2
- TfiI
- TseI
- Tsp45I
- TspMI
- TspRI
- Tth111I
- XbaI
- XcmI
- XhoI
- XmaI
- XmnI
- ZraI

High-Fidelity Restriction Enzymes

AgeI-HF, ApoI-HF, BamHI-HF, BbsI-HF, BclI-HF, BmtI-HF, BsaI-HFv2, BsiWI-HF, BsrGI-HF, BstEII-HF, BstZ17I-HF, DraIII-HF, EagI-HF, EcoRI-HF, EcoRV-HF, HindIII-HF, KpnI-HF, MfeI-HF, NcoI-HF, NheI-HF, NotI-HF, NruI-HF, NsiI-HF, PstI-HF, PvuI-HF, PvuII-HF, SacI-HF, SalI-HF, SbfI-HF, ScaI-HF, SpeI-HF, SphI-HF, SspI-HF, StyI-HF
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Methylation Sensitive Restriction Enzymes for Epigenetics Studies

AbaSI, DpnI, DpnII, FspEI, HpaII, LpnPI, McrBC, MspI, MspJI
280–281

Nicking Endonucleases

Nb.BbvCI, Nb.BsmI, Nb.BsrDI, Nb.BssSI, Nb.BtsI, Nt.AlwI, Nt.BbvCI, Nt.BsmAI, Nt.BspQI, Nt.BstNBI, Nt.CviPII
55–57

Homing Endonucleases

I-CeuI, I-SceI, PI-PspI, PI-SceI
57–58

NEBuffers, Diluents, Gel Loading Dyes, BSA & Recombinant Albumin, Molecular Biology Grade, NEB Tube Opener
58–59

Enzymes in green are all 100% active in CutSmart Buffer (see page 309–314).

Looking to bring convenience to your workflow?

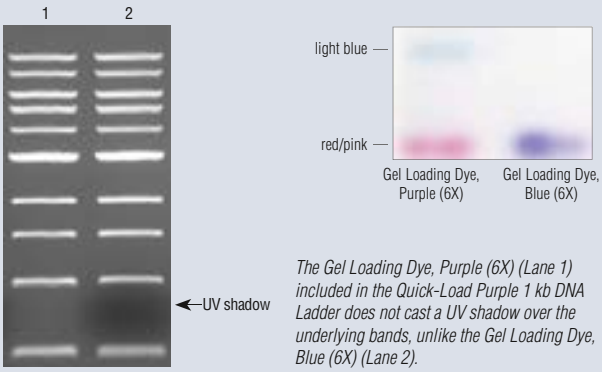
Speed up digestions with Time-Saver™ Qualified Restriction Enzymes

There are > 195 NEB restriction enzymes that can digest DNA in 5–15 minutes; many of which are supplied with rCutSmart or CutSmart 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.

For more information, visit **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 215 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 (see page 315). For more information, visit **www.NEBCutSmart.com**

Same high performance, now with BSA-free reaction buffer

BSA is commonly used to stabilize some proteins during reactions, and can also prevent adhesion to reaction tubes and pipette surfaces. We understand that there is an increased need for comparable performance using BSA-free reagents — beginning April 2021, we will be switching our current BSA-containing reaction buffers (NEBuffer 1.1, 2.1, 3.1 and CutSmart® Buffer) to Recombinant Albumin-containing buffers (NEBuffer r1.1, r2.1, r3.1 and rCutSmart™ Buffer). These buffers have been rigorously tested, and there is no difference in performance when using either buffer system. We anticipate this switch may take up to 6 months to complete. Over that time, you may receive product with BSA- or rAlbumin-containing buffer with your product – either will work for your reactions.

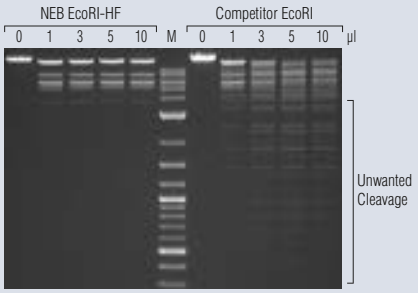
For more details visit **www.neb.com/BSA-free**

Looking to optimize performance in your reaction?

Choose a High-Fidelity (HF®) Restriction Enzyme

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 or CutSmart Buffer. Enjoy the improved performance of our engineered enzymes at the same price as the native enzymes!

For more information, visit **www.neb.com/HF**



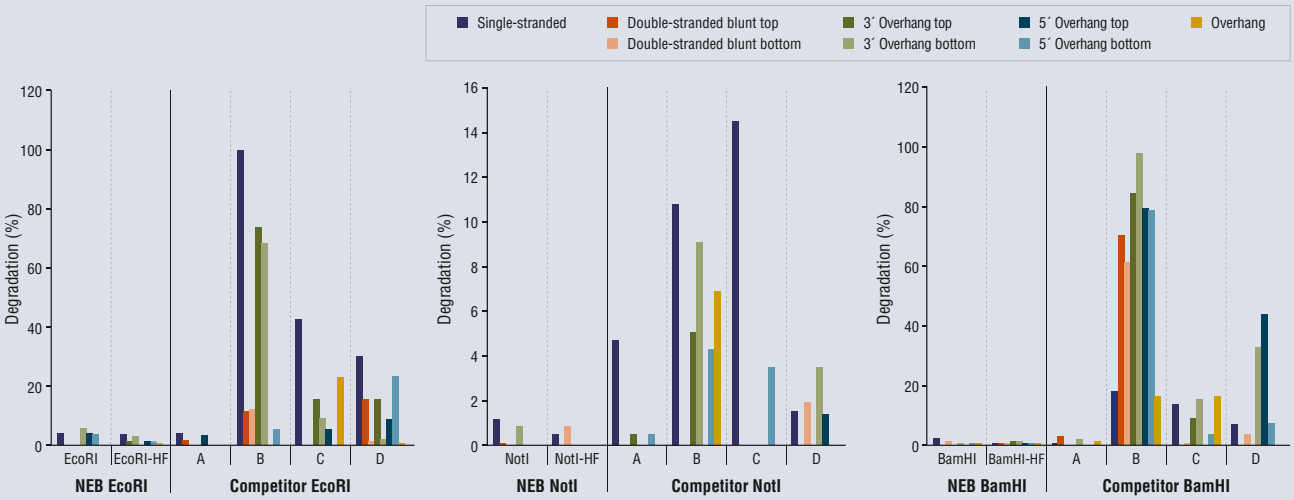
EcoRI-HF (NEB #R3101) shows no star activity in overnight digests, even when used at higher concentrations. 50 µl rxns were set up using 1 µg of Lambda DNA, the indicated amount of enzyme and the recommended reaction buffer. Rxns were incubated overnight at 37°C. Marker M is the 1 kb DNA Ladder (NEB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

For more information on quality at NEB, visit **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 · e · 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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

Note: *May exhibit star activity in this buffer.

AbaSI

rCutSmart · RR · Epi · dilC · 25° · 65°

#R0665S 1,000 units

See page 280 for more information.

AccI

rCutSmart · RR · e · 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: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

Acc65I

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

#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: Blocked by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

Note: *May exhibit star activity in this buffer.

AciI

rCutSmart · RR · e · 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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

AclI

rCutSmart · RR · e · dilB · 37° · No · 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

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

Concentration: 5,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

AcuI

rCutSmart · RR · e · dilB · 37° · 65°

#R0641S 300 units
#R0641L 1,500 units

5′... C T G A A G (N)$_{16}$▼... 3′
3′... G A C T T C (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	50	100	50	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

AfeI

rCutSmart · RR · e · 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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

AflII

rCutSmart · RR · e · 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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

AgeI

#R0552S 300 units
#R0552L 1,500 units

5′... A▼CCGGT...3′
3′... TGGCC▲A...5′

Reaction Conditions: NEBuffer r1.1, 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	25	75

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

Note: Star activity may result from extended digestion.

High-Fidelity

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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

AhdI

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

5′...GACNNNNNGTC...3′
3′...CTGNNNNNCAG...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: Cleavage of mammalian genomic DNA is impaired by some combinations of overlapping CpG methylation (see p. 321).

AleI-v2

#R0685S 500 units
#R0685L 2,500 units

5′...CACNNNNGTG...3′
3′...GTGNNNNCAC...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: Impaired by overlapping CpG methylation (see p. 321).

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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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: Blocked by *dam* methylation (see p. 321).

Note: Star activity may result from extended digestion, high enzyme concentration or 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.

Concentration: 10,000 units/ml

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

Methylation Sensitivity: Blocked by overlapping *dcm* methylation (see p. 321).

Note: Conditions of low ionic strength, high enzyme concentration, glycerol concentration > 5%, or pH > 8.0 may result in star activity.

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

ApaI

rCutSmart · RR · time-saver · dilA · 25° · 65° · dcm · CpG

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

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

Reaction Conditions: rCutSmart Buffer, 25°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 37°C: 100% However, the half-life of ApaI at 37°C is only 30 minutes.

Methylation Sensitivity: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

ApaLI

rCutSmart · RR · time-saver · dilA · 37° · No · CpG

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

5′... G▼T G C A C ...3′
3′... C A C G T▲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: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

ApeKI

RR · time-saver · NEB r3.1 · dilB · 75° · No · CpG

#R0643S 250 units
#R0643L 1,250 units

5′... G▼C W G C ...3′
3′... C G W C▲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: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

ApoI

RR · time-saver · NEB r3.1 · dilA · 50° · 80°

#R0566S 1,000 units

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

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

Concentration: 10,000 units/ml

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

Activity at 37°C: 50%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

High-Fidelity

ApoI-HF®

rCutSmart · butterfly · RR · e · time-saver · dilB · 37° · 80°

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

5′... R▼A A T T Y ...3′
3′... Y T T A A▲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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

AscI

rCutSmart · RR · time-saver · dilA · 37° · 80° · CpG

#R0558S 500 units
#R0558L 2,500 units

5′... G G▼C G C G C C ...3′
3′... C C G C G 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	<10	10	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

AseI

butterfly · RR · time-saver · NEB r3.1 · dilB · 37° · 65°

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

5′... A T▼T A A T ...3′
3′... T A A T▲T A ...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	10

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

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a glycerol concentration of > 5%. *May exhibit star activity in this buffer.

AsiSI

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

#R0630S 500 units
#R0630L 2,500 units

5′... G C G A T▼C G C ...3′
3′... C G C▲T A G C 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	100	25	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

Note: Star activity may result from extended digestion.

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 Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

AvaI

rCutSmart · RR · dIIA · 37° · 80° · CpG

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

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

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

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

AvaII

rCutSmart · RR · dIIA · 37° · 80° · dcm · CpG

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

5′... G▼G W C C ...3′
3′... C C W 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	75	10	100

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

Methylation Sensitivity: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

AvrII

rCutSmart · RR · dIIB · 37° · No

#R0174S 100 units
#R0174L 500 units

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

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 5,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BaeI

rCutSmart · RR · SAM · dIIA · 25° · 65° · CpG

#R0613S 250 units

5′...▼10(N) A C (N)4 G T A Y C (N)12▼...3′
3′...▲15(N) T G (N)4 C A T R G (N)7▲...5′

Reaction Conditions: rCutSmart Buffer + SAM, 25°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

Activity at 37°C: 100%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

BaeGI

RR · NEB r3.1 · dIIA · 37° · 80°

#R0708S 500 units

5′... G K G C M▼C ...3′
3′... C▲M C G K 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	75	75	100	25

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BamHI

RR · NEB r3.1 · dIIA · 37° · No

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

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

Reaction Conditions: NEBuffer r3.1, 37°C

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

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

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a glycerol concentration of > 5%. *May exhibit star activity in this buffer.

High-Fidelity

BamHI-HF®

rCutSmart · RR · e · dIIA · 37° · No

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

5′... G▼G A T C C ...3′
3′... C C T A G▲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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BanI

rCutSmart · RR · dIIA · 37° · 65° · dcm · CpG

#R0118S 5,000 units

5′... G▼G Y R C C ...3′
3′... C C R Y G▲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	25	<10	100

Methylation Sensitivity: Blocked by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

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

BanII

rCutSmart | RR | dilA | 37° | 80°

#R0119S 2,000 units

5′... G R G C Y▼C ...3′
3′... C▲Y C G R 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	100	100	50	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion.

BbsI

RR | NEB r2.1 | dilB | 37° | 65°

#R0539S 300 units
#R0539L 1,500 units

5′... G A A G A C (N)$_2$▼...3′
3′... C T T C T G (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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Store at -80°C.

BbsI-HF®

High-Fidelity

rCutSmart | RR | e | dilB | 37° | 65°

#R3539S 300 units
#R3539L 1,500 units

5′... G A A G A C (N)$_2$▼...3′
3′... C T T C T G (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	10	10	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BbvI

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

#R0173S 300 units

5′... G C A G C (N)$_8$▼...3′
3′... C G T C G (N)$_{12}$▲...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	25	100

Concentration: 2,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

BbvCI

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

#R0601S 100 units
#R0601L 500 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.

Concentration: 2,000 units/ml

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

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation (see p. 321).

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

BccI

rCutSmart | RR | dilA | 37° | 65°

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

5′... C C A T C (N)$_4$▼...3′
3′... G G T A G (N)$_5$▲...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

BceAI

RR | NEB r3.1 | dilA | 37° | 65° | CpG

#R0623S 50 units
#R0623L 250 units

5′... A C G G C (N)$_{12}$▼...3′
3′... T G C C G (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	100*	100*	100	100*

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

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

BcgI

RR | 2*site | NEB r3.1 | dilA | 37° | 65° | dam | CpG

#R0545S 250 units

5′...▼$_{10}$(N) C G A (N)$_6$ T G C (N)$_{12}$▼...3′
3′...$_{12}$▲(N) G C T (N)$_6$ A C G (N)$_{10}$▲...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: Impaired by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

Note: *May exhibit star activity in this buffer.

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

BciVI

rCutSmart · RR · dilC · 37° · 80°

#R0596S 200 units
#R0596L 1,000 units

5′... G T A T C C (N)$_6$▼... 3′
3′... C A T A G G (N)$_5$▲... 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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BclI

RR · NEB r3.1 · dilA · 50° · 65° · dam

#R0160S 3,000 units

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

Reaction Conditions: NEBuffer r3.1, 50°C

Concentration: 10,000 units/ml

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

Activity at 37°C: 50%

Methylation Sensitivity: Blocked by *dam* methylation (see p. 321).

BclI-HF®

High-Fidelity

rCutSmart · RR · dilB · 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: Blocked by *dam* methylation (see p. 321).

BcoDI

rCutSmart · RR · dilB · 37° · 80° · 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

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by some combinations of overlapping CpG methylation (see p. 321).

BfaI

rCutSmart · RR · dilB · 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.

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion. Store at -80°C.

BfuAI

RR · 2*site · NEB r3.1 · dilB · 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

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

Activity at 37°C: 25%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation (see p. 321).

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

BglI

RR · NEB r3.1 · dilB · 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.

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

BglII

RR · NEB r3.1 · dilA · 37° · 80°

#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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BlpI

#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

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BmgBI

#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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

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

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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

BmtI

#R0658S 300 units

5′... G C T A G▼C ...3′
3′... C▲G A T 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	100	100	100	100+

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion. +For added flexibility, NEB offers an isoschizomer or HF enzyme, supplied with rCutSmart Buffer.

High-Fidelity

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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Store at -80°C.

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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a extended digestion. *May exhibit star activity in this buffer.

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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

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 | Heat Inactivation | dam Methylation Sensitivity

BsaI

BsaI has been replaced by BsaI-HFv2 (engineered for performance).

High-Fidelity

BsaI-HF®v2

rCutSmart | RR | e | dilB | 37° | 80° | dcm | CpG

#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: Impaired by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

BsaAI

rCutSmart | RR | dilC | 37° | No | CpG

#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

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

Concentration: 5,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

BsaBI

rCutSmart | RR | dilB | 60° | 80° | dam | CpG

#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: Blocked by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

Note: Star activity may result from extended digestion.

BsaHI

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

#R0556S 2,000 units

5′... G R▼C G Y C ... 3′
3′... C Y G C▲R 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 units/ml

Methylation Sensitivity: Blocked by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

BsaJI

rCutSmart | RR | dilA | 60° | 80°

#R0536S 1,000 units

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

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

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

Concentration: 10,000 units/ml

Activity at 37°C: 25%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BsaWI

rCutSmart | RR | dilA | 60° | 80°

#R0567S 250 units

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

Reaction Conditions: rCutSmart Buffer, 60°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

Activity at 37°C: 50%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BsaXI

rCutSmart | dilC | 37° | No

#R0609S 100 units
#R0609L 500 units

5′... ▼$_9$(N) A C (N)$_6$ C T C C (N)$_{10}$▼... 3′
3′... ▲$_{12}$(N) T G (N)$_5$ G A G G (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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

BseRI

rCutSmart | RR | dilA | 37° | 80°

#R0581S 200 units
#R0581L 1,000 units

5′... G A G G A G (N)$_{10}$▼... 3′
3′... C T C C T C (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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BseYI

#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: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

BsgI

#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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BsiEI

#R0554S 1,000 units

5′...CGRY▼CG...3′
3′...GC▲YRGC...5′

Reaction Conditions: rCutSmart Buffer, 60°C.

Concentration: 10,000 units/ml

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

Activity at 37°C: 10%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

BsiHKAI

#R0570S 1,000 units

5′...GWGCW▼C...3′
3′...C▲WCGWG...5′

Reaction Conditions: rCutSmart Buffer, 65°C.

Concentration: 10,000 units/ml.

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

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

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

Activity at 37°C: 25%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

Note: *May exhibit star activity in this buffer.

High-Fidelity

BsiWI-HF®

#R3553S 300 units
#R3553L 1,500 units

5′...C▼GTACG...3′
3′...GCATG▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C.

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

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

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

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

Activity at 37°C: 50%

Methylation Sensitivity: Blocked by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

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.

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

Concentration: 10,000 units/ml

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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 · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

BsmAI

rCutSmart · RR · dinB · 55° · Mo · CpG

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

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

Reaction Conditions: rCutSmart Buffer, 55°C

Concentration: 5,000 units/ml

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

Activity at 37°C: 50%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

BsmBI

BsmBI has been replaced by BsmBI-v2 (engineered for performance).

NEW

BsmBI-v2

RR · e · NEB r3.1 · dinB · 55° · 80° · CpG

#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.

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

Concentration: 10,000 units/ml

Activity at 37°C: 10%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

BsmFI

rCutSmart · RR · dinA · 65° · 80° · dcm · CpG

#R0572S 100 units
#R0572L 500 units

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

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

Concentration: 2,000 units/ml

Activity at 37°C: 100%

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

Methylation Sensitivity: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

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

BsoBI

rCutSmart · RR · dinA · 37° · 80°

#R0586S 10,000 units

5′...CYCGRG...3′
3′...GRGCYC...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Bsp1286I

rCutSmart · RR · dinA · 37° · 65°

#R0120S 500 units

5′...GDGCH▼C...3′
3′...C▲HCGDG...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

BspCNI

rCutSmart · RR · dinA · 37° · 80°

#R0624S 100 units

5′...CTCAG(N)$_{10}$▼...3′
3′...GAGTC(N)$_8$▲...5′
and
5′...CTCAG(N)$_9$▼...3′
3′...GAGTC(N)$_7$▲...5′

Note: The cleavage site of BspCNI varies. Two equally represented species of fragments are produced from BspCNI cleavage.

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

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

Concentration: 2,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BspDI

rCutSmart · RR · dinA · 37° · 80° · dam · CpG

#R0557S 2,000 units

5′...AT▼CGAT...3′
3′...TAGC▲TA...5′

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

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

BspEI

RR · e · NEB r3.1 · dinB · 37° · 80° · dam · CpG

#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.

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is impaired by CpG methylation (see p. 321).

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

BspHI

rCutSmart · RR · ♦ · dilA · 37° · 80° · dam

#R0517S 500 units
#R0517L 2,500 units

5′...T▼CATGA...3′
3′...AGTAC▲T...5′

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

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Impaired by overlapping *dam* methylation (see p. 321).

BsrBI

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

#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.

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

BspMI

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

#R0502S 100 units

5′...ACCTGC(N)$_4$▼...3′
3′...TGGACG(N)$_8$▲...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	10

Concentration: 2,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

BsrDI

RR · ♦ · NEB r2.1 · dilA · 65° · 80°

#R0574S 200 units
#R0574L 1,000 units

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

Reaction Conditions: NEBuffer r2.1, 65°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

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

Activity at 37°C: 50%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

BspQI

RR · ♦ · NEB r3.1 · dilB · 50° · 80°

#R0712S 500 units
#R0712L 2,500 units

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

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

Concentration: 10,000 units/ml

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

Activity at 37°C: 50%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

BsrFI-v2

rCutSmart · RR · e · ♦ · dilC · 37° · No · CpG

#R0682S 1,000 units

5′...R▼CCGGY...3′
3′...YGGCC▲R...5′

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

BsrI

♦ · NEB r3.1 · dilB · 65° · 80°

#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.

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

Concentration: 10,000 units/ml

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BsrGI

RR · ♦ · NEB r2.1 · dilA · 37° · 80°

#R0575S 1,000 units
#R0575L 5,000 units

5′...T▼GTACA...3′
3′...ACATG▲T...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	100	25

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

BsrGI-HF®

High-Fidelity

rCutSmart · r1.1 · r2.1 · r3.1 · dirA · 37° · 80°

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

5′... T▼G T A C A ...3′
3′... A C A T G▲T ...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	100	100

Concentration: 20,000 units/ml.

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BstBI

rCutSmart · r1.1 · r2.1 · dirA · 65° · No · CpG

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

5′... T T▼C G A A ...3′
3′... A A G C▲T T ...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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

BssHII

rCutSmart · r1.1 · r2.1 · dirB · 50° · 65° · CpG

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

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

Reaction Conditions: rCutSmart Buffer, 50°C. Heat inactivation: 65°C for 20 minutes.

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

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

Activity at 37°C: 100%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

BstEII

r1.1 · r2.1 · NEB r3.1 · dirA · 60° · No

#R0162S 2,000 units
#R0162L 10,000 units

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

Reaction Conditions: NEBuffer r3.1, 60°C

Concentration: 10,000 units/ml

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

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from a glycerol concentration of > 5%. *May exhibit star activity in this buffer.

BssSI-v2

rCutSmart · r1.1 · r2.1 · dirB · 37° · No

#R0680S 200 units
#R0680L 1,000 units

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

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BstEII-HF®

High-Fidelity

rCutSmart · r1.1 · r2.1 · r3.1 · dirA · 37° · No

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

5′... G▼G T N A C C ...3′
3′... C C A N T G▲G ...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BstAPI

rCutSmart · r1.1 · r2.1 · dirA · 60° · 80° · CpG

#R0654S 200 units
#R0654L 1,000 units

5′... G C A N N N N▼N T G C ...3′
3′... C G T N▲N N N N A C G ...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: 10%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

BstNI

r1.1 · r2.1 · NEB r3.1 · dirA · 60° · No

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

5′... C C▼W G G ...3′
3′... G G W▲C C ...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

BstUI

rCutSmart · dilA · 60° · Heat Inactivation · CpG

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

5′...C G▼C G...3′
3′...G C▲G C...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: 25%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

Bsu36I

rCutSmart · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · dilC · 37° · Heat Inactivation 80°

#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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BstXI

Recommended Buffer r3.1 · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · dilB · 37° · Heat Inactivation 80° · dcm

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

5′...CCANNNNN▼NTGG...3′
3′...GGTN▲NNNNNACC...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	25

Concentration: 10,000 units/ml

Methylation Sensitivity: Blocked by some combinations of overlapping *dcm* methylation (see p. 321).

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

BtgI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · dilB · 37° · Heat Inactivation 80°

#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.

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BstYI

Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · NEB r2.1 · dilA · 60° · Heat Inactivation

#R0523S 2,000 units

5′...R▼GATCY...3′
3′...YCTAG▲R...5′

Reaction Conditions: NEBuffer r2.1, 60°C

Concentration: 10,000 units/ml

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

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BtgZI

rCutSmart · Cloned at NEB · Recombinant Enzyme · dilA · 60° · Heat Inactivation 80° · CpG

#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

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

Activity at 37°C: 50%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by CpG methylation (see p. 321).

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

High-Fidelity

BstZ17I-HF®

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

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

5′...GTA▼TAC...3′
3′...CAT▲ATG...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: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

BtsI-v2

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · dilA · 37° · Heat Inactivation

#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

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

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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 · 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

BtsIMutI

rCutSmart · RR · e · dirA · 55° · 80°

#R0664S 100 units

5′...CAGTGNN▼...3′
3′...GTCAC▲NN...5′

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

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

Concentration: 1,000 units/ml

Activity at 37°C: 50%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

BtsCI

rCutSmart · RR · dirB · 50° · 80°

#R0647S 2,000 units

5′...GGATGNN▼...3′
3′...CCTAC▲NN...5′

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

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

Concentration: 20,000 units/ml

Activity at 37°C: 25%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Cac8I

rCutSmart · dirB · 37° · 65° · CpG

#R0579L 500 units

5′...GCN▼NGC...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: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

Note: Store at -80°C.

ClaI

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

#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: Blocked by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

CspCI

rCutSmart · RR · 2*site · dirA · 37° · 65°

#R0645S 500 units

5′...▼10-11(N)CAA(N)5GTGG(N)12-13▼...3′
3′...▲12-13(N)GTT(N)5CACC(N)10-11▲...5′

Note: The cleavage point may shift one base pair depending on the DNA sequence context before and after the recognition site. For a given sequence, one site will predominate. For details, see www.neb.com.

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

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

Concentration: 5,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

CviAII

rCutSmart · RR · dirC · 25° · 65°

#R0640S 200 units
#R0640L 1,000 units

5′...C▼ATG...3′
3′...GTA▲C...5′

Reaction Conditions: rCutSmart Buffer, 25°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

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

CviKI-1

rCutSmart · RR · dirA · 37° · No

#R0710S 250 units

5′...RG▼CY...3′
3′...YC▲GR...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 5,000 units/ml

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

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

CviQI

RR · NEB r3.1 · dirC · 25° · No

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

5′...G▼TAC...3′
3′...CAT▲G...5′

Reaction Conditions: NEBuffer r3.1, 25°C

Concentration: 10,000 units/ml

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

Activity at 37°C: 25%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

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

DdeI

rCutSmart · RR · ♦ · dilB · 37° · 65°

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

5′...C▼TNAG...3′
3′...GANT▲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: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

DpnI

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

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

CH3
5′... GA▼TC...3′
3′... CT▲AG...5′
CH3

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: 20,000 units/ml

Methylation Sensitivity: DpnI cleaves only when its recognition site is methylated. DNA purified from a *dam*+ strain will be a substrate for DpnI. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

DpnII

RR · Epi · ♦ · NEBU · dilB · 37° · 65° · dam

#R0543S 1,000 units
#R0543L 5,000 units

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

5′...▼GATC...3′
3′...CTAG▲...5′

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

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

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

Methylation Sensitivity: Blocked by *dam* methylation (see p. 321).

Note: *May exhibit star activity in this buffer.

DraI

rCutSmart · RR · ♦ · dilA · 37° · 65°

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

5′...TTT▼AAA...3′
3′...AAA▲TTT...5′

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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

High-Fidelity

DraIII-HF®

rCutSmart · RR · e · ♦ · dilB · 37° · No · CpG

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

5′...CACNNN▼GTG...3′
3′...GTG▲NNNCAC...5′

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by some combinations of overlapping CpG methylation (see p. 321).

DrdI

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

#R0530S 300 units
#R0530L 1,500 units

5′...GACNNNNN▼NGTC...3′
3′...CTGNN▲NNNNCAG...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	10	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

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

EaeI

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

#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: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

EagI

EagI has been replaced by EagI-HF (engineered for performance).

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

High-Fidelity

EagI-HF®

rCutSmart · RR · e · dIIB · 37° · 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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

Note: Store at -80°C.

EarI

rCutSmart · RR · dIIB · 37° · 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: Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation (see p. 321).

EciI

rCutSmart · RR · dIIA · 37° · 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: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

Note: Star activity may result from extended digestion.

Eco53kI

rCutSmart · RR · dIIA · 37° · 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

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

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

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

EcoNI

rCutSmart · RR · dIIA · 37° · 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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

EcoO109I

rCutSmart · RR · dIIA · 37° · 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: Blocked by overlapping *dcm* methylation (see p. 321).

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

EcoP15I

RR · 2*site · NEB r3.1 · dIIA · 37° · 65°

#R0646S 500 units

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

Reaction Conditions: NEBuffer r3.1 + ATP, 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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

EcoRI

RR · NEB U · dIIC · 37° · 65° · CpG

#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: Cleavage of mammalian genomic DNA is blocked by some combination of overlapping CpG methylation (see p. 321).

Note: *May exhibit star activity in this buffer.

High-Fidelity

EcoRI-HF®

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · dilC · 37° Incubation Temperature · 65° Heat Inactivation · CpG Methylation Sensitivity

#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 100,00 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

EcoRV

Cloned at NEB · Recombinant Enzyme · Engineered for Performance · NEB r3.1 · dilA · 37° Incubation Temperature · 80° Heat Inactivation · CpG Methylation Sensitivity

#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: Cleavage of mammalian genomic DNA is impaired by some combinations of overlapping CpG methylation (see p. 321).

High-Fidelity

EcoRV-HF®

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · dilB · 37° Incubation Temperature · 65° Heat Inactivation · CpG Methylation Sensitivity

#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: Cleavage of mammalian genomic DNA is impaired by some combinations of overlapping CpG methylation (see p. 321).

Esp3I

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · dilB · 37° Incubation Temperature · 65° Heat Inactivation · CpG Methylation Sensitivity

#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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

FatI

Cloned at NEB · Recombinant Enzyme · NEB r2.1 · dilA · 55° Heat Inactivation · 80° Methylation Sensitivity

#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.

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

Concentration: 2,000 units/ml

Activity at 37°C: 100%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

FauI

rCutSmart · Cloned at NEB · Recombinant Enzyme · dilA · 55° Heat Inactivation · 65° Methylation Sensitivity · CpG Methylation Sensitivity

#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

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

Activity at 37°C: 20%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

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

Fnu4HI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · dilA · 37° Incubation Temperature · No Heat Inactivation · CpG Methylation Sensitivity

#R0178S	200 units
#R0178L	1,000 units

5′...GC▼NGC...3′
3′...CGN▲CG...5′

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

FokI

rCutSmart · Cloned at NEB · Recombinant Enzyme · 2*site Multiple Recognition Site · dilA · 37° Incubation Temperature · 65° Heat Inactivation · dcm Methylation Sensitivity · CpG Methylation Sensitivity

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

5′...GGATG(N)$_9$▼...3′
3′...CCTAC(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: Impaired by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation (see p. 321).

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 · 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

FseI

rCutSmart · dilB · 37° · 65° · dcm · CpG

#R0588S 100 units
#R0588L 500 units

5′...GGCCGG▼CC...3′
3′...CC▲GGCCGG...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: Impaired by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

Note: Store at -80°C.

FspI

rCutSmart · dilC · 37° · NO · CpG

#R0135S 500 units
#R0135L 2,500 units

5′...TGC▼GCA...3′
3′...ACG▲CGT...5′

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 10,000 units/ml.

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

FspEI

rCutSmart · Epi · dilB · 37° · 80°

#R0662S 200 units

See page 280 for more information.

HaeII

rCutSmart · dilA · 37° · 80° · CpG

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

5′...RGCGC▼Y...3′
3′...Y▲CGCGR...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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

HaeIII

rCutSmart · dilA · 37° · 80°

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

5′...GG▼CC...3′
3′...CC▲GG...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

HgaI

r1.1 · dilA · 37° · 65° · CpG

#R0154S 100 units

5′...GACGC(N)$_5$▼...3′
3′...CTGCG(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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

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

HhaI

rCutSmart · dilA · 37° · 65° · CpG

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

5′...GCG▼C...3′
3′...C▲GCG...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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

HincII

r3.1 · dilB · 37° · 65° · CpG

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

5′...GTY▼RAC...3′
3′...CAR▲YTG...5′

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

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

HindIII

r2.1 · 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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion.

High-Fidelity

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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

HpaII

rCutSmart · RR · Epi · dilA · 37° · 60° · CpG

#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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

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: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

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: This enzyme is blocked by *dam* and *dcm* methylation (see p. 321).

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

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.

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

Hpy99I

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

#R0615S 100 units
#R0615L 500 units

5′...CGWCG▼...3′
3′...▲GCWGC...5′

Reaction Conditions: rCutSmart Buffer 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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

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

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

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

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

Hpy166II

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

#R0616S 1,000 units

5′...GTN▼NAC...3′
3′...CAN▲NTG...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	50	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

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

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: Blocked by overlapping *dam* methylation (see p. 321).

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

Hpy188III

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

#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: Blocked by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

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

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: Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation. (see p. 321).

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

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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

KasI

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

#R0544S 250 units
#R0544L 1,250 units

5′...GGCGCC...3′
3′...CCGCGG...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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

Note: Star activity may result from a glycerol concentration of > 5%. Store at -80°C.

KpnI

RR · r1.1 · dilA · 37° · No

#R0142S 4,000 units
#R0142L 20,000 units
for high (5X) concentration
#R0142M 20,000 units

5′...GGTACC...3′
3′...CCATGG...5′

Reaction Conditions: NEBuffer r1.1, 37°C

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

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

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

KpnI-HF®

High-Fidelity

#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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

MfeI-HF®

High-Fidelity

#R3589S 500 units
#R3589L 2,500 units

5′...C▼AATTG...3′
3′...GTTAA▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 20,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

LpnPI

#R0663S 200 units

See page 280 for more information.

MboI

#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.

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

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

Methylation Sensitivity: Blocked by *dam* methylation. Its isoschizomer Sau3AI is not. Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation (see p. 321).

MboII

#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.

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

Concentration: 5,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dam* methylation (see p. 321).

Note: *May exhibit star activity in this buffer.

MfeI

#R0589S 500 units
#R0589L 2,500 units

5′...C▼AATTG...3′
3′...GTTAA▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

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

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion.

MluI

#R0198S 1,000 units
#R0198L 5,000 units

5′...A▼CGCGT...3′
3′...TGCGC▲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	25

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

MluI-HF®

High-Fidelity

#R3198S 1,000 units
#R3198L 5,000 units

5′...A▼CGCGT...3′
3′...TGCGC▲A...5′

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

MluCI

#R0538S 1,000 units
#R0538L 5,000 units

5′...▼AATT...3′
3′...TTAA▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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 · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

MseI

rCutSmart · 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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

MmeI

rCutSmart · 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: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

MslI

rCutSmart · 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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

MnlI

rCutSmart · 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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

MspI

rCutSmart · Epi · dilA · 37° · No

#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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

MscI

rCutSmart · 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.

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

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

Methylation Sensitivity: Blocked by overlapping *dcm* methylation (see p. 321). 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.

MspA1I

rCutSmart · 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: Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

MspJI

rCutSmart | RR | Epi | diluent B | 37°C | 65°C

#R0661S 200 units
#R0661L 1,000 units

See page 280 for more information.

MwoI

rCutSmart | RR | e | diluent B | 60°C | No | CpG

#R0573S 500 units
#R0573L 2,500 units

5′...GCNNNNNNNGC...3′
3′...CGNNNNNNNCG...5′

Reaction Conditions: rCutSmart Buffer, 60°C

Concentration: 5,000 units/ml

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

Activity at 37°C: 25%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

NaeI

rCutSmart | RR | 2*site | diluent A | 37°C | No | CpG

#R0190S 500 units
#R0190L 2,500 units

5′...GCCGGC...3′
3′...CGGCCG...5′

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

NarI

rCutSmart | RR | 2*site | diluent A | 37°C | 65°C | CpG

#R0191S 500 units
#R0191L 2,500 units

5′...GGCGCC...3′
3′...CCGCGG...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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

NciI

rCutSmart | RR | e | diluent A | 37°C | No | CpG

#R0196S 2,000 units
#R0196L 10,000 units

5′...CCSGG...3′
3′...GGSCC...5′

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation (see p. 321).

NcoI

RR | e | NEB r3.1 | diluent A | 37°C | 80°C

#R0193S 1,000 units
#R0193L 5,000 units
for high (5X) concentration
#R0193T 1,000 units
#R0193M 5,000 units

5′...CCATGG...3′
3′...GGTACC...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation. +For added flexibility, NEB offers an isoschizomer or HF enzyme, supplied with rCutSmart Buffer.

High-Fidelity

NcoI-HF®

rCutSmart | RR | e | e | diluent B | 37°C | 80°C

#R3193S 1,000 units
#R3193L 5,000 units
for high (5X) concentration
#R3193M 5,000 units

5′...CCATGG...3′
3′...GGTACC...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

NdeI

rCutSmart | RR | e | diluent A | 37°C | 65°C

#R0111S 4,000 units
#R0111L 20,000 units

5′...CATATG...3′
3′...GTATAC...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

NgoMIV

rCutSmart | RR | 2*site | e | diluent A | 37°C | No | CpG

#R0564S 1,000 units
#R0564L 5,000 units

5′...GCCGGC...3′
3′...CGGCCG...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

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

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 334).

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 | diluent A Diluent Buffer | 37°C Incubation Temperature | 65°C Heat Inactivation | dam Methylation Sensitivity

NheI

NheI has been replaced by NheI-HF (engineered for performance).

NheI-HF®

High-Fidelity

rCutSmart · 80° · CpG

#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: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

NlaIII

rCutSmart · dirB · 37° · 65°

#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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Store at -80°C.

NlaIV

rCutSmart · dirB · 37° · 65° · dcm · CpG

#R0126S 200 units

5′...GGN▼NCC...3′
3′...CCN▲NGG...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: 2,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

NmeAIII

rCutSmart · 2*site · dirB · 37° · 65°

#R0711S 250 units

5′...GCCGAG(N)$_{20\text{-}21}$▼...3′
3′...CGGCTC(N)$_{18\text{-}19}$▲...5′

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 site will predominate. For details, see www.neb.com.

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.

Concentration: 2,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

NotI

r3.1 · dirC · 37° · 65° · CpG

#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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

NotI-HF®

High-Fidelity

rCutSmart · dirA · 37° · 65° · CpG

#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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

NruI

r3.1 · dirA · 37° · No · dam · CpG

#R0192S 1,000 units
#R0192L 5,000 units

5′...TCG▼CGA...3′
3′...AGC▲GCT...5′

Reaction Conditions: NEBuffer r3.1, 37°C

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

NruI-HF®

High-Fidelity

rCutSmart · RR · e · ◷ · dilA · 37° · 80° · dam · CpG

#R3192S 1,000 units
#R3192L 5,000 units

5′...TCG▼CGA...3′
3′...AGC▲GCT...5′

Reaction Conditions: rCutSmart Buffer, 37°C.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	0	25	50	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Blocked by overlapping *dam* methylation. Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

PacI

rCutSmart · RR · ◷ · 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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

NsiI

RR · ◷ · NEB r3.1 · dilB · 37° · 65°

#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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

PaeR7I

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

#R0177S 2,000 units

5′...C▼TCGAG...3′
3′...GAGCT▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

NsiI-HF®

High-Fidelity

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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

NEW 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. Heat inactivation: 65°C for 20 minutes.

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

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

NspI

rCutSmart · RR · ◷ · 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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

PciI

RR · NEB r3.1 · 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.

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

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

PflFI

rCutSmart | r1.1 | r2.1 | r3.1 | dilA | 37° | 65°

#R0595S 2,000 units

5′...GACNNNGTC...3′
3′...CTGNNNCAG...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

PflMI

r1.1 | r2.1 | NEB r3.1 | dilA | 37° | 65° | dcm

#R0509S 1,000 units
#R0509L 5,000 units

5′...CCANNNNNTGG...3′
3′...GGTNNNNNACC...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: Blocked by overlapping *dcm* methylation (see p. 321).

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

PleI

rCutSmart | r1.1 | r2.1 | 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: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

PluTI

rCutSmart | r1.1 | r2.1 | 2*site | dilA | 37° | 65° | CpG

#R0713S 500 units

5′...GGCGCC...3′
3′...CCGCGG...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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

PmeI

rCutSmart | r1.1 | r2.1 | dilA | 37° | 65° | CpG

#R0560S 500 units
#R0560L 2,500 units

5′...GTTTAAAC...3′
3′...CAAATTTG...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: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

PmlI

rCutSmart | r1.1 | r2.1 | dilA | 37° | 65° | CpG

#R0532S 2,000 units
#R0532L 10,000 units

5′...CACGTG...3′
3′...GTGCAC...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	100	50	<10	100

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

PpuMI

rCutSmart | r1.1 | r2.1 | dilB | 37° | W1 | dcm

#R0506S 500 units
#R0506L 2,500 units

5′...RGGWCCY...3′
3′...YCCWGGR...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: Blocked by overlapping *dcm* methylation (see p. 321).

PshAI

rCutSmart | r1.1 | r2.1 | dilA | 37° | 65° | CpG

#R0593S 1,000 units
#R0593L 5,000 units

5′...GACNNNGTC...3′
3′...CTGNNNCAG...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: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

PsiI

PsiI has been replaced by PsiI-v2 (optimized for performance).

NEW

PsiI-v2

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

#R0744S 400 units
#R0744L 2,000 units

5′...T T A ▼T A A...3′
3′...A A T ▲A T T...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	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

PspGI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Diluent A · 75°C · No Heat Inactivation · dcm

#R0611S 1,000 units

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

Reaction Conditions: rCutSmart Buffer, 75°C

Concentration: 10,000 units/ml

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

Activity at 37°C: 25%

Methylation Sensitivity: Blocked by *dcm* methylation (see p. 321).

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

PspOMI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Diluent B · 37°C · Heat Inactivation 65°C · dcm · CpG

#R0653S 1,500 units
#R0653L 7,500 units

5′...G▼G G C C C...3′
3′...C C C G G▲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	10	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity: Impaired by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

PspXI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Diluent B · 37°C · No Heat Inactivation · CpG

#R0656S 200 units
#R0656L 1,000 units

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

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 5,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by CpG methylation (see page 321).

PstI

Cloned at NEB · Recombinant Enzyme · Engineered for Performance · NEB r3.1 · Diluent C · 37°C · Heat Inactivation 80°C

#R0140S 10,000 units
#R0140L 50,000 units
for high (5X) concentration
#R0140T 10,000 units
#R0140M 50,000 units

5′...C T G C A▼G...3′
3′...G▲A C G T 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	75	75	100	50*

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

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: *May exhibit star activity in this buffer.

High-Fidelity

PstI-HF®

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Diluent C · 37°C · No Heat Inactivation

#R3140S 10,000 units
#R3140L 50,000 units
for high (5X) concentration
#R3140T 10,000 units
#R3140M 50,000 units

5′...C T G C A▼G...3′
3′...G▲A C G T C...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

PvuI

Cloned at NEB · Recombinant Enzyme · NEB r3.1 · Diluent B · 37°C · No Heat Inactivation · CpG

#R0150S 500 units
#R0150L 2,500 units

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

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

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

High-Fidelity

PvuI-HF®

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Diluent B · 37°C · No Heat Inactivation · CpG

#R3150S 500 units
#R3150L 2,500 units

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

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 20,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

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 · 37°C Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

PvuII

#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

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

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

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

*May exhibit star activity in this buffer.

SacI

#R0156S 2,000 units
#R0156L 10,000 units
for high (5X) concentration
#R0156M 10,000 units

5′...GAGCT▼C...3′
3′...C▲TCGAG...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: 20,000 and 100,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: +For added flexibility, NEB offers an isoschizomer or HF enzyme, supplied with rCutSmart Buffer.

PvuII-HF®

High-Fidelity

#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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

SacI-HF®

High-Fidelity

#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: Not sensitive to *dam* or *dcm* methylation. Blocked by some combinations of overlapping CpG methylation.

RsaI

#R0167S 1,000 units
#R0167L 5,000 units

5′...GT▼AC...3′
3′...CA▲TG...5′

Reaction Conditions: rCutSmart Buffer, 37°C

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

SacII

#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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

RsrII

#R0501S 500 units
#R0501L 2,500 units

5′...CGGWCCG...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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

SalI

#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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

SalI-HF®

High-Fidelity

rCutSmart · RR · e · ⏱ · diluA · 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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

SbfI

rCutSmart · RR · ⏱ · diluA · 37° · 80°

#R0642S 500 units
#R0642L 2,500 units

5′...CCTGCA▼GG...3′
3′...GG▲ACGTCC...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	25	<10	100

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

SapI

rCutSmart · RR · ⏱ · diluB · 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: Not sensitive to *dam, dcm* or mammalian CpG methylation.

SbfI-HF®

High-Fidelity

rCutSmart · RR · e · ⏱ · diluB · 37° · 80°

#R3642S 500 units
#R3642L 2,500 units

5′...CCTGCA▼GG...3′
3′...GG▲ACGTCC...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: Not sensitive to *dam, dcm* or mammalian CpG methylation.

Sau3AI

RR · NEB r1.1 · diluA · 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.

Concentration: 5,000 units/ml

NEBuffer	r1.1	2.1	3.1	rCutSmart
% Activity	100	50	10	100+

Methylation Sensitivity: Unlike DpnII and MboI, Sau3AI is not blocked by *dam* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

Note: +For added flexibility, NEB offers an isoschizomer or HF enzyme, supplied with rCutSmart Buffer.

ScaI-HF®

High-Fidelity

rCutSmart · RR · e · ⏱ · diluB · 37° · 80°

#R3122S 1,000 units
#R3122L 5,000 units
for high (5X) concentration
#R3122M 5,000 units

5′...AGT▼ACT...3′
3′...TCA▲TGA...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: Not sensitive to *dam, dcm* or mammalian CpG methylation.

Sau96I

rCutSmart · RR · diluA · 37° · 65° · dcm · CpG

#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: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

ScrFI

rCutSmart · RR · diluC · 37° · 65° · dcm · CpG

#R0110S 1,000 units

5′...CC▼NGG...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: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation (see p. 321).

Note: Star activity may result from extended digestion.

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 · diluA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

SexAI

rCutSmart · dcm

#R0605S 200 units
#R0605L 1,000 units

5′...A▼CCWGGT...3′
3′...TGGWCC▲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	75	50	100

Concentration: 5,000 units/ml

Methylation Sensitivity: Blocked by *dcm* methylation (see p. 321).

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	1.1	2.1	r3.1	rCutSmart
% Activity	<10	75	100	25

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by some combinations of overlapping CpG methylation (see p. 321).

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′...GAYRT▲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	50	25	100

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

SfiI

rCutSmart · 2*site · dilC · 50° · dcm · CpG

#R0123S 3,000 units
#R0123L 15,000 units

5′...GGCCNNNNN▼NGGCC...3′
3′...CCGGN▲NNNNNCCGG...5′

Reaction Conditions: rCutSmart Buffer, 50°C

Concentration: 20,000 units/ml

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

Activity at 37°C: 10%

Methylation Sensitivity: Impaired by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

SfoI

rCutSmart · dilB · 37° · dcm · CpG

#R0606S 500 units
#R0606L 2,500 units

5′...GGC▼GCC...3′
3′...CCG▲CGG...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: Blocked by some combinations of overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

SgrAI

rCutSmart · 2*site · dilA · 37° · 65° · CpG

#R0603S 1,000 units
#R0603L 5,000 units

5′...CR▼CCGGYG...3′
3′...GYGGC▲CRC...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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

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

SmaI

rCutSmart · dilB · 25° · 65° · CpG

#R0141S 2,000 units
#R0141L 10,000 units

5′...CCC▼GGG...3′
3′...GGG▲CCC...5′

Reaction Conditions: rCutSmart Buffer, 25°C. Heat inactivation: 65°C for 20 minutes.

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

Concentration: 20,000 units/ml

Activity at 37°C: 25%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

SmlI

rCutSmart · dilA · 55°

#R0597S 500 units
#R0597L 2,500 units

5′...CTYRAG...3′
3′...GARYT▲C...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

SnaBI

rCutSmart · 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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

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

SpeI

rCutSmart · RR · dilC · 37° · 80°

#R0133S 500 units
#R0133L 2,500 units
for high (5X) concentration
#R0133M 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	75	100	25	100

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

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

SpeI-HF®

High-Fidelity

rCutSmart · RR · e · 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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

SphI

RR · r2.1 · 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	1.1	r2.1	3.1	rCutSmart
% Activity	100	100	50	100+

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion. +For added flexibility, NEB offers an isoschizomer or HF enzyme, supplied with rCutSmart Buffer.

SphI-HF®

High-Fidelity

rCutSmart · RR · e · 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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

SrfI

rCutSmart · RR · 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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p 321).

SspI

RR · NEBU · dilC · 37° · 65°

#R0132S 1,000 units
#R0132L 5,000 units
for high (5X) concentration
#R0132M 5,000 units

5′...AAT▼ATT...3′
3′...TTA▲TAA...5′

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

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

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

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

SspI-HF®

High-Fidelity

rCutSmart · RR · e · dilB · 37° · 65°

#R3132S 1,000 units
#R3132L 5,000 units
for high (5X) concentration
#R3132M 5,000 units

5′...AAT▼ATT...3′
3′...TTA▲TAA...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

StuI

rCutSmart · RR · e · dIIA · 37° · No · dcm

#R0187S 1,000 units
#R0187L 5,000 units
for high (10X) concentration
#R0187M 5,000 units

5′...AGG▼CCT...3′
3′...TCC▲GGA...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: Blocked by overlapping *dcm* methylation (see p. 321).

StyI

StyI has been replaced by StyI-HF (engineered for performance).

High-Fidelity

StyI-HF®

rCutSmart · RR · e · dIIA · 37° · No

#R3500S 3,000 units
#R3500L 15,000 units

5′...C▼CWWGG...3′
3′...GGWW▲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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

StyD4I

rCutSmart · RR · e · dIIB · 37° · No · dcm · CpG

#R0638S 200 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	10	100	100	100

Methylation Sensitivity: Blocked by overlapping *dcm* methylation. Cleavage of mammalian genomic DNA is impaired by overlapping CpG methylation. (see p. 321).

SwaI

RR · e · NEB r3.1 · dIIB · 25° · No

#R0604S 2,000 units
#R0604L 10,000 units

5′...ATTT▼AAAT...3′
3′...TAAA▲TTTA...5′

Reaction Conditions: NEBuffer r3.1, 25°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	1.1	2.1	r3.1	rCutSmart
% Activity	10	10	100	10

Activity at 37°C: 25%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

TaqI-v2

rCutSmart · RR · e · dIIB · 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▼CGA...3′
3′...AGC▲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: Blocked by overlapping *dam* methylation (see p. 321).

TfiI

rCutSmart · RR · e · dIIC · 65° · No · CpG

#R0546S 500 units

5′...G▼AWTC...3′
3′...CTWA▲G...5′

Reaction Conditions: rCutSmart Buffer, 65°C

Concentration: 10,000 units/ml

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

Activity at 37°C: 10%

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

TseI

rCutSmart · RR · e · dIIB · 65° · No · CpG

#R0591L 500 units

5′...G▼CWGC...3′
3′...CGW▲CG...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: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

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

Tsp45I

rCutSmart · RR · dIIA · 65° · No

#R0583S 400 units
#R0583L 2,000 units

5′...▼GTSAC...3′
3′...CAST▲G...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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

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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

Note: *May exhibit star activity in this buffer.

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

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

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Tth111I

rCutSmart · RR · dilB · 65° · No

#R0185S 400 units

5′...GACNNNGTC...3′
3′...CTGNN▲NCAG...5′

Reaction Conditions: rCutSmart Buffer, 65°C

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

Concentration: 5,000 units/ml

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

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: Blocked by overlapping *dam* methylation (see p. 321).

XcmI

RR · r2.1 · dilC · 37° · 65°

#R0533S 1,000 units
#R0533L 5,000 units

5′...CCANNNNN▼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	1.1	r2.1	3.1	rCutSmart
% Activity	10	100	25	100*

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Note: Star activity may result from extended digestion. *May exhibit star activity in this buffer.

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: Cleavage of mammalian genomic DNA is impaired by CpG methylation (see p. 321).

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.

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

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

Methylation Sensitivity: Cleavage of mammalian genomic DNA is impaired by CpG methylation (see p. 321).

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

XmnI

rCutSmart · RR · dilA · 37° · 65°

#R0194S 1,000 units
#R0194L 5,000 units

5′...GAANNNNTTC...3′
3′...CTTNN▲NNAAG...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

rCutSmart or 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

ZraI

rCutSmart · dIB · 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: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

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.

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(3) Van Ness, J. et al. (2003) *Proc. Natl. Acad. Sci. USA*, 89, 4504–4509.
(2) Chan, S.H. et al. (2004) *Nucl. Acids Res.*, 32, 6187–6199.

Nb.BbvCI

rCutSmart · dIA · 37° · 80°

#R0631S 1,000 units
#R0631L 5,000 units

5′...CCTCAGC...3′
3′...GGAGT▲CG...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: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Nb.BsmI

r3.1 · dIA · 65° · 80°

#R0706S 1,000 units

5′...GAATGCN...3′
3′...CTTAC▲GN...5′

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

NEBuffer	1.1	2.1	r3.1	rCutSmart
% Activity	<10	50	100	10

Concentration: 10,000 units/ml

Activity at 37°C: 25%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Nb.BsrDI

rCutSmart · RR · dilA · 65° · 80°

#R0648S 1,000 units
#R0648L 5,000 units

5′...GCAATGNN...3′
3′...CGTTACNN...5′

Reaction Conditions: rCutSmart Buffer, 65°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

Activity at 37°C: 50%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Nt.BbvCI

rCutSmart · RR · 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.

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by some combinations of overlapping CpG methylation (see p. 321).

Nb.BssSI

RR · r3.1 · dilB · 37° · No

#R0681S 1,000 units
for high (5X) concentration
#R0681T 5,000 units

5′...CACGAG...3′
3′...GTGCTC...5′

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

NEBuffer	1.1	2.1	r3.1	rCutSmart
% Activity	10	100	100	25

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

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Nt.BsmAI

rCutSmart · RR · 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.

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

Concentration: 5,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

Nb.BtsI

rCutSmart · RR · dilA · 37° · 80°

#R0707S 1,000 units

5′...GCAGTGNN...3′
3′...CGTCACNN...5′

Reaction Conditions: rCutSmart Buffer, 37°C.

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Nt.BspQI

RR · 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.

NEBuffer	1.1	2.1	r3.1	rCutSmart
% Activity	<10	25	100	10

Concentration: 10,000 units/ml

Activity at 37°C: 80%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

Nt.AlwI

rCutSmart · RR · 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.

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

Concentration: 10,000 units/ml

Methylation Sensitivity: Cleavage is blocked by *dam* methylation (see p. 321).

Nt.BstNBI

RR · 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.

NEBuffer	1.1	2.1	r3.1	rCutSmart
% Activity	0	10	100	10

Concentration: 10,000 units/ml

Activity at 37°C: 10%

Methylation Sensitivity: Not sensitive to *dam*, *dcm* or mammalian CpG methylation.

rCutSmart or 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

Nt.CviPII

rCutSmart dilA 37° 65° CpG

#R0626S 40 units

5´...▼CCD...3´
3´...GGH...5´

D = A or G or T (not C)
H = A or C or T (not G)

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

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

Concentration: 2,000 units/ml

Methylation Sensitivity: Cleavage of mammalian genomic DNA is blocked by CpG methylation (see p. 321).

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:

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I-CeuI

rCutSmart dilB 37° 65°

#R0699S 500 units
#R0699L 2,500 units

(Supplied with 5 µg of plasmid DNA)

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´...TAACTATAACGGTCCTAA▼GGTAGCGAA...3´
3´...ATTGATATTGCCAGGA▲TTCCATCGCTT...5´

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

Concentration: 5,000 units/ml

Note: Homing endonucleases do not have stringently-defined recognition sequences.

I-SceI

rCutSmart dilB 37° 65°

#R0694S 500 units
#R0694L 2,500 units

(Supplied with 5 µg of plasmid DNA)

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´...TAGGGATAA▼CAGGGTAAT...3´
3´...ATCCCTATTGTCCCATTA▲...5´

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

Concentration: 5,000 units/ml

Note: Homing endonucleases do not have stringently-defined recognition sequences. Store at -80°C.

PI-PspI

#R0695S 500 units

(Supplied with 5 µg of plasmid DNA)

Reaction Conditions: NEBuffer PI-PspI + Recombinant Albumin, 65°C

Specificity: The homing or recognition site for this endonuclease is shown below:

```
5´...TGGCAAACAGCTATTATGGGTATTATGGGT...3´
3´...ACCGTTTGTCGATAATACCCATAATACCCA...5´
```

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

Concentration: 5,000 units/ml

Activity at 37°C: 5%

Note: Homing endonucleases do not have stringently-defined recognition sequences.

PI-SceI

#R0696S 250 units

(Supplied with 5 µg of plasmid DNA)

Reaction Conditions: NEBuffer PI-SceI + Recombinant Albumin, 37°C. 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´
```

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

Concentration: 5,000 units/ml

Note: Homing endonucleases do not have stringently-defined recognition sequences.

Recombinant Albumin, Molecular Biology Grade

#B9200S 12 mg

Reaction Conditions: 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.

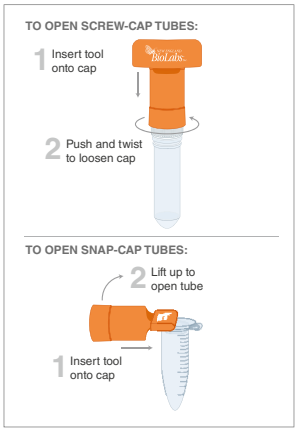
Companion Product:

BSA, Molecular Biology Grade
#B9000S 12 mg

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™ 1.1 (10X)
#B7201S 5.0 ml

NEBuffer 2.1 (10X)
#B7202S 5.0 ml

NEBuffer 3.1 (10X)
#B7203S 5.0 ml

CutSmart Buffer (10X)
#B7204S 5.0 ml

NEBuffer Set (EcoRI/SspI, DpnII)
#B7006S 2.5 ml of each

NEBuffer Set (1.1, 2.1, 3.1 & CutSmart)
#B7200S 1.25 ml of each

NEW
NEBuffer Set (r1.1, r2.1, r3.1 & rCutSmart™)
#B7030S 1.25 ml of each

S-adenosylmethionine (SAM)
#B9003S (32 mM) 0.5 ml

Nuclease-free Water
#B1500S 25 ml
#B1500L 100 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 (1.1, 2.1, 3.1 & CutSmart) is formulated with BSA, while 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.

For more information about NEBuffer compatibility, please turn to page 309.

Reaction Buffer Compositions: Visit www.neb.com for details.

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · Recombinant Enzyme · EpiMark Validated · Engineered for Performance · Multiple Recognition Site · Time-Saver Qualified · Diluent Buffer · Incubation Temperature · Heat Inactivation · Methylation Sensitivity

Diluent Buffers

Diluent A
#B8001S 5.0 ml

Diluent B
#B8002S 5.0 ml

Diluent C
#B8003S 5.0 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; a complete listing of all restriction endonucleases and their diluents can be found on page 309.

Storage Conditions:
Store at –20°C.

Diluent Buffer Compositions: 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.

Scott has been with NEB for 2 years as a Purification Scientist in our Rowley Production Facility. He primarily works on downstream processing of products, but also helps with enzyme purification. In his spare time, Scott enjoys cooking and spending time with friends and family outdoors.

Aim AR camera at this page
to see image in motion.

Conserving Biodiversity

Biodiversity is a broad concept that refers to the "web of life". It describes all the organisms on Earth (species diversity), the variety of organisms in an ecosystem (ecosystem diversity), and the genetic make-up of those organisms (genetic diversity). Biodiversity is responsible for sustaining life as we know it — from producing oxygen, to pollinating our food sources and cleaning our water supplies.

It is estimated that 80% of the world's biodiversity exists underwater, within the just over 70% of Earth's surface that is covered by ocean. And, if you have had the opportunity to explore a coastline or to gaze underwater, this likely comes as no surprise. Teeming with creatures from microscopic to the largest known on Earth (the blue whale), ocean life is amazingly diverse.

It is easy to understand why Don Comb's curiosity was piqued by the marine ecosystems he visited as he pursued his Ph.D. at the University of Michigan. His graduate research first took him to the Bermuda Biological Research Station, where he studied sea urchin development. Firsthand experience observing rich and diverse marine organisms and their interactions within the ecosystem inspired Don to make protecting biodiversity a priority.

In the 1970s, Don supported the Conservation Law Foundation's efforts to stop offshore drilling on George's Bank, an area recognized for its diverse marine biology. Not long after, he helped establish a marine sanctuary in the waters off the island of Saint Barthélemy through the New England Biolabs Foundation (NEBF), which was established shortly after NEB became profitable. This sanctuary protects breeding grounds for local fish, as well as a diversely inhabited coral reef ecosystem.

In 2000 Don read an opinion piece in *Science*, which asked the question: Why aren't we saving DNA from the world's threatened and endangered species? Don realized that it wouldn't require new technology to do so, just someone with the initiative. In 2001, he founded The Ocean Genome Legacy (OGL), a non-profit environmental research organization and DNA bank dedicated to promoting new methods for studying and conserving marine species through preservation and genome analysis. As part of its mission, OGL created the Ocean Genome Resource Collection, a publicly accessible genome biorepository containing samples of DNA from species around the world, abundant and rare, who call the ocean home. In 2013, this priceless collection was moved to Northeastern University's Marine Science Center, located in Nahant, MA. Here it continues to help uncover some of our oceans' deepest mysteries and reveal genomic information that can help cure diseases, improve the sustainability of global food and energy supplies, and protect the environment.

Don challenged everyone to consider their impact on the planet's biodiversity. In the 2002/2003 NEB Catalog, he included a personal note, urging readers to "learn to live well on less and remember that small is beautiful. We all need to recognize that the earth's biodiversity is our future." Through the NEBF, Don has encouraged individuals to do just that, all over the world, by providing support and funding to communities in biodiversity-rich areas and the organizations working to conserve and protect them.

View the calming motions of the sea urchin.

Sea Urchin moving its parts.
Credit: Uw.art, Adobe Stock

DNA Polymerases & Amplification Technologies

NEB has pursued the discovery & development of DNA polymerases for over 25 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.

Find an overview of PCR.

Featured Products

66 Q5® High-Fidelity DNA Polymerase

68 One*Taq*® DNA Polymerase

72 Luna® qPCR & RT-qPCR Products

78 WarmStart® Colorimetric LAMP/ RT-LAMP 2X Master Mix

Featured Tools & Resources

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322 Guidelines for PCR Optimization

323 PCR Troubleshooting Guide

Visit www.neb.com/PCR to find additional online tools, video tech tips and tutorials to help you in setting up your PCR experiments.

RI Recombinant Enzyme

Amplification-based Molecular Diagnostics

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 diagnostics. The table below summarizes products available from NEB for diagnostic applications. Bulk 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 AVAILABLE
PCR APPLICATIONS	qPCR/RT-qPCR	DNA, Dye **Luna Universal qPCR Master Mix** (NEB #M3003) DNA, Probe **Luna® Universal Probe qPCR Master Mix** (NEB #M3004)	• Sensitive, reproducible and reliable performance • Compatible with automated liquid handling and reaction miniaturization • Room temperature stable for ≥ 24 hours	• ROX-free • 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 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)	• 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 • High conc. ideal for viral targets (NEB #M3019) • Includes carryover prevention (NEB #M3019)	• Rox-free • Blue-dye-free • Lyo-compatible
		RNA (2-step) **LunaScript® RT SuperMix Kit** (NEB #E3010)	• Novel thermostable RT • Single-tube format • 13-minute 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
		Hemo KlenTaq (NEB #M0332)	• Amplification direct from blood	• +/- Hot Start • High conc.
		Hot Start *Taq* DNA Polymerase (NEB #M0495) **Hot Start *Taq* 2X Master Mix** (NEB #M0496)	• Unique aptamer-based enzyme control supports fast protocols • Compatible with automation and reaction miniaturization	• 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)	• Fast, clear pink-to-yellow visible detection of amplification • Results in approximately 30 minutes	
		SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit (NEB #E2019)	• Simple, colorimetric detection of amplification of SARS-CoV-2 nucleic acid • Automation-compatible when coupled with absorbance plate reader	
		WarmStart Fluorescent LAMP/RT-LAMP Kit (NEB #E1700) **WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG)** (NEB #E1708) **WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)** (NEB #M1708)	• Master mix for LAMP and RT-LAMP workflows • Supports multiple detection methods, including fluorescence and turbidity • Automation compatible	• Lyo-compatible • High conc.
		***Bst* 2.0 WarmStart DNA Polymerase** (NEB #M0538) ***Bst* 2.0 DNA Polymerase** (NEB #M0537)	• 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.
		WarmStart RTx Reverse Transcriptase (NEB #M0380)	• *In-silico* designed RT for RT-LAMP with reversibly-bound aptamer that inhibits activity below 40°C	• Glycerol-free • High conc.
	Strand Displacement	**Nt.BstNBI** (NEB #R0607)	• High purity, high quality nicking endonuclease	• Glycerol-free • High conc.
	Helicase-dependent Amplification	***Tte* UvrD Helicase** (NEB #M1202)	• Thermostable • Improves specifically of problematic fluorescent LAMP reactions	• High conc.
		IsoAmp II Universal tHDA Kit (NEB #H0110)	• Requires only two primer • Produces short, discrete DNA products	
	Other	***Bsu* DNA Polymerase Large Fragment** (NEB #M0330)	• Enables low temperature isothermal applications	• High conc.
		T4 Gene 32 Protein (NEB #M0300)	• Can increase yield and efficiency of amplification reactions	• Glycerol-free
		Deoxynucleotide (dNTP) Solution Mix (NEB #N0447)	• Highly pure • Individual mixes available	• Custom conc.
		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.

PCR Polymerase Selection Chart

For over 45 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.

High-fidelity polymerases benefit from a Tm+3 annealing temp. Use the **NEB Tm Calculator** to ensure successful PCR at **TmCalculator.neb.com**

★ **indicates recommended choice for application**

	STANDARD PCR		HIGH-FIDELITY PCR			SPECIALTY PCR			
			HIGHEST FIDELITY		MODERATE FIDELITY	LONG AMPLICONS	dU TOLERANCE		BLOOD DIRECT PCR
	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*	Epimark® Hot Start *Taq*	Q5U™	Hemo KlenTaq®
PROPERTIES									
Fidelity vs. *Taq*	2X	1X	~280X(2)	> 39X	5–6X	2X	1X	ND	ND
Amplicon Size	< 6 kb	≤ 5 kb	≤ 20 kb	≤ 20 kb	≤ 6 kb	≤ 30 kb	≤ 1 kb	app-specific	≤ 2 kb
Extension Time	1 kb/min	1 kb/min	6 kb/min	4 kb/min	1 kb/min	1.2 kb/min	1 kb/min	2 kb/min	0.5 kb/min
Resulting Ends	3´ A/Blunt	3´ A	Blunt	Blunt	Blunt	3´ A/Blunt	3´ A	Blunt	3´ A
3´→5´ exo	Yes	No	Yes	Yes	Yes	Yes	No	Yes	No
5´→3´ exo	Yes	Yes	No	No	No	Yes	Yes	No	No
Units/50 µl Reaction	1.25	1.25	1.0	1.0	0.5–1.0	5.0	1.25	1.0	N/A
Annealing Temperature	Tm-5	Tm-5	Tm+3	Tm+3	Tm-5	Tm-5	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. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific.

(5) Use NEBNext Enzymatic Methyl-seq Kit (EM-seq™) for Illumina.

Why choose Q5 for your PCR?

Q5® High-Fidelity DNA Polymerase

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 rxns (50 µl vol)
#M0492L 500 rxns (50 µl vol)

Q5 Hot Start High-Fidelity DNA Polymerase
#M0493S 100 units
#M0493L 500 units

Q5 Hot Start High-Fidelity 2X Master Mix
#M0494S 100 rxns (2 x 1.25 ml)
#M0494L 500 rxns (10 x 1.25 ml)
#M0494X 500 rxns (1 x 12.5 ml)

NOW AVAILABLE: NEBNext Ultra II Q5 Master Mix – see page 169.

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)

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**

Q5U™ Hot Start High-Fidelity DNA Polymerase
#M0515S 100 units
#M0515L 500 units

Q5 High-Fidelity PCR Kit
#E0555S 50 rxns (50 µl vol)
#E0555L 200 rxns (50 µl vol)

Q5 Site-Directed Mutagenesis Kit
#E0554S 10 rxns

Q5 Site-Directed Mutagenesis Kit (without competent cells)
#E0552S 10 rxns

For a complete listing of Deoxynucleotide Solutions, see page 85.

For a complete listing of Reaction Buffers, see pages 85.

Description: Q5 High-Fidelity DNA Polymerase sets a new standard for both fidelity and performance. With the highest fidelity amplification available (~ 280 times higher than *Taq*), Q5 DNA Polymerase results in ultra-low error rates. 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 of performance.

The Q5 buffer system is designed to provide 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.

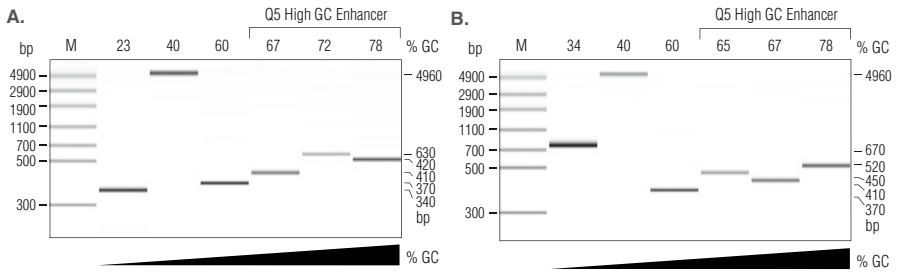
Q5 Hot Start DNA Polymerase: In contrast to chemically-modified or antibody-based hot start polymerases, NEB's Q5 Hot Start utilizes a unique synthetic aptamer. This structure binds to the polymerase through non-covalent interactions, blocking activity during the reaction setup. The polymerase is activated 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 degredation, 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.

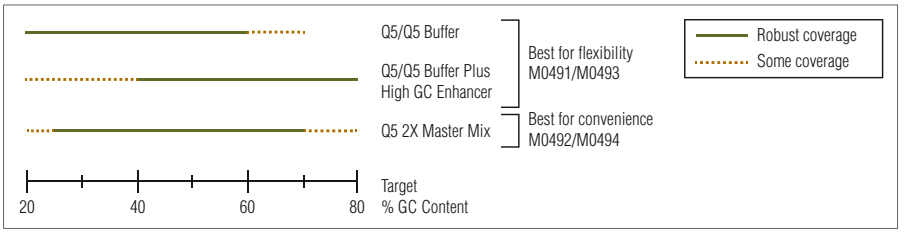
Additional Formats: For added convenience, Q5 DNA Polymerase is available in master mix format or as a kit. Master mix formulations include dNTPs, Mg^{++} and all necessary buffer components. The Q5 High-Fidelity PCR Kit contains the Q5 High-Fidelity 2X Master Mix, nuclease-free water and the Quick-Load Purple 1 kb Plus DNA Ladder. For information on the Q5 Site-Directed Mutagenesis Kit, with or without competent cells, see page 98.

Concentration: 2,000 units/ml

Visit **Q5PCR.com** for more information.



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.*



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.

LABCHIP® is a registered trademark of Caliper Life Sciences, Inc.

Cloned at NEB | Recombinant Enzyme | High-Fidelity | PCR Enzyme | Hot Start/WarmStart | Requires BSA | Heat Inactivation | Annealing Temperature | EpiMark | Extraction Free

Phusion® High-Fidelity DNA Polymerase

RR HiFi PCR No Tm+3

Phusion Hot Start Flex DNA Polymerase

RR 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 rxns (50 µl vol)
#M0531L 500 rxns (50 µl vol)

Phusion High-Fidelity PCR Master Mix with GC Buffer
#M0532S 100 rxns (50 µl vol)
#M0532L 500 rxns (50 µl vol)

Phusion Hot Start Flex DNA Polymerase
#M0535S 100 units
#M0535L 500 units

Phusion Hot Start Flex 2X Master Mix
#M0536S 100 rxns (50 µl vol)
#M0536L 500 rxns (50 µl vol)

Phusion High-Fidelity PCR Kit
#E0553S 50 rxns (50 µl vol)
#E0553L 200 rxns (50 µl vol)

For a complete listing of Deoxynucleotide Solutions, see page 85.

For a complete listing of Reaction Buffers, see pages 85.

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 Activation Required	Yes 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

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.

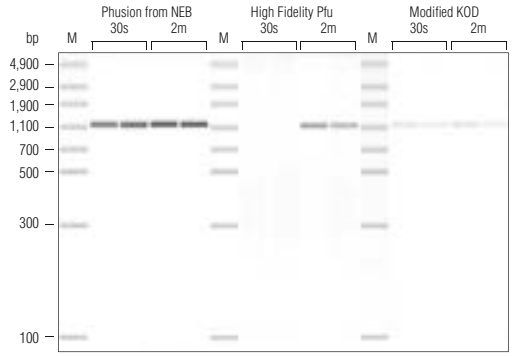
The Phusion PCR Kit contains Phusion Polymerase, Phusion HF and GC buffers, deoxynucleotides, $MgCl_2$, DMSO and DNA size standards.

Concentration: 2,000 units/ml

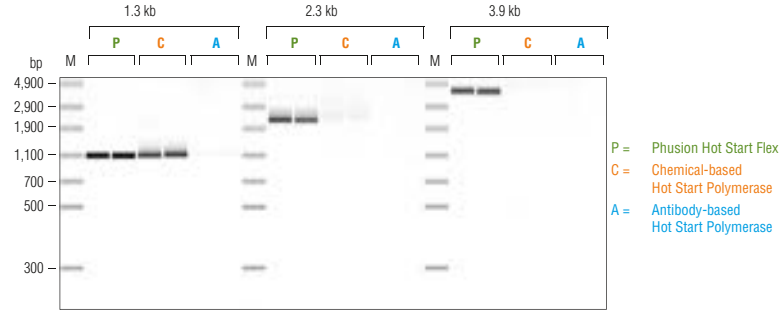
* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

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**



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.*

One*Taq*® DNA Polymerase

One*Taq* Hot Start DNA Polymerase

One*Taq* Products

One*Taq* DNA Polymerase
#M0480S 200 units
#M0480L 1,000 units
#M0480X 5,000 units

One*Taq* 2X Master Mix with Standard Buffer
#M0482S 100 rxns (50 µl vol)
#M0482L 500 rxns (50 µl vol)

One*Taq* Quick-Load® DNA Polymerase
#M0509S 100 units
#M0509L 500 units
#M0509X 2,500 units

One*Taq* Quick-Load 2X Master Mix with Standard Buffer
#M0486S 100 rxns (50 µl vol)
#M0486L 500 rxns (50 µl vol)

One*Taq* RT-PCR Kit
#E5310S 30 rxns

One*Taq* One-step RT-PCR Kit
#E5315S 30 rxns

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 - One*Taq* 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

One*Taq* Hot Start Products

One*Taq* Hot Start DNA Polymerase
#M0481S 200 units
#M0481L 1,000 units
#M0481X 5,000 units

One*Taq* Hot Start 2X Master Mix with Standard Buffer
#M0484S 100 rxns (50 µl vol)
#M0484L 500 rxns (50 µl vol)

One*Taq* Hot Start 2X Master Mix with GC Buffer
#M0485S 100 rxns (50 µl vol)
#M0485L 500 rxns (50 µl vol)

One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer
#M0488S 100 rxns (50 µl vol)
#M0488L 500 rxns (50 µl vol)

One*Taq* Hot Start Quick-Load 2X Master Mix with GC Buffer
#M0489S 100 rxns (50 µl vol)
#M0489L 500 rxns (50 µl vol)

For a complete listing of Deoxynucleotide Solutions, see page 85.

For a complete listing of Reaction Buffers, see pages 85.

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.

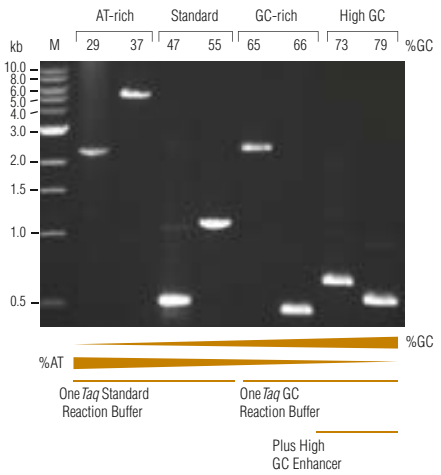
Quick-Load formats offer direct loading of PCR products onto gels, eliminating the need for a PCR clean-up step.

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. For information on the One*Taq* RT-PCR Kit or One*Taq* One-step RT-PCR Kit, see page 81.

Concentration: 5,000 units/ml

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.



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).*

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/WarmStart · Requires BSA · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

Taq DNA Polymerase

Hot Start *Taq* DNA Polymerase

Taq Polymerases

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 Std *Taq* Buffer
#M0273S 400 units
#M0273L 2,000 units
#M0273X 4,000 units
#M0273E 20,000 units

Taq DNA Polymerase with Std *Taq* (Mg-free) Buffer
#M0320S 400 units
#M0320L 2,000 units

Taq PCR Kit
#E5000S 200 rxns

Taq 2X Master Mix
#M0270L 500 rxns (50 µl vol)

Quick-Load *Taq* 2X Master Mix
#M0271L 500 rxns (50 µl vol)

Taq 5X Master Mix
#M0285L 500 rxns (50 µl vol)

Multiplex PCR 5X Master Mix
#M0284S 100 rxns (50 µl vol)

Hot Start *Taq* Products

Hot Start *Taq* DNA Polymerase
#M0495S 200 units
#M0495L 1,000 units

Hot Start *Taq* 2X Master Mix
#M0496S 100 rxns (50 µl vol)
#M0496L 500 rxns (50 µl vol)

For a complete listing of Deoxynucleotide Solutions, see page 85.

For a complete listing of Reaction Buffers, see pages 85.

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

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* from NEB is an ideal choice for molecular diagnostics and other 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

Taq Buffer Selection Chart

CHOICE OF BUFFER	AVAILABLE PRODUCTS	NEB #
ThermoPol Reaction Buffer: Designed for optimal yield and specificity	*Taq* DNA Polymerase with ThermoPol Buffer	M0267
Standard *Taq* Reaction Buffer: Detergent-free and designed to be compatible with existing assay systems	*Taq* DNA Polymerase with Standard *Taq* Buffer	M0273
	Taq DNA Polymerase with Standard *Taq* (Mg-free) Buffer	M0320

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 rxns (50 µl vol)
#M0287L 500 rxns (50 µl vol)

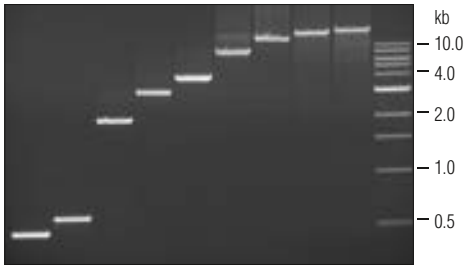
LongAmp Hot Start *Taq* 2X Master Mix
#M0533S 100 rxns (50 µl vol)
#M0533L 500 rxns (50 µl vol)

LongAmp *Taq* PCR Kit
#E5200S 100 rxns (50 µl vol)

For a complete listing of Deoxynucleotide Solutions, see page 85.

For a complete listing of Reaction Buffers, see pages 85.

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), $MgSO_4$ (100 mM) and nuclease-free water.

Concentration: 2,500 units/ml

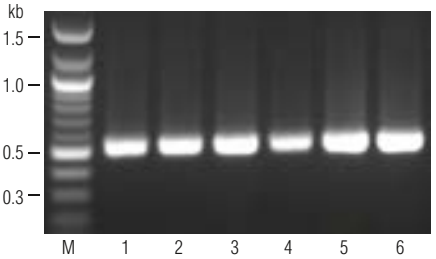
Hemo KlenTaq®

#M0332S 200 rxns (25 µl vol)
#M0332L 1,000 rxns (25 µl vol)

For a complete listing of Deoxynucleotide Solutions, see page 85.

HEMO KLENTaq 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 KlenTaq 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 H_2O 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´ flap endonuclease domain and the gene has three internal point mutations.

Reaction Buffer: 1X Hemo KlenTaq Reaction Buffer

EpiMark® Hot Start *Taq* DNA Polymerase

#M0490S 100 rxns (50 µl vol)
#M0490L 500 rxns (50 µl vol)

For a complete listing of Deoxynucleotide Solutions, see page 85.

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.

Companion Product:

EpiMark Bisulfite Conversion Kit
#E3318S 48 reactions

Source: An *E. coli* strain that carries the *Taq* DNA Polymerase gene from Thermus aquaticus YT-1.

Concentration: 5,000 units/ml

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

For a complete listing of Deoxynucleotide Solutions, see page 85.

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.

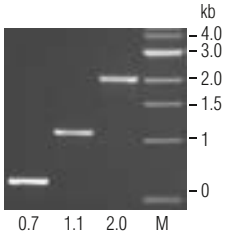
Deep Vent DNA Polymerase is a modified version of Vent DNA Polymerase. Deep Vent has similar fidelity with even more stability than Vent at temperatures of 95 to 100°C, with a half-life of 8 hours at 100°C.

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.

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)
Two-Step RT-qPCR	LunaScript® RT SuperMix Kit (NEB #E3010) + Luna Universal qPCR Master Mix (NEB #M3003)	LunaScript RT SuperMix Kit (NEB #E3010) + 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)

Avoiding pipetting errors with Luna

Find an overview of qPCR.

Cloned at NEB | Recombinant Enzyme | High-Fidelity | PCR Enzyme | Hot Start/ WarmStart | Requires BSA | Heat Inactivation | Annealing Temperature

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*

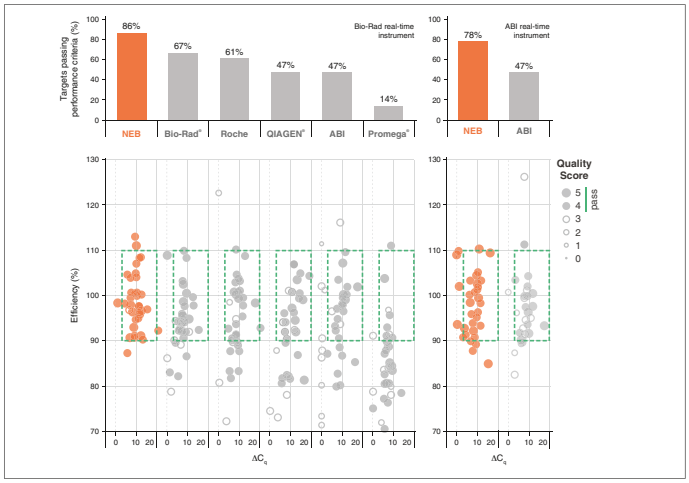
Learn more about our comprehensive qPCR/RT-qPCR testing and "dots in boxes" data visualization at LUNAqPCR.com.

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.

SYBR® is a registered trademark of Thermo Fisher Scientific.



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 Kit & LunaScript RT Master Mix Kit (Primer-free)

LunaScript RT SuperMix Kit

#E3010S	25 reactions
#E3010L	100 reactions

NEW

LunaScript RT Master Mix Kit (Primer-free)

#E3025S	25 reactions
#E3025L	100 reactions

- *Less than 15 minute first-strand cDNA synthesis protocol*
- *Combine with Luna qPCR master mixes for robust RT-qPCR results*

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. The LunaScript RT SuperMix Kit is available with or without random hexamer and poly-dT primers, which allow 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.

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

How can we ensure best in class performance with Luna?

Epi EpiMark

Extraction Free

Luna One-Step RT-qPCR Products

NEW
Luna Probe One-Step RT-qPCR Kit (No ROX)
#E3007E 2,500 reactions

NEW
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 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

Companion Product:

Antarctic Thermolabile UDG
#M0372S 100 units
#M0372L 500 units

- *Novel, thermostable reverse transcriptase (RT) improves performance*
- *WarmStart RT 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*

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.

The NEB Luna Universal One-Step RT-qPCR Kit is optimized for dye-based real-time quantitation of target RNA sequences via the SYBR/FAM fluorescence channel of most real-time instruments.

The NEB Luna Universal Probe One-Step RT-qPCR Kit is optimized for real-time quantitation of target RNA sequences using hydrolysis probes.

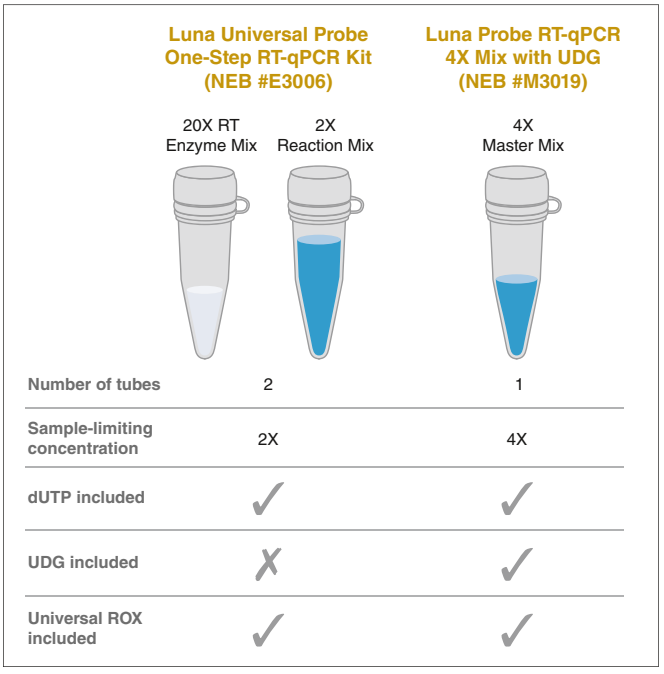
For instruments that do not utilize ROX normalization, the Luna Probe One-Step RT-qPCR Kit (No ROX) (NEB #E3007) contains no reference dye. If ROX normalization is desired, ROX can be added; this is only necessary with the E3007 product.

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



A new product choice for one-step RT-qPCR assays

Cloned at NEB | Recombinant Enzyme | High-Fidelity | PCR Enzyme | Hot Start/ WarmStart | Requires BSA | Heat Inactivation | Annealing Temperature | EpiMark | Extraction Free

Luna Cell Ready One-Step RT-qPCR Kit

Luna Cell Ready Probe One-Step RT-qPCR Kit

NEW
Luna Cell Ready One-Step RT-qPCR Kit
#E3030S 100+500 reactions

NEW
Luna Cell Ready Probe One-Step RT-qPCR Kit
#E3031S 100+500 reactions

NEW
Luna Cell Ready Lysis Module
#E3032S 100 rxns (50 µl vol)

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 to 100,000 cells across numerous cell lines*
- *Features Luna WarmStart RT paired with Hot Start 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.

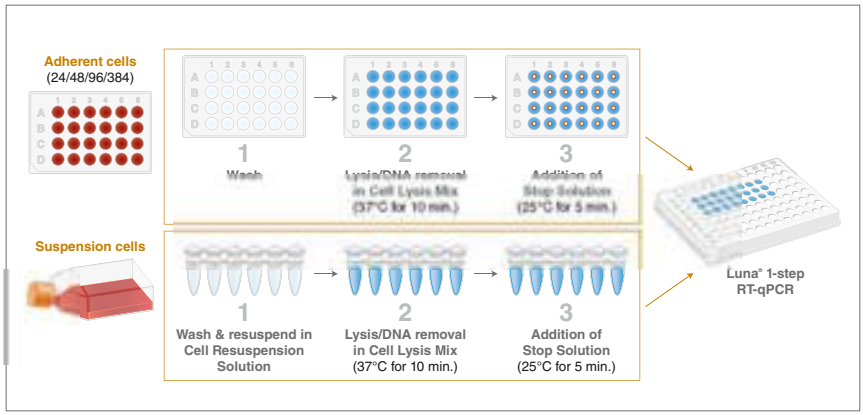
Cell cultures are often analyzed for gene expression or treatment responses as a proxy for a living organism. Traditionally, RNA is extracted and purified from treated cells via column-based or chemical methods. Coordinating the actions of DNase I and the Luna Cell Ready Protease, the Luna Cell Ready 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.

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.

NEW

SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit

#E2019S 96 reactions

Companion Product:

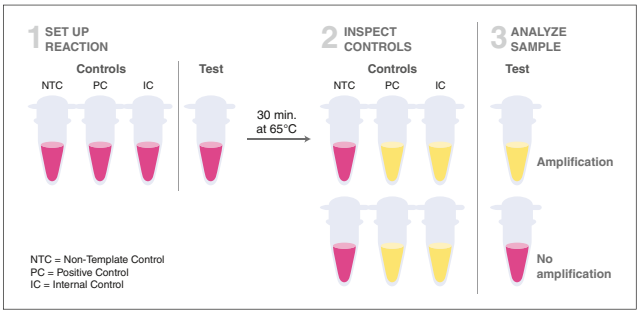
SARS-CoV-2 Positive Control (N gene)
#N2117S 0.05 ml

- *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.

The SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit Includes:

- WarmStart Colorimetric LAMP 2X Master Mix with UDG
- SARS-CoV-2 Positive Control (N gene)
- Internal Control LAMP Primer Mix
- 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. Using the provided protocol, a Non-Template Control (NTC) reaction will contain all materials of the test sample (master mix, primers, etc) except for the test input nucleic acid itself and serves as a measure of reaction contamination and primer-based mis-amplification. The NTC sample should not amplify and should stay pink throughout the experiment. The Positive Control (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 in the PC and the sample should become yellow after incubation. The Internal Control (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.

View our loop mediated isothermal amplification tutorial.

Cloned at NEB | Recombinant Enzyme | High-Fidelity | PCR Enzyme | Hot Start/ WarmStart | Requires BSA | Heat Inactivation | Annealing Temperature

NEW

WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG)

#E1708S	100 reactions
#E1708L	500 reactions

Companion Products:

LAMP Fluorescent Dye

#B1700S	500 reactions

WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)

#M1708S	100 reactions
#M1708L	500 reactions

- *LAMP amplification of DNA or RNA targets*
- *Reduce risk of carryover contamination with UDG and dUTP included in the master mix*
- *Improve LAMP specificity and sensitivity with optimzed 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) (NEB #M1708) supports a variety of detection methods including turbidity, visual detection, and electrophoresis*

Description: The WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG) is designed to provide a simple, one-step solution for Loop-Mediated Isothermal Amplification (LAMP) of DNA or RNA (RT-LAMP) targets. This kit is supplied with the WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG), which contains a blend of *Bst* 2.0 WarmStart DNA Polymerase, WarmStart RTx Reverse Transcriptase, Antarctic Thermolabile UDG and dUTP in an optimized LAMP buffer solution. Both *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx Reverse Transcriptase have been engineered for improved performance in LAMP and RT-LAMP reactions. The inclusion of dUTP and UDG in the master mix reduces the possibility of carryover contamination between reactions. A fluorescent dye is also supplied to enable real-time fluorescence measurement of LAMP. The WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG) is compatible with multiple detection methods, including turbidity detection, real-time fluorescence detection (when used with LAMP fluorescent dye) and end-point visualization.

The WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG) Includes:

– WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)

– LAMP Fluorescent Dye

NEW

Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit

#E3019S	96 reactions
#E3019L	480 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.

The Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit Includes:

– Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019)

– SARS-CoV-2 Positive Control (N gene)

– SARS-CoV-2 Primer/Probe Mix (N1/N2/RP)

– Nuclease-free Water

For the isolation of viral RNA, try our Monarch RNA purification kits. See pages 137-138 for details.

Epi EpiMark

Extraction Free

WarmStart® Colorimetric LAMP/RT-LAMP 2X Master Mix

NEW

WarmStart Colorimetric LAMP/RT-LAMP 2X Master Mix (with UDG)

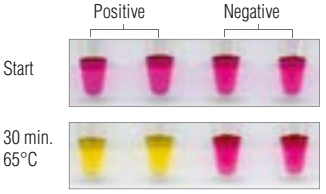
WarmStart Colorimetric LAMP/RT-LAMP 2X Master Mix
#M1800S 100 reactions
#M1800L 500 reactions

WarmStart Colorimetric LAMP/RT-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.



WarmStart Fluorescent LAMP/RT-LAMP Kit

#E1700S 100 reactions
#E1700L 500 reactions

Companion Product:

LAMP Fluorescent Dye
#B1700S 500 reactions

Tte UvrD Helicase
#M1202S 50 reactions

- *LAMP amplification of RNA and DNA targets*
- *Improve LAMP specificity and sensitivity with optimized master mixes*
- *Set up reactions at room temperature with our unique dual WarmStart formulation*
- *Use with a variety of detection methods including fluorescence, turbidity, visual detection and electrophoresis*

Description: The WarmStart Fluorescent LAMP/RT-LAMP Kit is 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 amplification techniques that provides rapid detection of a target nucleic acid using LAMP-specific primers (supplied by the user) and a strand-displacing DNA polymerase. This kit is supplied with the WarmStart LAMP 2X Master Mix, which contains a blend of *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx Reverse Transcriptase in an optimized LAMP buffer solution. Both *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx Reverse Transcriptase have been engineered for improved performance in LAMP and RT-LAMP reactions. A fluorescent dye is also supplied to enable real-time fluorescence measurement of LAMP. The WarmStart Fluorescent LAMP/RT-LAMP Kit is compatible with multiple detection methods, including turbidity detection, real-time fluorescence detection (when used with LAMP fluorescent dye) and end-point visualization.

The WarmStart Fluorescent LAMP/RT-LAMP Kit Includes:

– WarmStart LAMP 2X Master Mix
– LAMP Fluorescent Dye (50X)

How is colorimetric LAMP used in point of care?

Cloned at NEB | Recombinant Enzyme | High-Fidelity | PCR Enzyme | Hot Start/ WarmStart | Requires BSA | Heat Inactivation | Annealing Temperature

Bst DNA Polymerase-based Products for Isothermal DNA Amplification

	5´→ 3´ EXO ACTIVITY	AMPLIFICATION SPEED	ROOM TEMPERATURE SETUP	REVERSE TRANSCRIPTASE ACTVITY	INHIBITOR TOLERANCE	APPLICATIONS
***Bst* DNA Polymerase, Full Length**	★★	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

★★★ 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

Bst 3.0 DNA Polymerase
#M0374S 1,600 units
#M0374L 8,000 units
for high (15X) concentration
#M0374M 8,000 units

Companion Products:

WarmStart RTx Reverse Transcriptase
#M0380S 50 reactions
#M0380L 250 reactions

Tte UvrD Helicase
#M1202S 0.5 µg

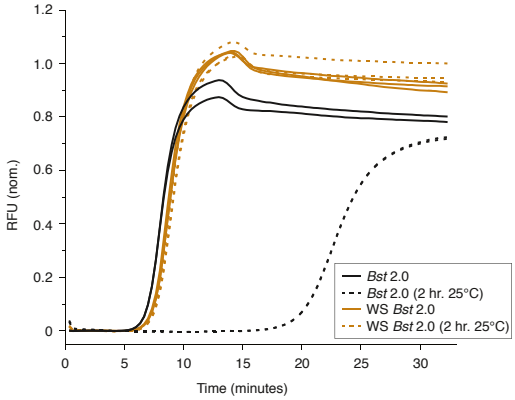
For a complete listing of Deoxynucleotide Solutions, see page 85.

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 I, 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.



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 increase reverse transcriptase activity compared to *Bst* DNA Polymerase.

Concentration: *Bst* DNA Polymerase, Full Length: 5,000 units/ml. All others: 8,000 and 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. Generally, reaction temperatures above 72°C are not recommended for any *Bst* DNA Polymerase-based product.

Epi EpiMark
Extraction Free

IsoAmp® II Universal tHDA Kit

#H0110S 50 reactions

- *Easy-to-use for assay development*
- *Helicase eliminates need for thermocycler*
- *Reactions performed at constant temp*
- *Amplify & detect short DNA sequences (70–120 bp)*
- *Use with a variety of templates (microbial genomic DNA, viral DNA, plasmid DNA and cDNA)*
- *Amplify a single copy of target DNA by tHDA when optimized primers and buffer are used*

Description: Thermophilic Helicase-Dependent Amplification (tHDA) is a novel method for isothermal amplification of nucleic acids. Like PCR, the tHDA reaction selectively amplifies a target sequence defined by two primers. However, unlike PCR, tHDA uses an enzyme called a helicase to separate DNA, rather than heat. This allows DNA amplification without the need for thermocycling. The tHDA reaction can also be coupled with reverse transcription for RNA analysis.

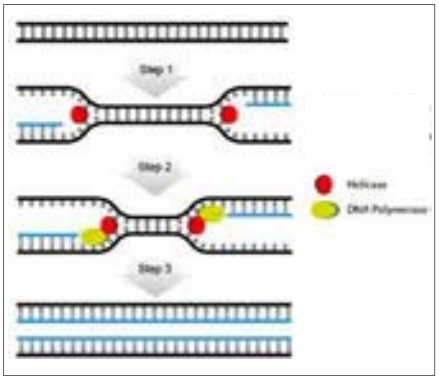
IsoAmp II Universal tHDA Kit is based on a second-generation thermophilic Helicase-Dependent Amplification platform. The reactions supported by IsoAmp II Universal tHDA Kit include tHDA, reverse transcription HDA (RT-HDA), real-time quantitative HDA (qHDA) and real-time quantitative RT-HDA (qRT-HDA), from a single reaction buffer.

The IsoAmp II Universal tHDA Kit Includes:

– IsoAmp dNTP solution and IsoAmp Enzyme Mix

– 10X Annealing Buffer II, 100 mM $MgSO_4$ and 500 mM NaCl

– Control template and amplification primers



HDA technology. Helicase Dependent Amplification: Step 1: Helicase unwinding and primer binding. Step 2: DNA polymerization. Step 3: DNA amplification.

Developed by BioHelix Corporation a NEB-affiliated company, now part of Quidel Corporation. ISOAMP® is a registered trademark of BioHelix Corporation

phi29 DNA Polymerase

#M0269S 250 units
#M0269L 1,250 units

For a complete listing of Deoxynucleotide Solutions, see page 85.

- *Extreme processivity*
- *Extreme strand displacement*
- *Replication requiring a high degree of strand displacement and/or processive synthesis*
- *High-fidelity replication at moderate temperatures*

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.

Concentration: 10,000 units/ml

Heat Inactivation: 65°C for 10 minutes

As a member of our Marketing Team, Michelle provides support to our various ordering channels. This can include our Freezer Programs, as well as other third-party platforms used at various academic institutions. Prior to taking this role, Michelle was a member of our Technical Support Group.

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/WarmStart · Requires BSA · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

One*Taq* One-Step RT-PCR Kit

#E5315S 30 reactions

Companion Products:

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

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

For a complete listing of One*Taq* products, see page 68.

- *Combine cDNA synthesis and PCR in a single reaction*
- *Detect at little as 0.1 pg of a GAPH target*
- *Robust amplification from 100 bp to 9 kb*
- *Faster protocols with less hands-on time*
- *Quick-Load Reaction Mix allows instant gel loading*

Description: 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.

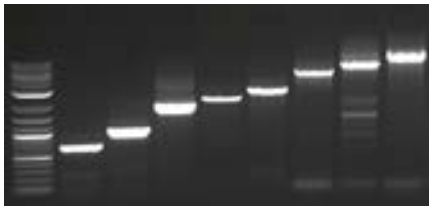
The kit combines three optimized mixes: One*Taq* One-Step Enzyme Mix, One*Taq* One-Step Reaction Mix and One*Taq* One-Step Quick-Load Reaction Mix. The Enzyme Mix combines ProtoScript II Reverse Transcriptase, Murine RNase Inhibitor and One*Taq* Hot Start DNA Polymerase. ProtoScript II Reverse Transcriptase is a mutant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. One*Taq* Hot Start DNA Polymerase is mixture of a Hot Start *Taq* DNA Polymerase combined with a proof-reading DNA polymerase, resulting in high-yield amplification with minimal optimization. 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* One-Step 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 One*Taq* One-Step RT-PCR Kit is capable of multiplex detection of two or three targets.

The One*Taq* One-Step RT-PCR 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
#M0314L 15,000 units

M-MuLV Reverse Transcriptase
#M0253S 10,000 units
#M0253L 50,000 units

One*Taq* Hot Start 2X Master Mix with Standard Buffer
#M0484S 100 rxns (50 µl vol)
#M0484L 500 rxns (50 µl vol)

For a complete listing of One*Taq* products, see page 68.

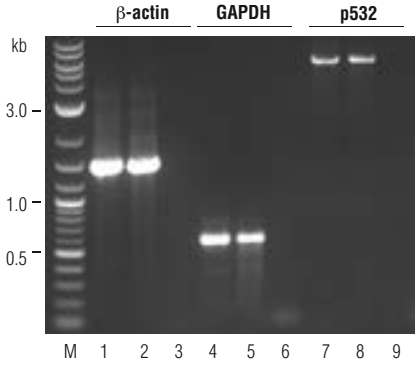
- *Robust RT-PCR reactions*
- *Convenient master mix formats*
- *OneTaq DNA Polymerase offers robust amplification for a wide range of templates*

Description: 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.

The One*Taq* RT-PCR Kit Includes:

– 10X M-MuLV Enzyme Mix
– 2X M-MuLV Reaction Mix
– One*Taq* Hot Start 2X Master Mix with Standard Buffer
– Random Primer Mix (60 µM)
– Oligo $d(T)_{23}VN$ Primer (50 µM)**
– 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 $dT_{23}VN$ (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).*

** Oligo $d(T)_{23}$ VN and Random Primer Mix contain 1 mM dNTP

PreCR® Repair Mix

#M0309S 30 reactions
#M0309L 150 reactions

Companion Product:

beta-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?
See page 152 for more information.

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.

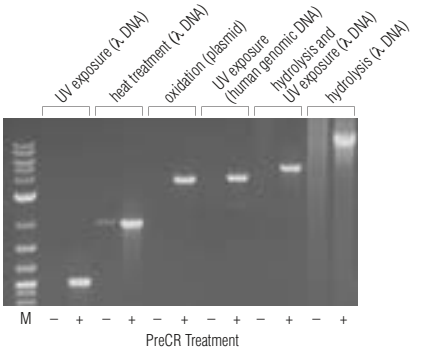
Applications:

- Repair DNA prior to its use as a template in PCR or other DNA technologies

Reagents Supplied:
1X PreCR Repair Mix
10X ThermoPol Reaction Buffer
100X NAD+ solution
Control Template (UV damaged λ DNA)
PCR primers for control template
Purified BSA

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



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).*

Sulfolobus DNA Polymerase IV

#M0327S 100 units

For a complete listing of Deoxynucleotide Solutions, see page 85.

- *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.

Concentration: 2,000 units/ml

Therminator™ DNA Polymerase

#M0261S 200 units
#M0261L 1,000 units

For a complete listing of Deoxynucleotide Solutions, see page 85.

- *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

Usage Notes: Amplification of extended regions may require optimization of reaction conditions.

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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.

Concentration: 10,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Usage Notes: DNase I is not included with this enzyme and must be added for nick translation reactions.

DNA Polymerase I, Large (Klenow) Fragment

#M0210S 200 units
#M0210L 1,000 units
for high (10X) concentration
#M0210M 1,000 units

For a complete listing of Deoxynucleotide Solutions, see page 85.

- *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

Usage Notes: Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times will 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

For a complete listing of Deoxynucleotide Solutions, see page 85.

- *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

Usage Notes: Klenow Fragment (3´→ 5´ exo⁻) is not suitable for generating blunt ends because it lacks the 3´→ 5´ exonuclease activity 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

For a complete listing of Deoxynucleotide Solutions, see page 85.

- *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.

Concentration: 3,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Usage Notes: Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times will result in recessed ends due to the 3´→ 5´ exonuclease activity of the enzyme.

Note: This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

T7 DNA Polymerase (unmodified)

RR NEBU BSA 37° 75°

#M0274S 300 units
#M0274L 1,500 units

For a complete listing of Deoxynucleotide Solutions, see page 85.

- *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 kDa) and *E. coli* thioredoxin (12 kDa). Each protein is cloned and overexpressed in a T7 expression system in *E. coli*.

Reaction Conditions: 1X T7 DNA Polymerase Reaction Buffer. Supplement with BSA and dNTPs (not included). Incubate at 37°C. Heat inactivation: 75°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 37°C.

Concentration: 10,000 units/ml

Usage Notes: The high polymerization rate of the enzyme makes long incubations unnecessary. T7 DNA Polymerase is not suitable for DNA sequencing.

Bsu DNA Polymerase, Large Fragment

RR NEB2 37° 75°

#M0330S 200 units
#M0330L 1,000 units

For a complete listing of Deoxynucleotide Solutions, see page 85.

- *Random primer labeling*
- *Second strand cDNA synthesis*
- *Single dA tailing*
- *Strand displacement DNA synthesis*

Description: *Bsu* DNA Polymerase I, Large Fragment retains the 5´→ 3´ polymerase activity of the *Bacillus subtilis* DNA polymerase I, 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 I gene (starting from codon 297 thus lacking the 5´→ 3´ exonuclease domain).

Concentration: 5,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Usage Notes: *Bsu* DNA Polymerase, Large Fragment is not suitable for generating blunt ends because it lacks the 3´→ 5´ exonuclease activity 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

RR NEBU 37° 75°

#M0315S 500 units
#M0315L 2,500 units

For a complete listing of Deoxynucleotide Solutions, see page 85.

- *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.

Concentration: 20,000 units/ml

Heat Inactivation: 75°C for 20 minutes

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Polymerase Reaction Buffers

Q5

Q5 Reaction Buffer Pack [Q5 Reaction Buffer (5X), Q5 High GC Enhancer (5X)]
#B9027S 6.0 ml

Phusion*

Phusion HF Buffer Pack [Phusion HF Reaction Buffer (5X), $MgCl_2$ (50 mM), DMSO]
#B0518S 6.0 ml

Phusion GC Buffer Pack [Phusion GC Reaction Buffer (5X), $MgCl_2$ (50 mM), DMSO]
#B0519S 6.0 ml

Taq

Standard *Taq* Reaction Buffer [Standard *Taq* Reaction Buffer (10X), $MgCl_2$ (25 mM)]
#B9014S 6.0 ml

Standard *Taq* (Mg-free) Reaction Buffer Pack [Standard *Taq* (Mg-free) Reaction Buffer (10X), $MgCl_2$ (25 mM)]
#B9015S 6.0 ml

Other

ThermoPol Reaction Buffer Pack [ThermoPol Reaction Buffer (10X), $MgSO_4$ (100 mM)]
#B9004S 6.0 ml

Isothermal Amplification Buffer Pack [Isothermal Amplification Buffer (10X)]
#B0537S 6.0 ml

Isothermal Amplification Buffer II Pack [Isothermal Amplification Buffer II (10X)]
#B0374S 6.0 ml

* 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.

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 $MgCl_2$.

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 $MgSO_4$ 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.

Nucleotides

Acyclonucleotide Set
#N0460S 0.5 µmol of each

Deoxynucleotide (dNTP) Solution Set
#N0446S 25 µmol of each

Deoxynucleotide (dNTP) Solution Mix
#N0447S 8 µmol of each
#N0447L 40 µmol of each

Ribonucleotide Solution Set
#N0450S 10 µmol of each
#N0450L 50 µmol of each

Ribonucleotide Solution Mix
#N0466S 10 µmol of each
#N0466L 50 µmol of each

7-deaza-dGTP
#N0445S 0.3 µmol of each
#N0445L 1.5 µmol of each

Adenosine 5´-Triphosphate (ATP)
#P0756S 1.0 ml
#P0756S 5.0 ml

5-methyl-dCTP
#N0356S 1 µmol

dATP Solution
#N0440S 25 µmol

NEW
dUTP Solution
#N0459S 25 µmol

NEW
dGTP Solution
#N0442S 25 µmol

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:
dm5CTP supplied as 10 mM solution at pH 7.0.

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 as a dry powder. 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.

For RNA Cap Analogs, see page 204.

MILLI-Q® is a registered trademark of Millipore, Inc.

Products for cDNA Synthesis

cDNA SYNTHESIS	FEATURES	SIZE
KITS		
LunaScript RT SuperMix Kit (NEB #E3010)	• Ideal for cDNA synthesis in a two-step RT-qPCR workflow • 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	25/100 rxns
NEW **LunaScript RT Master Mix Kit (Primer-free)** (NEB #E3025)	• Ideal for first strand cDNA synthesis • Master mix contains dNTPs, Murine RNase Inhibitor and Luna Reverse Transcriptase • Visible blue tracking dye for easy reaction setup • Flexible choice of primers	25/100 rxns
ProtoScript® II First Strand cDNA Synthesis Kit (NEB #E6560)	• Generates cDNA at least 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	30/150 rxns
ProtoScript First Strand cDNA Synthesis Kit (NEB #E6300)	• 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	30/150 rxns
Template Switching RT Enzyme Mix (NEB #M0466)	• Incorporates a universal adaptor sequence at the 3´ end of cDNA during the RT reaction • Enzyme mix and buffer are optimzed 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	20/100 rxns
STANDALONE REAGENTS		
ProtoScript II Reverse Transcriptase (NEB #M0368) An alternative to SuperScript® II	• RNase H⁻ mutant of M-MuLV Reverse Transcriptase with increased thermostability and reduced RNase H activity • Increased reaction temperatures (37–50°C)	4,000/10,000/40,000 units
M-MuLV Reverse Transcriptase (NEB #M0253)	• Robust reverse transcriptase for a variety of templates • Standard reaction temperatures (37–45°C)	10,000/50,000 units
AMV Reverse Transcriptase (NEB #M0277)	• Robust reverse transcriptase for a broad temperature range (37–52°C) • Can be used for templates requiring higher reaction temperatures	200/1,000 units
WarmStart RTx Reverse Transcription (NEB #M0380)	• Permits room temperature reaction setup • Increased reaction temperatures (50–65°C) • Optimized for RT-LAMP isothermal detection	50/250 rxns

More information about our reverse transcriptases and cDNA synthesis kits can be found in the RNA analysis chapter, pages 206–209.

Cloned at NEB · RR Recombinant Enzyme · HiFi High-Fidelity · PCR PCR Enzyme · Hot Start/WarmStart · BSA Requires BSA · 65° Heat Inactivation · Tm-5 Annealing Temperature · Epi EpiMark · Extraction Free

Monarch PCR & DNA Cleanup Kit (5 µg)

#T1030S 50 preps
#T1030L 250 preps

Companion Products:

Monarch DNA Cleanup Columns (5 µg)
#T1034L 100 columns + tubes

Monarch DNA Cleanup Binding Buffer
#T1031L 175 ml

Monarch DNA Wash Buffer
#T1032L 25 ml

Monarch DNA Elution Buffer
#T1016L 25 ml

Monarch Plasmid Miniprep Kit
#T1010S 50 preps
#T1010L 250 preps

Monarch DNA Gel Extraction Kit
#T1020S 50 preps
#T1020L 250 preps

- *Elute in as little as 6 µl*
- *Prevent buffer retention and salt carry-over with optimized column design*
- *Purify small DNA and oligos with a slight protocol modification*
- *Save time with fast, user-friendly protocol*
- *Purchase optimized kit formats or buffers & columns separately for your convenience*

Description: The Monarch PCR & DNA Cleanup Kit (5 µg) is a rapid and reliable method for the purification and concentration of up to 5 µg of high-quality, double-stranded DNA from enzymatic reactions such as PCR, restriction digestion, ligation and reverse transcription. This method employs a bind/wash/elute workflow with minimal incubation and spin times, resulting in purification in less than 5 minutes. DNA Cleanup Binding Buffer is used to dilute the samples and ensure they are compatible for loading onto the proprietary silica matrix under high salt conditions. The DNA Wash Buffer ensures enzymes, short primers (≤ 40 nt), detergents and other low-molecular weight reaction components (e.g., nucleotides, DMSO, betaine) are removed, thereby allowing low-volume elution of concentrated, high-purity DNA. Eluted DNA is ready for use in restriction digests, DNA sequencing, ligation and other enzymatic manipulations. The unique column design ensures no buffer retention and no carryover of contaminants, allowing elution of sample in volumes as low as 6 µl. A slight protocol modification enables purification of small DNA and oligonucleotides.

Applications:

- PCR cleanup
- Enzymatic reaction cleanup
- cDNA cleanup
- Labeling cleanup
- Plasmid cleanup
- Oligo cleanup

The Monarch PCR & DNA Cleanup Kit Includes:

– Monarch DNA Cleanup Columns (5 µg)
– Monarch DNA Cleanup Binding Buffer
– Monarch DNA Wash Buffer
– Monarch DNA Elution Buffer
– Monarch Collection Tubes

With Monarch PCR & DNA Cleanup Kit, you can purify your DNA in as little as 5 minutes.

Exo-CIP™ Rapid PCR Cleanup Kit

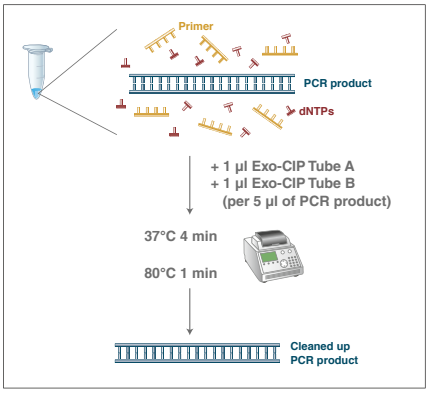
#E1050S 100 reactions
#E1050L 400 reactions

- *5 minute protocol for enzymatic cleanup of primers and dNTPs*
- *Improves sequencing results, allowing longer reads*

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.

The Exo-CIP Rapid PCR Cleanup Kit Includes:

– Exo-CIP Tube A (thermolabile Exo I)
– Exo-CIP Tube B (thermolabile CIP)



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.*

Ask Big Questions

Voltaire said, “Judge a man by his questions rather than by his answers.”

Asking questions is easy. What’s for dinner? Where’s the library? But asking big questions is much harder. It takes courage to challenge the status quo, and to ask questions that take time, energy and exploration to answer. But aren’t these questions, the tough ones with no definitive answer, the ones worth asking? These are the questions that drive discovery and innovation.

Questions can be powerful tools. The art of asking impactful questions can be innate in curious and emotionally intelligent individuals, but it can also be learned. The first step toward asking better questions is to avoid “yes” or “no” questions — these often lead to short answers with little detail. Anyone with young children knows this well! Instead, ask questions that start with “what,” “why,” “how,” or “if” — these types of questions are open-ended, and can lead to a more thoughtful discussion and new ideas to explore. This brings us to the second step, asking follow-up questions that dig deeper. Scientists know that asking one great question can get the thought process rolling, and that one great question often leads to two more, which can easily lead to four more, and so on.

Don Comb’s passion was reflected in the intensity of his questions. New England Biolabs’ CEO Jim Ellard recalls a dinner some 30 years ago when Don leaned across the table and asked, “What if you could come up with a way to keep algae, seaweed and barnacles from sticking to the bottom of boats? You would reduce drag, conserve fuel and revolutionize the shipping industry!” — always the inquisitor!

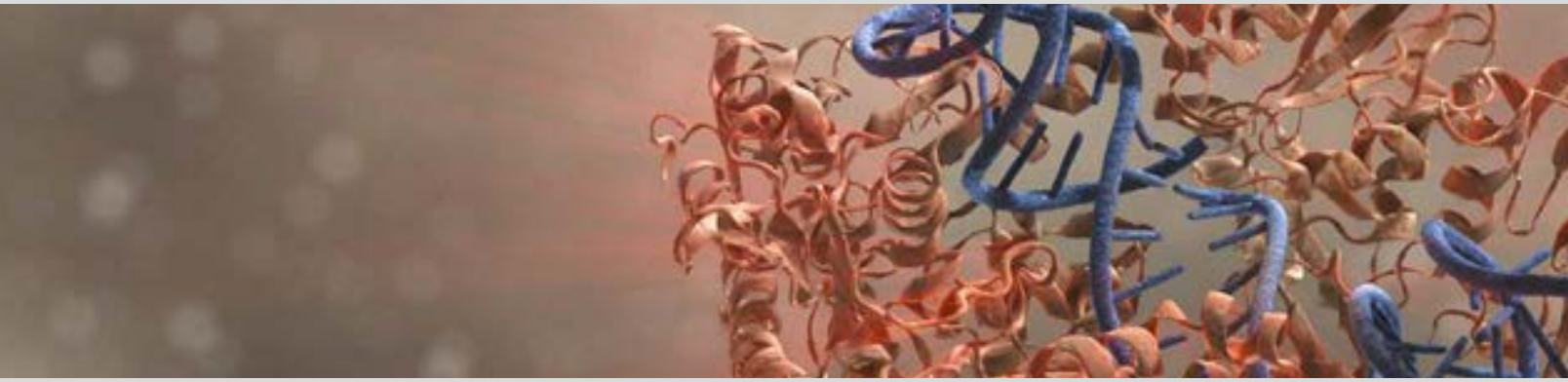
Over the years, New England Biolabs’ culture has been shaped by great questions that Don asked early on — for example, “How can we run a company that does great things without impacting the environment?” A company can take responsibility for the inevitable footprint it creates by offsetting negative impacts through the use of solar energy, managing treatment of its own wastewater, supporting the reforestation of land to improve global carbon capture, practicing extensive recycling and composting, and investing in conservation efforts around the world. Another big question Don asked was, “How can we help underdeveloped countries that are struggling with filariasis?” For 40 years, NEB scientists have been dedicated to research on neglected tropical diseases caused by parasitic worms, such as lymphedema and elephantiasis. These diseases are the second leading cause of disability worldwide, and our scientists continue to improve understanding and detection, and freely share their findings with the research community.

Asking provocative questions has stimulated many exciting conversations at NEB, and has helped nurture the curiosity of its employees. In 2012, NEB introduced the Innovation Awards to emphasize the importance of “blue-sky” thinking, the courage to take risks, and the drive to implement new ideas. These awards are given out annually, and recognize employees who contribute and bring to fruition novel ideas that help make NEB a better place and help to carry on Don’s legacy of provocative curiosity.

View of the moody sky over Thailand at twilight.
Credit: Boy Anupong, Getty Images

Learn more about NEB’s Innovation Awards.

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 Golden Gate Assembly.

Over 45 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
- **ExoSelector** – find the right exonuclease for your workflows
- **NEB® Golden Gate Assembly Tool** – use this tool for help with construct design for Golden Gate Assembly
- **Ligase Fidelity Tools** – utilize ligation preferences for the design of high-fidelity Golden Gate Assembly

To view the full list of online tools available, see page 304.

Featured Products

94 NEBuilder HiFi DNA Assembly Master Mix & Cloning Kit

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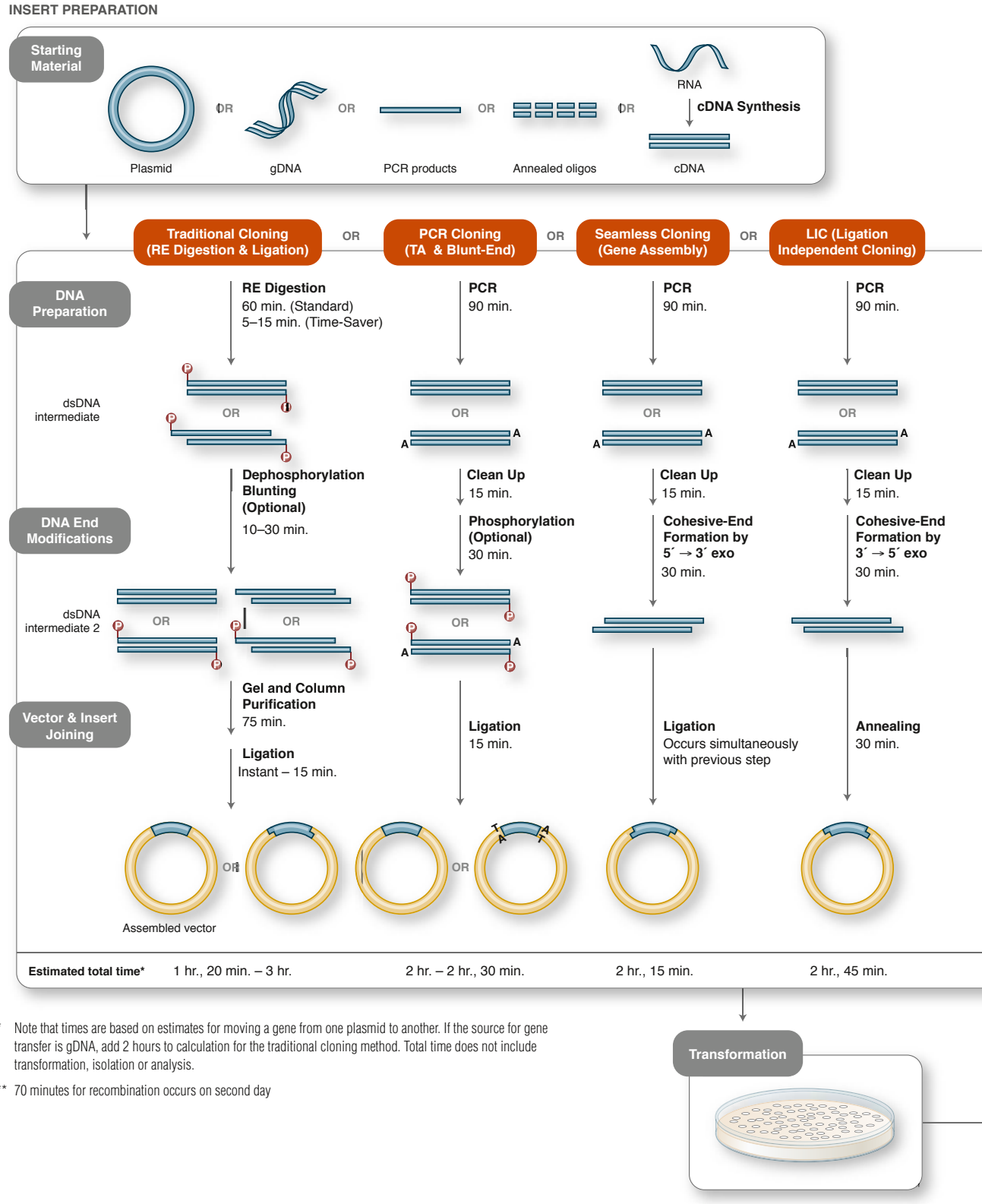
Visit www.neb.com/FastCloning to accelerate your cloning experiments with reagents from NEB.

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.

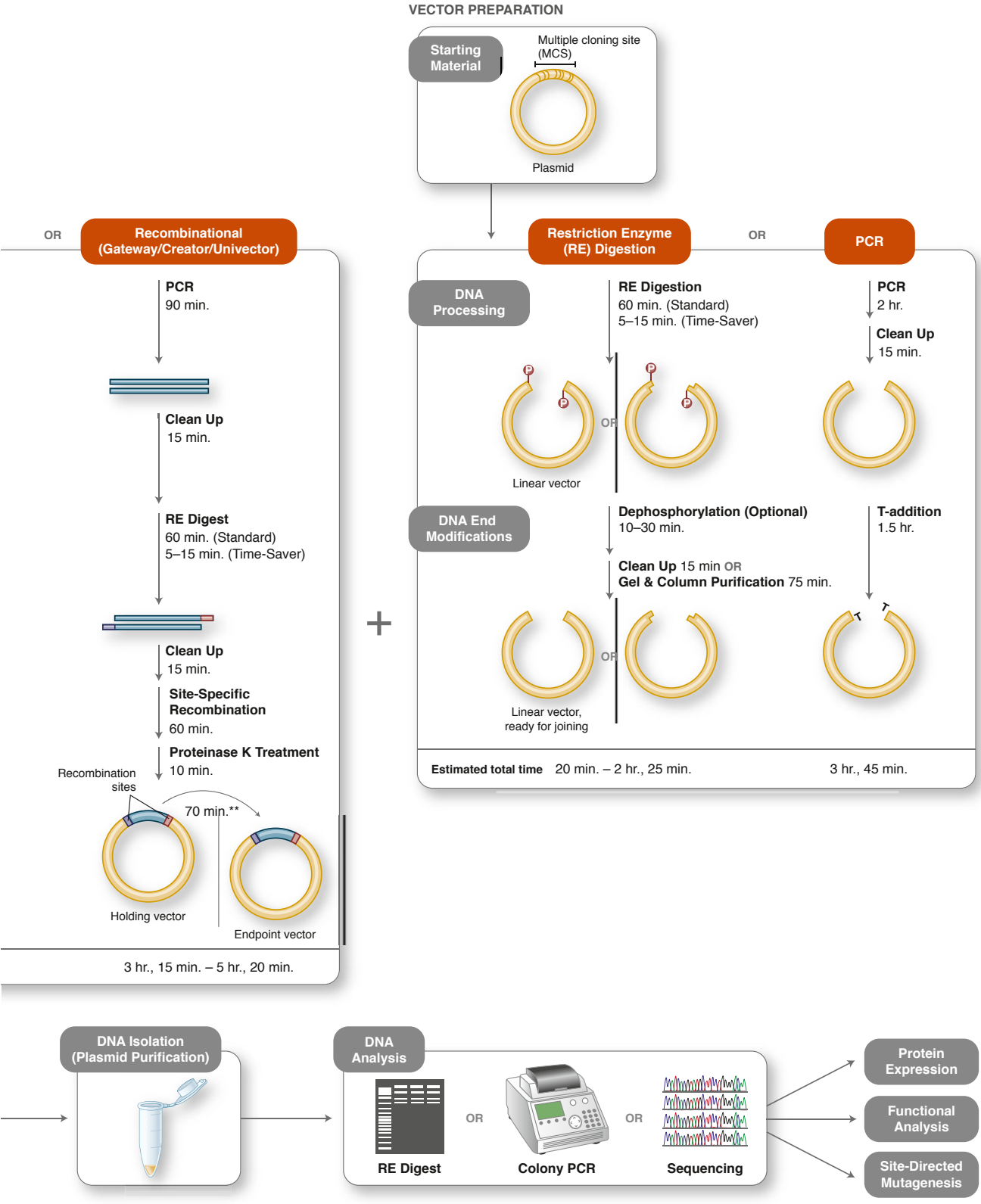


* Note that times are based on estimates for moving a gene from one plasmid to another. If the source for gene transfer is gDNA, add 2 hours to calculation for the traditional cloning method. Total time does not include transformation, isolation or analysis.

** 70 minutes for recombination occurs on second day

NEBcloner®

For help with choosing the right product for each step in the cloning workflow, try NEBcloner at **NEBcloner.neb.com**



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)*
- *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 licensing fee requirements from NEB for NEBuilder products*
- *NEBuilder HiFi DNA Assembly Cloning Kit includes the NEBuilder HiFi DNA Assembly Master Mix and NEB 5-alpha Competent E. coli*
- *NEBuilder HiFi DNA Assembly Bundle for Large Fragments includes the NEBuilder HiFi DNA Assembly Master Mix and NEB 10-beta Competent E. coli for assemblies larger than 15 kb*

To learn how simple NEBuilder HiFi is, visit **NEBuilderHiFi.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:

- Exonuclease creates single-stranded 3´ overhangs that facilitate the annealing of fragments that share complementarity at one end (the overlap region)
- The polymerase fills in gaps within each annealed fragment
- The DNA ligase seals nicks in the assembled DNA

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*.

The NEBuilder HiFi DNA Assembly Cloning Kit combines the power of the NEBuilder HiFi DNA Assembly Master Mix with NEB 5-alpha Competent *E. coli*, enabling fragment assembly and transformation in just under 2 hours.

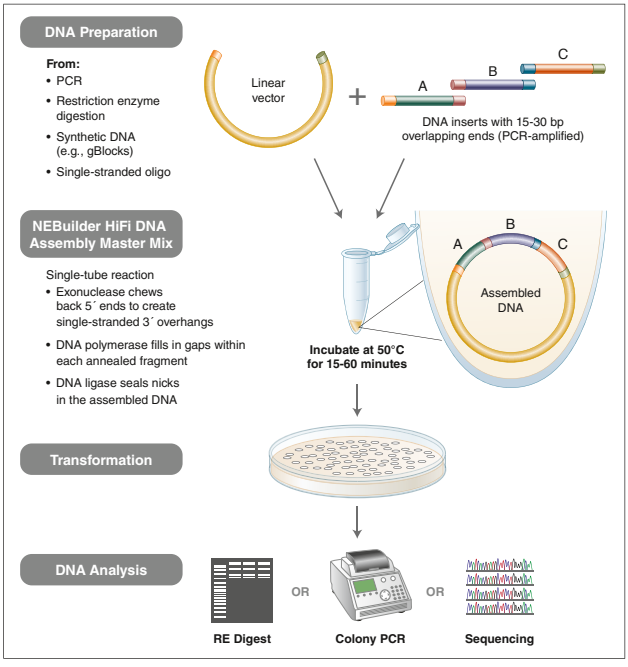
NEBuilder HiFi Kits can be purchased with NEB 5-alpha Competent *E. coli* (Cloning Kit, NEB #E5520) or as a bundle with NEB 10-beta Competent *E. coli* (Bundle for Large Fragments, NEB #E2623). NEB 5-alpha competent cells are excellent for routine assemblies of 15 kb or less. NEB recommends NEB 10-beta 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

NEBuilder® Assembly Tool
Speed up your experimental design with our primer design tool at **NEBuilder.neb.com**



Overview of the NEBuilder HiFi DNA Assembly Cloning Method.

How does NEBuilder HiFi DNA Assembly work?

Gibson Assembly® Master Mix & Cloning Kit

RR

Gibson Assembly Master Mix
#E2611S 10 reactions
#E2611L 50 reactions

Gibson Assembly Cloning Kit
#E5510S 10 reactions

- *Increased number of successful assembly products, 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 was developed by Dr. Daniel Gibson and his colleagues at the J. Craig Venter Institute and licensed to NEB by Synthetic Genomics, Inc. It 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 combines the power of the Gibson Assembly Master Mix with NEB 5-alpha Competent *E. coli*, enabling fragment assembly and transformation in just under 2 hours.

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

GIBSON ASSEMBLY® is a registered trademark of Synthetic Genomics Inc.

NEBuilder® Assembly Tool

Speed up your experimental design with our primer design tool at **NEBuilder.neb.com**

To learn how simple Gibson Assembly is, 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 #E5510) (NEB #E2611)	NEB® Golden Gate Assembly Kit (BsaI-HFv2, BsmBI-v2) (NEB #E1601) (NEB #E1602)	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 with High Efficiency with Low Amounts of DNA	★★★	★★	★★	★★
Accommodates Flexible Overlap Lengths	★★★	★★★	★	★★
APPLICATIONS				
Simple Cloning (1-2 Fragments)	★★★	★★★	★★★	★★★
4-6 Fragment Assembly (one pot)	★★★	★★★	★★★	★★★
7-11 Fragment Assembly (one pot)	★★★	★★	★★★	★★★
12-35 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 Cloning (shRNA)	★★★	★★	★	★
gRNA Library Generation	★★★	★★	★	★
Large Fragment (> 10 kb) Assembly	★★★	★★★	★★★	★★
Small Fragment (< 100 bp) Assembly	★★★	★	★★★	★★★
Use in Successive Rounds of Restriction Enzyme Assembly	★★★	★	NR	★

★★★ Optimal, recommended product for selected application
★★ Works well for selected application
★ Will perform for selected application, but is not recommended
N/A Not applicable to this application
NR Not recommended

(1) Please visit www.neb.com/GoldenGate for more information.

NEB Golden Gate Assembly Kits

NEB Golden Gate Assembly Kit (BsaI-HFv2)
#E1601S 20 reactions
#E1601L 100 reactions

NEW
NEB Golden Gate Assembly Kit (BsmBI-v2)
#E1602S 20 reactions
#E1602L 100 reactions

Companion Products:

NEB 5-alpha Competent *E. coli* (High Efficiency)
#C2987H 20 x 0.05 ml
#C2987I 6 x 0.2 ml
#C2987P 1 x 96 well plate
#C2987R 1 x 384 well plate
#C2987U 96 x 50 µl/tube

NEB 10-beta Competent *E. coli* (High Efficiency)
#C3019H 20 x 0.05 ml
#C3019I 6 x 0.2 ml

NEB Cloning Competent *E. coli* Sampler
#C1010S 8 x 0.05 ml

Q5 Hot Start High-Fidelity 2X Master Mix
#M0494S 100 rxns (50 µl vol)
#M0494L 500 rxns (50 µl vol)
#M0494X 500 rxns (50 µl vol)

- *Choose from BsaI-HFv2 or BsmBI-v2*
- *Seamless cloning – no scar remains following assembly*
- *Includes destination plasmid with T7/SP6 promoters*
- *Ordered assembly of multiple fragments (2-35+) 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)*

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?

Description: The NEB Golden Gate Assembly Kits (BsaI-HFv2 and BsmBI-v2) contain an optimized mix of restriction enzyme and T4 DNA Ligase. Together these enzymes can direct the assembly of multiple inserts/modules using the Golden Gate approach. Also included is the pGGAselect destination plasmid, which provides a backbone for your assembly, features convenient restriction enzyme sites for subcloning, and has T7/SP6 promoter sequences to enable *in vitro* transcription.

The efficient and seamless assembly of DNA fragments, is commonly referred to as Golden Gate Assembly. In this workflow, 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 be used to generate DNA fragments with unique overhangs. As an example, BsaI has a recognition site of GGTCTC(N1/ N5), where the GGTCTC represents the recognition/binding site, and the N1/ N5 indicates the cut site is one base downstream on the top strand, and five bases downstream on the bottom strand. Assembly of digested fragments proceeds through annealing of complementary four base overhangs on adjacent fragments. The digested fragments and the final assembly no longer contain Type IIS restriction enzyme recognition sites, so no further cutting is possible. The assembly product accumulates with time.

***Note:** Complexities up to 24 fragments have been routinely achieved with both precloned and amplicon insert test systems. Complexities of 35^{+} fragments have only used amplicon inserts to date, as we have not yet constructed a 35 fragment precloned insert test system.

NEB Golden Gate Assembly Tool

Speed up your experimental design with our assembly tool at **GoldenGate.neb.com**

While particularly useful for multi-fragment assemblies such as Transcription Activator Like Effectors (TALEs) and TALEs fused to a FokI nuclease catalytic domain (TALENs), the Golden Gate method can also be used for cloning of single inserts and inserts from diverse populations that enable library creation.

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.

The NEB Golden Gate Assembly Kit (BsaI-HFv2) Includes:

– NEB Golden Gate Assembly Mix
– T4 DNA Ligase Reaction Buffer (10X)
– pGGAselect Destination Plasmid

The NEB Golden Gate Assembly Kit (BsmBI-v2) Includes:

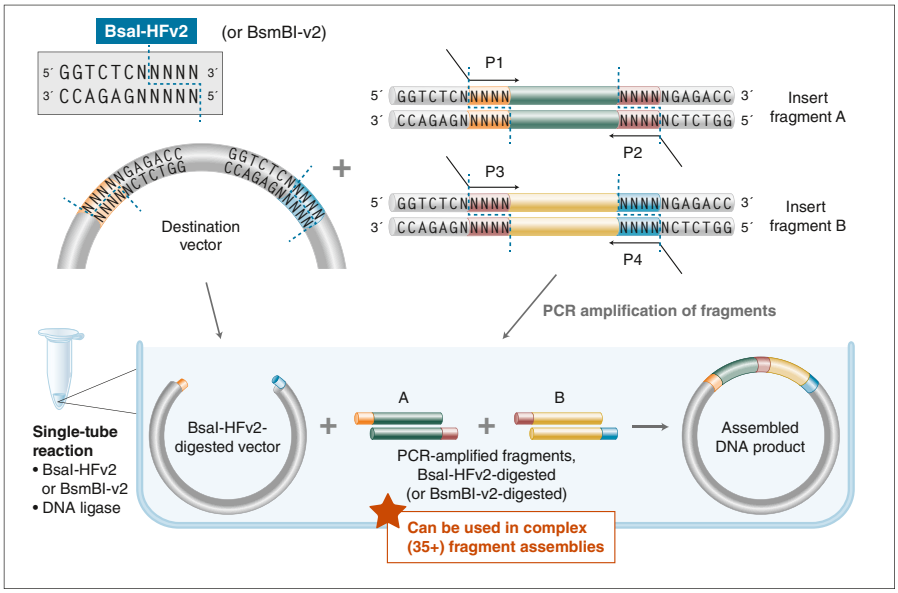
– NEB Golden Gate Assembly Mix
– T4 DNA Ligase Reaction Buffer (10X)
– pGGAselect Destination Plasmid

Ligase Fidelity Tools

For the design of high-fidelity Golden Gate Assemblies

- Ligase Fidelity Viewer™ (v2) – visualize overhang ligation junctions
- GetSet™ – predict high-fidelity junction sets
- SplitSet™ – split DNA sequence for scarless high-fidelity assembly

Access these tools at **www.neb.com/research/nebeta-tools**.



*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.**

rCutSmart or NEB r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | BSA Requires BSA | SAM Requires SAM

BioBrick® Assembly Kit

#E0546S 50 reactions

The BioBrick Assembly Kit was developed in partnership with Ginkgo BioWorks. For more details and for technical questions, please see: **ginkgobioworks.com/support**

BIOBRICK® is a registered trademark of The BioBricks Foundation. (http://www.biobricks.org)

Description: The BioBrick Assembly Kit provides a streamlined method for assembly of BioBrick parts into multi-component genetic systems. BioBrick parts are DNA sequences that encode a defined biological function and can be readily assembled with any other BioBrick part. The process for assembling any two BioBrick parts is identical and results in a new composite BioBrick part.

Please refer to the individual datacards for each reagent's recommended use and storage conditions.

Note: This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

The BioBrick Assembly Kit Includes:

- EcoRI-HF
- XbaI
- SpeI
- PstI
- 10X NEBuffer r2.1
- T4 DNA Ligase
- 10X T4 DNA Ligase Buffer

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

The PCR Cloning 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.

USER® Enzyme

Thermolabile USER II Enzyme

USER Enzyme
#M5505S 50 units
#M5505L 250 units

Thermolabile USER II Enzyme
#M5508S 50 units
#M5508L 250 units

- *USER Cloning*
- *Directional RNA-Seq*
- *NEBNext adaptor cleavage*

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.

Reaction Conditions: rCutSmart Reaction Buffer. Incubate at 37°C. Heat Inactivation of Thermolabile USER II Enzyme: 65°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to nick 10 pmol of a 34-mer 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

Note: This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

How does the NEB PCR Cloning Kit work?

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

- *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*

For help with primer design, try **NEBaseChanger** at **NEBaseChanger.neb.com**

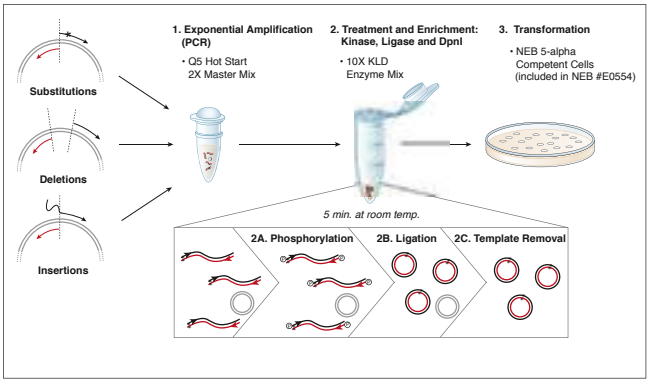
Description: The Q5 Site-Directed Mutagenesis Kit allows rapid site-specific mutagenesis of double-stranded plasmid DNA in less than 2 hours. The kit utilizes the robust Q5 Hot Start High-Fidelity DNA Polymerase along with custom mutagenic primers to create substitutions, deletions and insertions in 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

The Q5 Site Directed Mutagenesis Kit Includes:

– Q5 Hot Start High-Fidelity Master Mix (2X)
– KLD Enzyme Mix (10X) and Reaction Buffer (2X)
– Control Primer Mix (10 µM each) and Template DNA (5 ng/µl)
– NEB 5-alpha Competent *E. coli* (High Efficiency), pUC19 Transformation Control Plasmid (5 pg/µl), SOC Outgrowth Medium (NEB #E0554 only)



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.

Quick Blunting™ Kit

#E1201S 20 reactions
#E1201L 100 reactions

Special Offer:
Quick Blunting and Quick Ligation Kits
#E0542S 20 reactions

See page 101 for details on the Quick Ligation Kit.

- *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.

Applications:

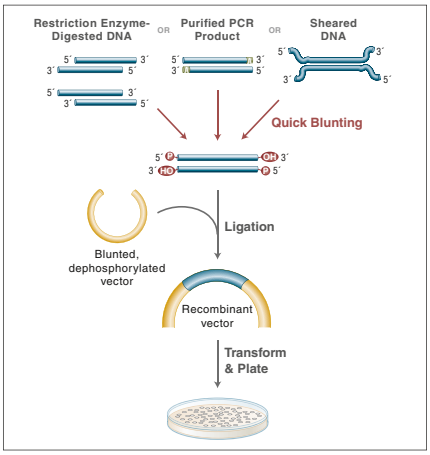
- Prepare sheared, nebulized or restriction enzyme-digested DNA for blunt-end ligation into a plasmid, cosmid, fosmid or BAC vector
- Prepare PCR products for efficient blunt-end cloning

The Quick Blunting Kit Includes:

– Blunting Enzyme Mix
– 10X Blunting Buffer
– 1 mM Deoxynucleotide (dNTP) Solution Mix

Heat Inactivation: 70°C for 10 minutes.

Notes: 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.



Learn more about the benefits of the Q5 SDM Kit.

rCutSmart or NEB r1.1 Recommended Buffer | Cloned at NEB | RR Recombinant Enzyme | BSA Requires BSA | SAM Requires SAM

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 Ligase Master Mix	Electro Ligase®	T4 DNA Ligase	Quick Ligation™ Kit	Salt-T4® DNA Ligase	Hi-T4™ DNA Ligase	Immo-bilized T4 DNA Ligase	T3 DNA Ligase	T7 DNA Ligase	HiFi *Taq* DNA Ligase	*E. coli* DNA Ligase	*Taq* DNA Ligase	9°N™ DNA Ligase	SplintR® Ligase
DNA APPLICATIONS															
Ligation of sticky ends	★★	★★★	★★	★★	★★★	★★	★★	★★★	★★	★★	★	★	★	★	
Ligation of blunt ends	★★★	★	★★	★★	★★★	★★	★★	★	★★						
T/A cloning	★★★	★	★★	★★	★★	★★	★★		★	★					
Electroporation			★★★	★★			★★								
Ligation of sticky ends only										★★★					
Repair of nicks in dsDNA	★★	★★	★★	★★★	★★	★★★	★★★		★★	★★	★★	★★	★★	★★	★★
High complexity library cloning	★★	★★	★★	★★★	★★										
Adaptor 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 of T4 DNA Ligase)						✔			✔						
Ligation in 15 min. or less	✔	✔		✔	✔	✔	✔		✔	✔	✔		✔	✔	✔
Master Mix Formulation	✔	✔													
Thermostable											✔		✔	✔	
Thermotolerant							✔				✔		✔	✔	
Recombinant	✔	✔	✔	✔	✔	✔	✔		✔	✔	✔	✔	✔	✔	✔

★★★ 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

Helpful Online Tools:

For help with choosing the best ligase for your experiment, try **NEBcloner** at **NEBcloner.neb.com**

For help with estimating incubation temperature when using thermostable ligases, try the **Thermostable Ligase Reaction Temperature Calculator** at **LigaseCalc.neb.com**

For help with scientific calculations and conversions, try **NEBioCalculator** at **NEBioCalculator.neb.com**

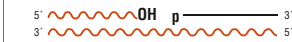
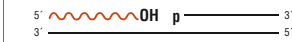
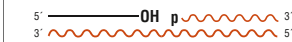
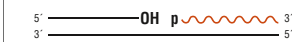
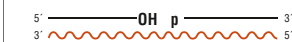
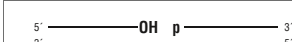
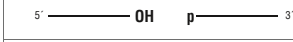
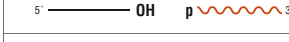
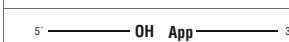
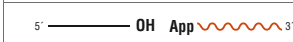
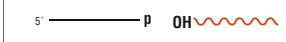
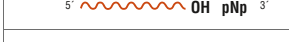
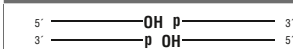
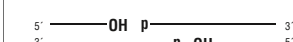
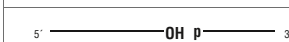
Find an overview of ligation.

Enzymes for Innovation 37° Incubation Temperature 65° Heat Inactivation

Substrate-based Ligase Selection Chart

DNA
RNA

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.

	RECOMMENDED LIGASE	COMMENTS
NICKED DNA/RNA		
	T4 RNA Ligase 2	
	T4 RNA Ligase 2	
	T4 RNA Ligase 2	
	T4 DNA Ligase	
	N/A	No ligase optimized for this activity
	N/A	No ligase optimized for this activity
	SplintR Ligase	100 – 1,000-fold higher efficiency than T4 DNA Ligase
	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	
	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/RNA		
	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 T3 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 T3 DNA Ligase. For ligation of cohesive ends ONLY, we recommend T7 DNA Ligase.

CIRCLIGASE™ is a trademark of EpiCentre Technologies Corp.

rCutSmart or r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · BSA Requires BSA · SAM Requires SAM · Enzymes for Innovation · 37° Incubation Temperature · 65° Heat Inactivation

T4 DNA Ligase Products

For help with molar ratio calculations, try **NEBioCalculator** at **NEBioCalculator.neb.com**

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	M0202S/L/T/M	• 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.	S/L Regular Concentration (400,000 cohesive end units/ml) 20,000/100,000 units T/M High Concentration (2,000,000 cohesive end units/ml) 20,000/100,000 units
MASTER MIXES				
Instant Sticky-end Ligase Master Mix	M0370S/L	• 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	50/250 rxns
Blunt/TA Ligase Master Mix	M0367S/L	• 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	50/250 rxns
FORMULATIONS				
Quick Ligation Kit	M2200S/L	• 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).	30/150 rxns
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 rxns
NEW **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.	1.1 mg
VARIANTS				
NEW **Hi-T4™ DNA Ligase**	M2622S/L	• 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.	20,000/100,000 units
NEW **Salt-T4® DNA Ligase**	M0467S/L	• 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°C. Heat inactivate at 65°C for 10 minutes.	20,000/100,000 units

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: 1X StickTogether™ DNA Ligase Buffer. Incubate at 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

Notes: ATP is an essential cofactor for the reaction.

T3 DNA Ligase is also active in buffers without PEG 6000, including T4 DNA Ligase Buffer for applications in which PEG 6000 is detrimental. Supplement with 1 mM ATP (final concentration). In these buffers T3 DNA Ligase exhibits ~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: 1X StickTogether DNA Ligase Buffer. Incubate at 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

Notes: ATP is an essential cofactor for the reaction.

T7 DNA Ligase is also active in buffers without PEG 6000, including T4 DNA Ligase Buffer for applications in which PEG 6000 is detrimental. Supplement the reaction with 1 mM ATP (final concentration). Using these buffers, the activity of T7 DNA Ligase is reduced ~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: 1X *E. coli* DNA Ligase Reaction Buffer. Optimal ligation occurs at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

Usage Notes:
Requires NAD^+ (nicotinamide adenine dinucleotide) as a cofactor.

Ligation of blunt-ended fragments is extremely inefficient. For ligation of blunt-ended fragments we recommend the Blunt/TA Ligase Master Mix (NEB #M0367) or the Quick Ligation Kit (NEB #M2200).

HiFi *Taq* DNA Ligase

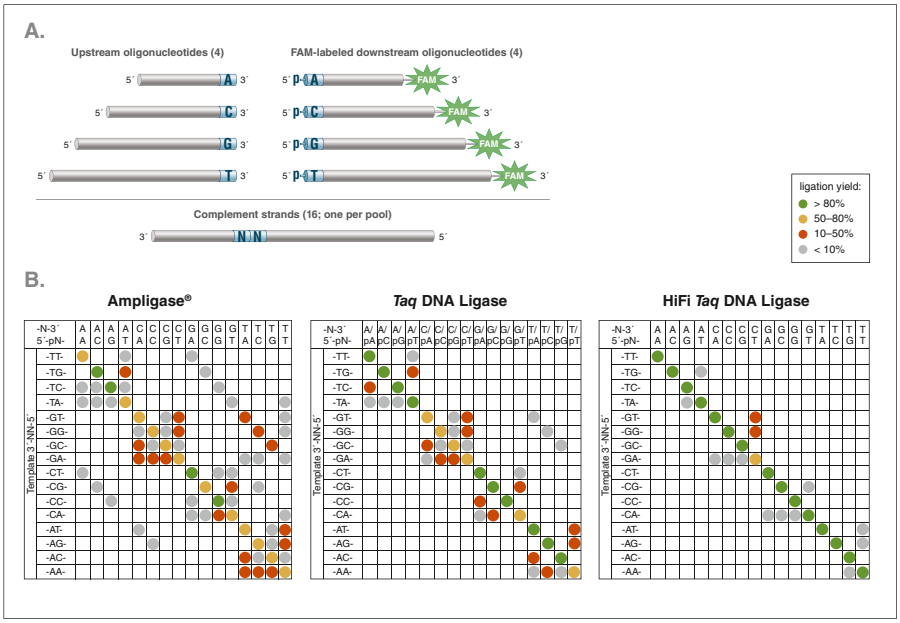
#M0647S 50 reactions

- *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*

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: 1X HiFi *Taq* DNA Ligase Reaction Buffer. Incubate at 25°C.

For help with calculating ligation temp, try our **Thermostable Ligase Reaction Temperature Calculator** at **LigaseCalc.neb.com**



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 µl of ligase in a 50 µl 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.*

Thermus aquaticus (Taq) DNA Ligase

#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: 1X *Taq* DNA Ligase Reaction Buffer. Incubate at 45°C.

For help with calculating ligation temp, try our **Thermostable Ligase Reaction Temperature Calculator** at **LigaseCalc.neb.com**

Requires NAD^+ as a cofactor. NAD^+ is supplied in the 10X *Taq* DNA Ligase Reaction Buffer; *the buffer should be stored at –70°C to extend the half life of the NAD^+ cofactor.*

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. Unit assay conditions can be found at www.neb.com.

Concentration: 40,000 units/ml

Notes: Will not ligate short 4-base overlaps (typical of restriction enzyme digests), while it efficiently ligates 12 base pair overlaps.

9°N™ DNA Ligase

RR NEBU 45° 65°

#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: 1X 9°N DNA Ligase Reaction Buffer. Incubate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 40,000 units/ml

Notes: Will not ligate short 4-base overlaps (typical of restriction enzyme digests), while it efficiently ligates 12 base pair overlaps.

SplintR® Ligase

BF RR 25° 65°

See page 214 for more information.

#M0375S 1,250 units
#M0375L 6,250 units

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.

T4 Polynucleotide Kinase & T4 Polynucleotide Kinase (3´ phosphatase minus)

BF RR NEBU 37° 65°

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.

Usage 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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

5-hydroxymethyluridine DNA Kinase

#M0659S 1,000 units

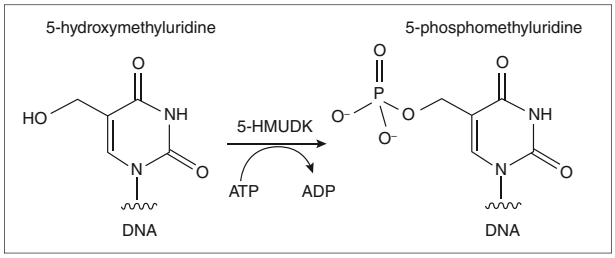
This is an **Enzyme for Innovation** (EFI). EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

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: 1X T4 DNA Ligase Reaction Buffer. Incubate at 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



5-HMUDK activity.

Phosphatase Selection Chart

	Quick CIP	Recombinant Shrimp Alkaline Phosphatase (rSAP)	Antarctic Phosphatase
FEATURES			
100% heat inactivation	2 minutes/80°C	5 minutes/65°C	2 minutes/80°C
High specific activity	●	●	
Improved stability	●	●	
Works directly in NEBuffers	●	●	●
Requires additive			● (Zn^{2+})
Quick Protocol	●		

NEW

Quick CIP

#M0525S 1,000 reactions
#M0525L 5,000 reactions

- *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: 1X rCutSmart Reaction Buffer. Incubate at 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

Note: This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

The Quick CIP Includes:

– rCutSmart Buffer
– Quick CIP

Find an overview of dephosphorylation.

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: 1X rCutSmart Reaction Buffer. Incubate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 1,000 units/ml

Note: This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

Antarctic Phosphatase

RR NEBU 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 70°C for 5 minutes, thereby making removal of Antarctic Phosphatase prior to ligation or end-labeling unnecessary.

Reaction Conditions: 1X Antarctic Phosphatase Reaction Buffer. Incubate at 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*. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

Jessica serves as a Technician in our Production Group, helping to prepare buffers for purification processes, assisting with enzyme formulation and fermentation as needed. When not a work, Jessica spends time rollerblading and listening to vinyl records.

Inorganic & Thermostable Inorganic Pyrophosphatases

See page 203 for more information.

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

NEW
NudC Pyrophosphatase
#M0607S 250 pmol

Apyrase

#M0398S 10 units
#M0398L 50 units

- *Highly efficient degradation of ATP 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*
- *Supplied at 10-fold higher concentration*

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 catalyse the conversion of 5´ triphosphorylated RNA to 5´ monophosphorylated RNA by sequential removal of γ and β phosphates.

Reaction Conditions: 1X Apyrase Reaction Buffer. 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 volume of 50 µl. Unit assay conditions can be found at www.neb.com.

Concentration: 500 units/ml

Notes: 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 1.1, 2.1, 3.1 and CutSmart Buffer.

Apyrase does not remove 5´ caps from eukaryotic mRNA.

Tte UvrD Helicase

#M1202S 0.5 µg

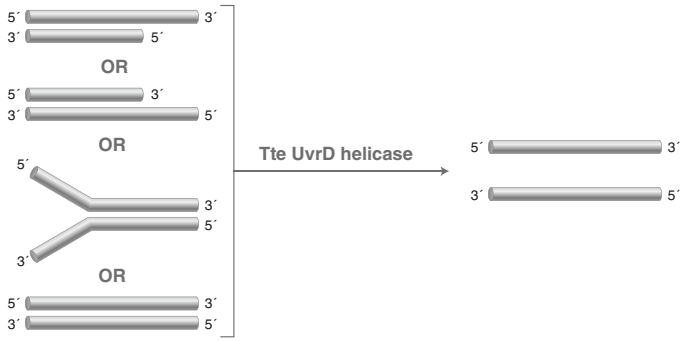
This is an **Enzyme for Innovation** (EFI). EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

- *Unwinds double-stranded DNA*
- *Thermostable to 65°C*
- *Reduces non-specific product formation in isothermal amplification (e.g. LAMP)*

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: 1X Isothermal Amplification Buffer. Supplement with 1 mM ATP. Heat inactivation: 80°C for 20 minutes.

Concentration: 20 µg/ml



Tte UvrD Helicase activity.

Properties of Exonucleases and Non-specific Endonucleases

ENZYME	POLARITY	ACTIVITY ON ssDNA		ACTIVITY ON dsDNA[1]:					PARTIAL DIGESTION TO GENERATE ss EXTENSION[2]	PRODUCTS PRODUCED[3]	INHIBITION BY PHOSPHO-ROTHIOATE[4]	NOTES
		LINEAR	CIRCULAR	LINEAR 5´ext	LINEAR 3´ext	LINEAR BLUNT	NICKED CIRCULAR/ LINEAR)	CIRCULAR (SUPER-COILED)				
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
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´	+	–	–[16]	–	–[5]	–	–	No	dNMP, ssDNA	Yes	5, 15
Mung Bean Nuclease	endonuclease	+	+	–	–	–	–	–	No	dNMP, dsDNA	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
Nuclease BAL-31	both[12]	+	+	+	+	+	+	–	Yes	dsDNA, dNMP	No	12
T5 Exonuclease	5´→3´	+	+	+	+	+	+	–	3´	dNMP to 6-mer	No	
DNase I (RNase-free)	endonuclease	+	+	+	+	+	+	+	NA	dinucleotides, trinucleotides, oligonucleotides, ssDNA, dsDNA	No	
Micrococcal Nuclease	endonuclease	+	+	+	+	+	+	+	NA	diphosphonucleotides, ssDNA, dsDNA 3´- monophosphonucleotides[13]	No	13

This table is intended to be used as a guide. Not all reported activities and properties for each exonuclease or endonuclease are listed. The amount of enzyme, substrate and time of incubation can have a dramatic effect upon the desired outcome of the experiment.

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

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 initate 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 and Exonuclease T may remove a few nucleotides from blunt termini.
6. Thermolabile Exonuclease I, 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 DNaseI 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 and 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.

Common Applications for Exonucleases and Endonucleases

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	Quick heat inactivation versus Exonuclease I
	Exonuclease VII	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	Lambda Exonuclease	Strand targeted for removal requires one 5´ end with phosphate
If 3´ → 5´ polarity required	Exonuclease III	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*)	Removes nicked-strand DNA from 3´ to 5´
	T7 Exonuclease	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	Degrades linear ss + dsDNA, nicked DNA
	Exonuclease V (RecBCD)	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	Only the un-nicked form of ligated circular double-stranded remains
	Exonuclease V (RecBCD)	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	
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	
Progressive shortening of both ends of double-stranded DNA	Nuclease BAL-31	
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	

What are exonucleases and their applications?

DNase I (RNase-Free)

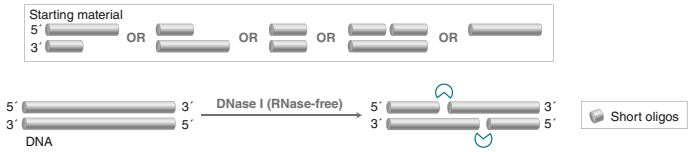
See page 217 for more information.

#M0303S 1,000 units
#M0303L 5,000 units

DNase I (RNase-free) is a DNA-specific endonuclease that degrades ds- and ss-DNA to release short oligos with 5´-phosphorylated and 3´-hydroxylated ends.

Note: GMP-grade reagent also available. See page 7.

- *Removal of contaminating genomic DNA from RNA samples*
- *Degradation of DNA templates in transcription reactions*



Lambda Exonuclease

#M0262S 1,000 units
#M0262L 5,000 units

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.

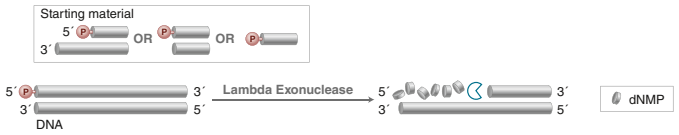
Reaction Conditions: 1X Lambda Exonuclease Reaction Buffer. Incubate at 37°C. Heat inactivation: 75°C for 10 minutes.

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. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

Note: 5´-OH ends are digested 10X slower than 5´-PO_4 ends. ssDNA is digested 100X slower than dsDNA.

- *Conversion of linear dsDNA to single-stranded DNA via preferred activity on 5'-phosphorylated ends*



Exonuclease I (*E. coli*)

#M0293S 3,000 units
#M0293L 15,000 units

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.

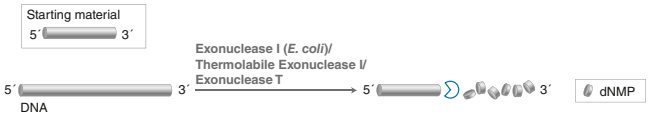
Reaction Conditions: 1X Exonuclease I Reaction Buffer. Incubate at 37°C. Heat inactivation: 80°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will catalyze the release of 10 nmol of acid-soluble nucleotide in 30 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 20,000 units/ml

Note: Thermolabile version also available (NEB #M0568).

- *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*



Thermolabile Exonuclease I

#M0568S 3,000 units
#M0568L 15,000 units

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: 1X NEBuffer r3.1. Incubate at 37°C. Heat inactivation: 80°C for 1 minute.

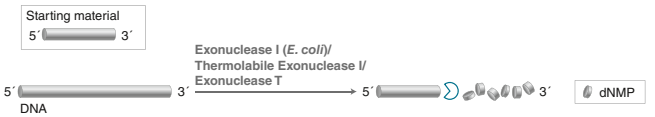
Unit Definition: One unit is defined as the amount of enzyme that will catalyze the release of 2 nmol of acid-soluble nucleotide in a total reaction volume of 100 µl in 6 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 20,000 units/ml

Note: Try Exo-CIP Rapid PCR Cleanup Kit (NEB #E1050).

This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

- *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*



Exonuclease III (*E. coli*)

RR NEB1 37° 70°

#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 is a dsDNA-specific exonuclease that catalyzes the removal of nucleotides from 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.

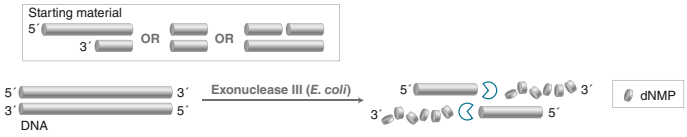
Exonuclease III has also been reported to have RNase H, 3´ phosphatase and AP-endonuclease activities.

Reaction Conditions: 1X NEBuffer 1. Incubate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 100,000 units/ml

Usage Notes: Phosphorothioate linkages are not cleaved by Exonuclease III. Unidirectional deletions can also be created by protecting one terminus by incorporation of an α-phosphorothioate-containing nucleotide.



Exonuclease V (RecBCD)

RR NEB4 37° 70°

#M0345S 1,000 units
#M0345L 5,000 units

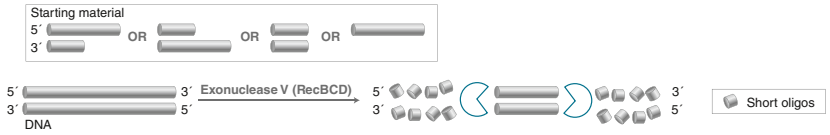
- *Degradation of contaminating linear DNA in plasmid samples*
- *Removal of residual gDNA after purification of low copy plasmid*

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: 1X NEBuffer 4. Supplement with 1 mM ATP. Incubate at 37°C. 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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml



Exonuclease VII

RR NEBU 37° 95°

#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: 1X Exonuclease VII Reaction Buffer. Incubate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

Exonuclease VIII, truncated

#M0545S 1,000 units

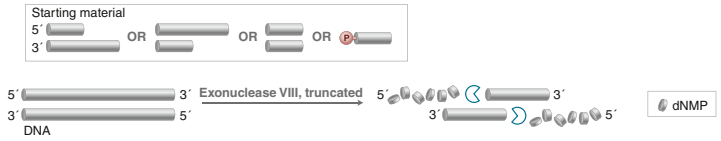
- *Removal of linear dsDNA, leaving behind circular DNA in the sample*

Description: Exonuclease VIII, truncated, is a dsDNA-specific exonuclease. 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: 1X NEBuffer 4. Incubate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml



Exonuclease T (RNase 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 or SNP analysis*
- *Removal of single-stranded primers for nested PCR reactions*
- *Removal of ssDNA, leaving behind dsDNA in the sample*

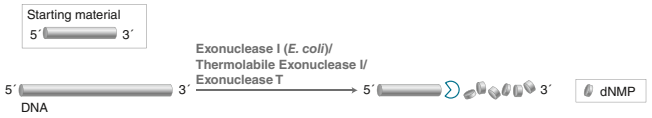
Description: Exonuclease T (Exo T), also known as RNase T, is a single-stranded RNA or DNA specific nuclease that requires a free 3´ terminus and removes nucleotides in the 3´→ 5´ direction. Exonuclease T can be used to generate blunt ends from RNA or DNA molecules that have 3´ extensions.

Reaction Conditions: 1X NEBuffer 4. Incubate at 25°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 0.1 nmol of TCA soluble nucleotides from 1 nmol of [^{3}H]-labeled polythymidine in a total reaction volume of 100 µl in 30 minutes at 25°C in 1X NEBuffer 4 with 1 nmol [^{3}H]-labeled polythymidine DNA.

Concentration: 5,000 units/ml

Usage Note: Exo T is has different activity on RNA vs. DNA. For RNA, 1 unit of Exo T is required to completely digest 1.0 pmol of rA20 under standard reaction conditions.



Thermostable FEN1

#M0645S 1,600 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. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

Description: Thermostable Flap Endonuclease 1, 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: 1X ThermoPol Reaction Buffer. Incubate at 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 in 10 minutes at 65°C. Unit assay conditions can be found at www.neb.com.

Concentration: 32,000 units/ml

Micrococcal Nuclease

RR NEBU BSA 37° No

#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 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.

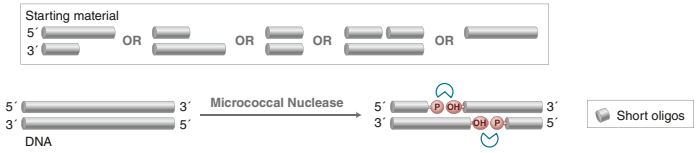
Reaction Conditions: 1X Micrococcal Nuclease Reaction Buffer. Supplement with 100 µg/ml BSA. Incubate at 37°C.

Unit Definition: (Agarose Gel Unit) One gel unit is defined as the amount of enzyme required to digest 1 µg of lambda DNA in 15 minutes at 37°C into molecular DNA fragments (100–400 base pairs) on a 1.2% agarose gel.

Note: 10,000 Gel Units are approximately equal to 1,000 Kunitz Units.

Concentration: 2 x 10^6 gel units/ml

Notes: 1–5 mM Ca^{2+} is required for activity. The enzyme is active in the pH range 7–10, with optimal activity at pH 9.2, as long as salt concentration is less than 100 mM. Enzyme can be inactivated by addition of excess EGTA.



Mung Bean Nuclease

NEBU 30° No

#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*

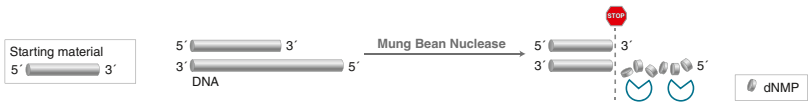
Description: Mung Bean Nuclease is a single-strand specific DNA and RNA endonuclease which will degrade single-stranded extensions from the ends of DNA and RNA molecules, leaving blunt, ligatable ends.

Reaction Conditions: 1X Mung Bean Nuclease Reaction Buffer. Incubate at 30°C.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 µg of acid-soluble total nucleotide in a total reaction volume of 50 µl in 1 minute at 37°C in 1X Mung Bean Nuclease Reaction Buffer with 0.5 mg/ml denatured Calf Thymus DNA. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

Usage Note: Do not attempt to heat inactivate, DNA will "breathe" before enzyme inactivates, causing undesirable degradation.



Nuclease BAL-31

NEBU 30° 65°

#M0213S 50 units

- *Progressive shortening of dsDNA*

Description: Nuclease BAL-31 is a DNA and RNA exonuclease that degrades linear dsDNA from both 5´ and 3´ termini. The enzyme is also a highly-specific single-stranded endonuclease which cleaves at nicks, gaps and single-stranded regions of duplex DNA and RNA.

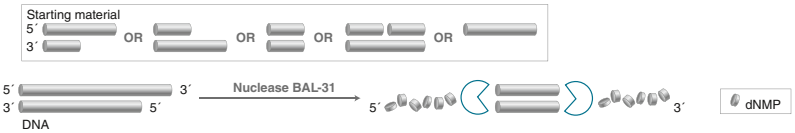
Reaction Conditions: 1X Nuclease BAL-31 Reaction Buffer. Incubate at 30°C. Heat inactivation: 65°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to remove 200 base pairs from each end of linearized double-stranded ϕX174 DNA (40 µg/ml) in 50 µl of 1X Nuclease BAL-31 Reaction Buffer in 10 minutes at 30°C.

Concentration: 1,000 units/ml

Usage Notes: Duplex products of the exonuclease are a mixture of blunt and staggered ends. This mixture can be cloned directly, although maximal ligation efficiency requires repairing the staggered ends with a suitable DNA polymerase.

If necessary, the enzyme may be diluted in reaction buffer just prior to use.



Nuclease P1

NEBU 37° 75°

#M0660S 10,000 units

- *Conversion of ssDNA or RNA to 5´-mono-nucleotides*
- *Analysis of the base composition of nucleic acids*
- *Studies of the potential damage and modification in 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 1.1, to limit activity on dsDNA while maintaining single-strand nuclease activity.

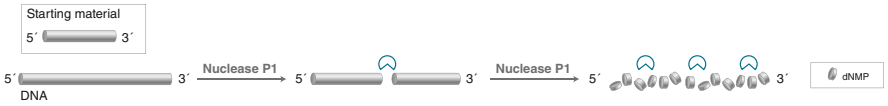
Reaction Conditions: 1X Nuclease P1 Reaction Buffer. Incubate at 37°C. Heat inactivation: 75°C for 10 minutes.

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 minute at 37°C in 1X Nuclease P1 Reaction Buffer.

Concentration: 100,000 units/ml

Usage Notes: 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.



$RecJ_f$

RR NEB2 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)*

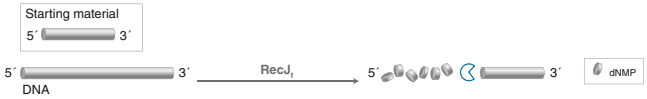
Description: $RecJ_f$ is a ssDNA-specific exonuclease that catalyzes the removal of nucleotides from linear ssDNA in the 5´ → 3´ direction. The preferred substrate is dsDNA with 5´ single-stranded overhangs > 6 nucleotides long.

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: 1X NEBuffer 2. Incubate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 30,000 units/ml



NEW

T5 Exonuclease

RR NEB4 37° NO

#M0663S 1,000 units
#M0663L 5,000 units

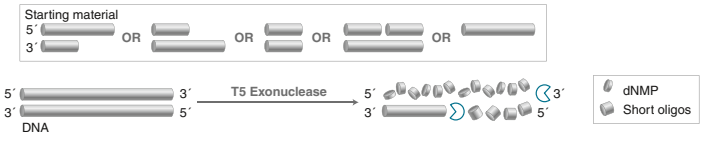
- *Removal of incomplete ligation products from ligated circular dsDNA*
- *Degradation of denatured DNA from alkaline-based plasmid purification methods for improved DNA cloning*
- *Degradation of contaminating linear and nicked DNA in plasmid samples*

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: 1X NEBuffer 4. Incubate at 37°C.

Unit Definition: One unit of T5 Exonuclease is defined as the amount of enzyme required to cause the change of 0.00032 A_{260} nm/min at 37°C in CutSmart Buffer.

Concentration: 10,000 units/ml



T7 Exonuclease

#M0263S 1,000 units
#M0263L 5,000 units

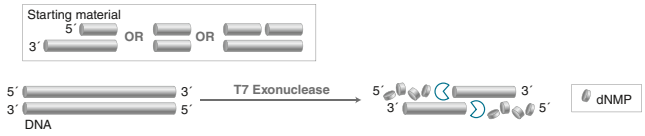
- *Site-directed mutagenesis*
- *Nick-site extension*

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: 1X NEBuffer 4. Incubate at 25°C.

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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml



Nucleoside Digestion Mix

See page 217 for more information.

#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*

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.

Annie has been with NEB for over 12 years as a member of our Marketing Communications Team. She helps coordinate and ship materials for vendor tabletops and tradeshows and supports our Sales Team. You may have spoken with her directly, as she sometimes works at the front desk!

Properties of DNA Repair Enzymes and Structure-specific Endonucleases

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	1nt gap	dR5P	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	
Tma Endo III	AP site, damaged pyrimidines, Tg	N-glycosidic bond, 1st phosphodiester bond 3´ to AP site	1nt gap	P	PA	Glycosylase & AP lyase	Yes
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	
Endo VIII	AP site[4]	phosphodiester bond 3´ and 5´ to AP site	1nt gap	P	P	AP lyase	
Fpg	8oxoG, 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	dU[4], 5-hmU, 5-hoU, 5fU	N-glycosidic bond	AP site	N/A	N/A	Glycosylase	
RNaseHII	rN in dsDNA	phosphodiester bond 5´ to ribo	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	
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
Antarctic Thermolabile UDG	dU[4]	N-glycosidic bond	AP site	N/A	N/A	Glycosylase	
T7 Endo I	Cruciforms, mismatches, Holliday junctions, across DNA nicks	phosphodiester bond 5´ to structure	nick	P	OH	Endonuclease	
Thermostable FEN1	5´ DNA flap[3]	phosphodiester bond at base of flap	nick	P	OH (on flap)	Endonuclease	Yes

Table Legend:

3mA	3-methyladenine	**dR5P**	deoxyribose-5´-phosphate
5fU	5-formyluridine	**dU**	deoxyuridine
5-hmU	5-hydroxymethyluridine	**dX**	deoxyxanthosine
5-hoU	5-hydroxyuridine	**NA**	not applicable
7mG	7-methylguanine	**OH**	Hydroxyl
8oxoG	8-oxo-7, 8-dihydroguanine	**P**	Phosphate
AP	apurinic/apyrimidinic sites	**PA**	3´ phospho-α, β-unsaturated aldehyde
CPDs	Cyclobutane pyrimidine dimers	**rN**	ribonucleotides
dI	deoxyinosine	**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)										
	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	THYMINE GLYCOL:A
REPAIR ENZYMES	APE 1	++++	+	–	–	–	–	–	–	–	–	–
	Endo III	++++	+	–	–	–	–	–	–	–	–	+
	Tma Endo III	++++	++	–	–	–	–	+	+	–	–	++
	Endo IV	++++	+	–	–	–	–	–	–	–	–	–
	Tth Endo IV	++++	+	–	–	–	–	–	+	–	–	–
	Endo V*	+++	+	+	+	++++	+	++	+	+	++++	++
	Endo VIII	++++	++	–	–	–	–	–	–	–	–	+++
	Fpg	+	+	–	–	–	–	++++	++++	–	–	+
	hAAG	–	–	–	–	++++	–	–	–	–	–	–
	hNEIL1	++++	++	–	–	–	–	+	+	–	–	++
	hOGG1	++	–	–	–	–	–	++++	+	–	–	–
	T4 PDG	++++	–	–	–	–	–	–	–	–	–	–
	UDG	N/A	–	–	–	–	–	–	–	++++	+	–
	Afu UDG	N/A	–	–	–	–	–	–	–	++++	+	–
	hSMUG1	N/A	–	+++	+++	–	–	–	–	++++	++++	–

Standard reaction conditions were used to titer the enzymes with the alternate base.
**Nicks only, does not remove damage*

		SINGLE-STRANDED DNA OLIGOS (34-MERS)							
	ENZYME	AP	DHT	5-hmU	I	6-MeA	8-OG	U	THYMINE GLYCOL:A
REPAIR ENZYMES	APE 1	++	–	–	–	–	–	–	–
	Endo III	++	–	–	–	–	–	–	–
	Tma Endo III	++	+	–	–	–	–	–	–
	Endo IV	–	–	–	–	–	–	–	–
	Tth Endo IV	–	–	–	–	–	–	–	–
	Endo V	+	–	–	++++	–	+	–	–
	Endo VIII	+++	–	–	–	–	–	–	–
	Fpg	+	+	–	–	–	+	–	+
	hAAG	–	–	–	+	–	–	–	–
	hNEIL1	+	+	–	–	–	–	–	+
	hOGG1	++	–	–	–	–	+	–	–
	T4 PDG	–	–	–	–	–	–	–	–
	UDG	N/A	–	–	–	–	–	++++	–
	Afu UDG	N/A	–	–	–	–	–	++++	–
	hSMUG1	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
U:A	uridine paired with an adenine
U:G	uridine paired with a guanine

Activity Level:

++++	100%
+++	50%
++	10% – 25%
+	< 10%
–	no detectable enzyme activity (< 0.7%)
N/A	not applicable

APE 1

#M0282S 1,000 units
#M0282L 5,000 units

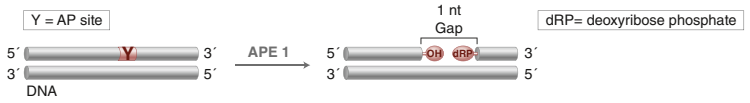
- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*
- *Modified nick translation*

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: 1X NEBuffer 4. Incubate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml



Endonuclease IV

#M0304S 1,000 units
#M0304L 5,000 units

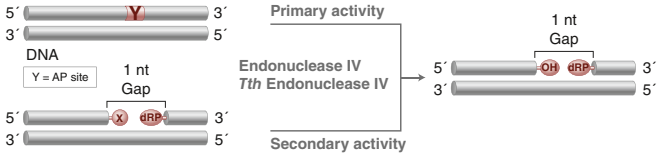
- Single-cell gel electrophoresis (Comet assay)
- Alkaline elution
- Alkaline unwinding

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: 1X NEBuffer 3. Incubate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml



X= Phosphate α, β-unsaturated aldehyde (UA)
dRP= deoxyribose phosphate

Tth Endonuclease IV

#M0294S 500 units

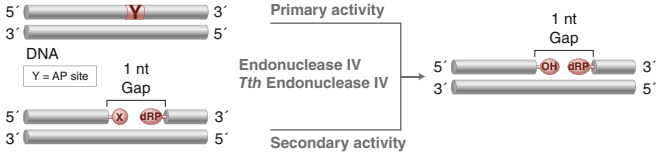
- *Thermostable*
- *Alkaline elution*
- *Alkaline unwinding*

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.

Reaction Conditions: 1X ThermoPol Reaction Buffer. Incubate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml



X= Phosphate α, β-unsaturated aldehyde (UA)
dRP= deoxyribose phosphate

Endonuclease III (Nth)

#M0268S 1,000 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

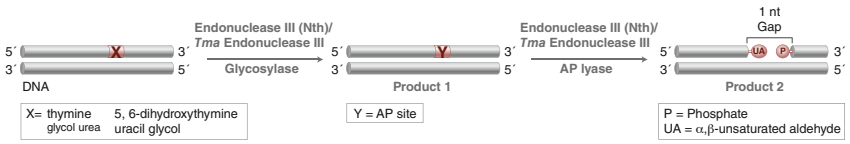
Description: Endonuclease III (Nth) protein from *E. coli* acts both as a *N*-glycosylase and an AP-lyase. The *N*-glycosylase activity releases damaged pyrimidines, including thymine glycol and 5, 6-dihydroxythymine, generating an AP site. The AP lyase activity cleaves an AP site via β-elimination, 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: 1X Endonuclease III (Nth) Reaction Buffer. Incubate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml



Tma Endonuclease III

#M0291S 500 units

- *Alkaline elution*
- *Alkaline unwinding*

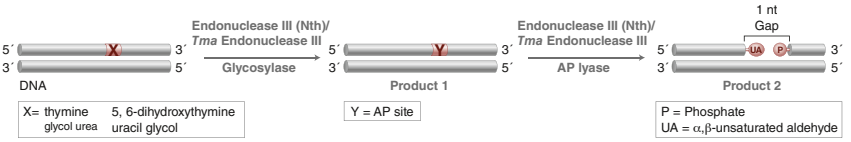
Description: A thermostable homolog of the *E. coli* Endonuclease III (Nth) that acts as an *N*-glycosylase and an AP-lyase. The *N*-glycosylase activity releases damaged pyrimidines, including thymine glycol and 5,6-dihydroxythymine, generating an AP site. The AP lyase activity cleaves an AP site via β-elimination, creating a 1-nucleotide gap with 3´-α, β-unsaturated aldehyde and 5´-phosphate termini.

Tma Endonuclease III recognizes abasic sites, 5,6 dihydroxythymine and thymine glycol in DNA.

Reaction Conditions: 1X ThermoPol Reaction Buffer. Incubate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml



Endonuclease V

#M0305S 250 units

- *Cleavage of oligonucleotides containing deoxyinosines*
- *Mismatch cleavage*

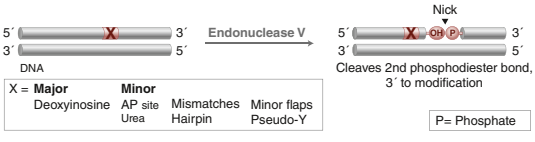
Description: Endonuclease V is a repair enzyme found in *E. coli* that recognizes deoxyinosine, a deamination product of deoxyadenosine in DNA. Endonuclease V, often called Deoxyinosine 3´ Endonuclease, recognizes DNA containing deoxyinosines (paired or not) on dsDNA, ssDNA with deoxyinosines and, to 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: 1X NEBuffer 4. Incubate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml



Endonuclease VIII

#M0299S 1,000 units
#M0299L 5,000 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

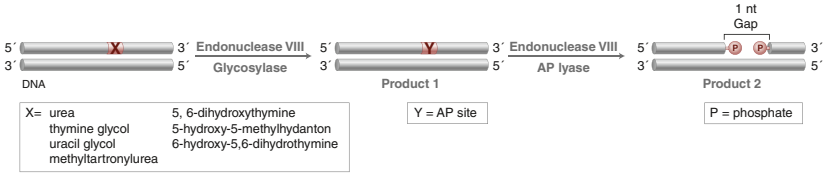
Description: Endonuclease VIII acts as both an *N*-glycosylase and an AP-lyase. The *N*-glycosylase activity releases damaged pyrimidines, including thymine glycol and uracil glycol. The AP lyase activity cleaves DNA phosphodiester backbone at AP sites via β and δ-elimination, creating a 1 nucleotide 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: 1X Endonuclease VIII Reaction Buffer. Incubate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml



Fpg

#M0240S 500 units
#M0240L 2,500 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

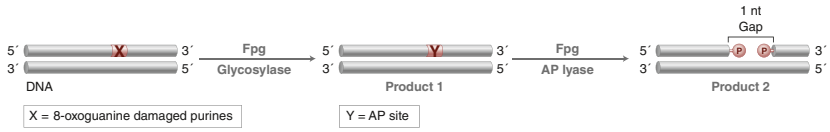
Description: Fpg (formamidopyrimidine [fapy]-DNA glycosylase), also known as 8-oxoguanine DNA glycosylase, acts both as an *N*-glycosylase and an AP-lyase. *N*-glycosylase activity releases damaged purines, including 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) and 8-oxo-7,8-dihydroguanine (8oxoG), 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: 1X NEBuffer 1. Supplement with 100 µg BSA. Incubate at 37°C. 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. Unit assay conditions can be found at www.neb.com.

Concentration: 8,000 units/ml



hAAG

#M0313S 500 units

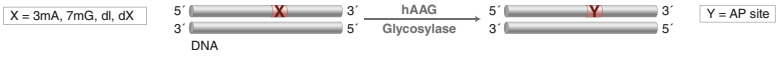
- *Single-cell gel electrophoresis (Comet Assay)*
- *Alkaline elution*
- *Alkaline unwinding*

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: 1X ThermoPol Reaction Buffer. Incubate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml



T4 PDG (T4 Endonuclease V)

RR NEBU BSA 37° NG

#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: 1X T4 PDG Reaction Buffer. Supplement with BSA. Incubate at 37°C.

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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 units/ml

Note: Incubation time should be ≤ 30 minutes for best results.

Uracil-DNA Glycosylase (UDG)

RR NEBU 37° NG

#M0280S 1,000 units
#M0280L 5,000 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*

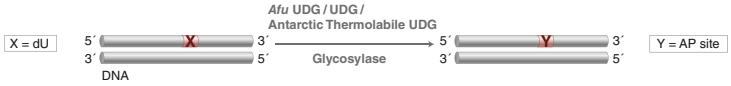
Description: *E. coli* Uracil-DNA Glycosylase (UDG) is a monofunctional DNA glycosylase that catalyzes the release of uracil from uracil-containing DNA. UDG efficiently hydrolyzes uracil from single-stranded or double-stranded DNA, but not from oligomers (6 or fewer bases).

Reaction Conditions: 1X UDG Reaction Buffer Incubate at 37°C.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the release of 60 pmol of uracil per minute from double-stranded, uracil-containing DNA. Activity is measured by release of [^{3}H]-uracil in a 50 µl reaction containing 0.2 µg DNA (10^4-10^5 cpm/µg) in 30 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

Usage Notes: UDG is active over a broad pH range with an optimum at pH 8.0, does not require divalent cation, and is inhibited by high ionic strength (> 200 mM).



Afu Uracil-DNA Glycosylase (UDG)

RR NEBU 65° NG

#M0279S 200 units

- *Thermostable*
- *Release of uracil from ss- or ds- DNA*

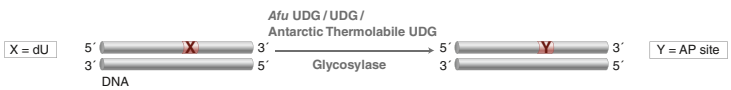
Description: A thermostable homolog of the *E. coli* Uracil-DNA Glycosylase (UDG) from *Archaeoglobus fulgidus*. *Afu* UDG catalyses the release of uracil from uracil-containing DNA. *Afu* UDG efficiently hydrolyzes uracil from ss- or ds-DNA.

Reaction Conditions: 1X ThermoPol II (Mg-free) Reaction Buffer. Incubate at 65°C.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the release of 60 pmol of uracil per minute from double-stranded uracil-containing DNA. Activity is measured by release of [^{3}H]-uracil in a 50 µl reaction containing 0.2 µg DNA (10^4–10^5 cpm/µg) in 30 minutes at 65°C. Unit assay conditions can be found at www.neb.com.

Concentration: 2,000 units/ml

Usage Notes: *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.



Antarctic Thermolabile UDG

#M0372S 100 units
#M0372L 500 units

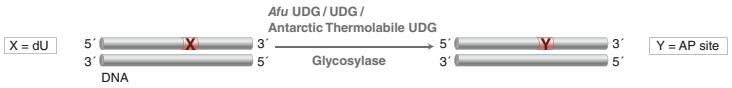
- *Eliminates PCR carry-over contamination*
- *Release of uracil from ss- or ds- DNA*
- *Component of Thermolabile User II Enzyme (NEB #M5508)*

Description: Antarctic Thermolabile UDG (Uracil-DNA Glycosylase) is a monofunctional DNA glycosylase that catalyzes the release of free uracil from uracil-containing ss-or ds-DNA. The resulting abasic sites are susceptible to the hydrolytic cleavage at the elevated temperature and high pH. This enzyme is sensitive to heat and can be rapidly and completely inactivated at temperatures above 50°C.

Reaction Conditions: 1X Standard *Taq* Reaction Buffer. Incubate at 37°C. Heat inactivation: 50°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the release of 60 pmol of uracil per minute from double-stranded, uracil-containing DNA. Activity is measured by release of [^{3}H]-uracil in a 50 µl Standard *Taq* reaction buffer containing 0.2 µg DNA (10^4-10^5 cpm/ug) in 30 minutes at 37°C.

Concentration: 1,000 units/ml



hSMUG1

#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-hydroxy-uracil, 5-hydroxymethyluracil, and 5-formyluracil.

Reaction Conditions: 1X NEBuffer 1. Supplement with 100 µg BSA. Incubate at 37°C.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. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

Usage Notes: hSMUG1 has 50% activity on 5-hydroxymethyluracil when compared to uracil. hSMUG1 has 50% activity on ssDNA compared to dsDNA.

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.

Source: An *E. coli* strain that carries a fusion of maltose binding protein (MBP) and T7 Endo I.

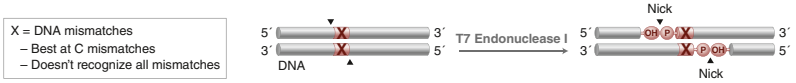
Reaction Conditions: 1X NEBuffer 2. Incubate at 37°C.

Unit Definition: One unit is defined as the amount of enzyme required to convert > 90% of 1 µg of super-coiled cruciform pUC(AT)* to > 90% linear form in a total reaction volume of 50 µl in 1 hour at 37°C. Unit assay conditions can be found at www.neb.com.

*pUC(AT) is derived from pUC19 with a modification of the polylinker between the EcoRI and PstI sites.

Concentration: 10,000 units/ml

Usage Note: It is important to control the amount of enzyme and the reaction time used for cleavage of a particular substrate. Temps. > 42°C increase nonspecific nuclease activity.



PreCR® Repair Mix

See page 82 for more information.

#M0309S 30 reactions
#M0309L 150 reactions

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.

Cre Recombinase

RR NEBU 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: 1X Cre Recombinase Reaction Buffer. Incubate at 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 a total reaction volume of 50 µl in 30 minutes at 37°C. Maximal recombination is determined by agarose gel analysis and by transformation of reactions followed by selection on ampicillin plates.

Concentration: 1,000 units/ml and 15,000 units/ml

TelN Protelomerase

RR NEBU 30° 75°

#M0651S 250 units

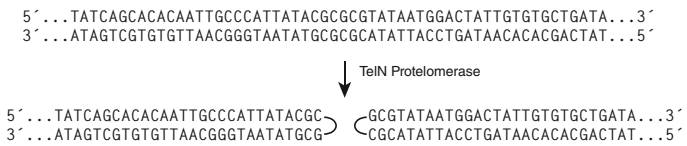
This is an **Enzyme for Innovation** (EFI). EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

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: 1X ThermoPol Reaction Buffer. Incubate at 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: 1X rCutSmart Buffer. Incubate at 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 negatively supercoiled pUC19 RF I DNA in a total reaction volume of 25 µl in 15 minutes at 37°C. DNA supercoiling is assessed by agarose gel electrophoresis in the absence of ethidium bromide.

Concentration: 5,000 units/ml

Note: This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

β-Agarase I

RR NEBU 42° 95°

#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: 1X β-Agarase I Reaction Buffer. Incubate at 42°C.

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.

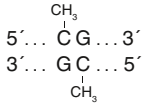
Concentration: 1,000 units/ml

Heat Inactivation: 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)

RR NEB2 SAM 37° 65°

#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: 1X NEBuffer 2 + SAM. Supplement with 160 µM S-adenosylmethionine (supplied). Incubate at 37°C. Heat inactivation: 65° for 20 minutes.

Note: $MgCl_2$ is not required as a cofactor. In the presence of Mg^{2+}, methylation becomes distributive rather than processive and exhibits topoisomerase activity.

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.

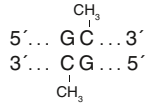
Concentration: 4,000 units/ml and 20,000 units/ml. Assayed on λ DNA.

See page 321 for more information on CpG methylation.

GpC Methyltransferase (M.CviPI)

RR NEBU SAM 37° 65°

#M0227S 200 units
#M0227L 1,000 units



- *Blocking restriction enzyme cleavage*
- *Altering the physical properties of DNA*
- *Uniform [^{3}H]-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: 1X GC Reaction Buffer. Supplement with 160 µM SAM (supplied). Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Note: $MgCl_2$ is not required as a cofactor.

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.

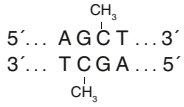
Concentration: 4,000 units/ml. Assayed on λ DNA.

For more information on products to study DNA methylation, see pages 276–289.

AluI Methyltransferase

RR NEBU SAM 37° 65°

#M0220S 100 units



Description: AluI Methyltransferase modifies the cytosine residue (C^5) in the sequence to the left.

Reaction Conditions: 1X AluI Methyltransferase Reaction Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

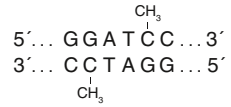
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by AluI restriction endonuclease.

Concentration: 5,000 units/ml

BamHI Methyltransferase

RR NEBU SAM 37° NO

#M0223S 100 units



Description: BamHI Methyltransferase modifies the internal cytosine residue (N^4) in the sequence to the left.

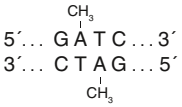
Reaction Conditions: 1X BamHI Methyltransferase Reaction Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C.

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

#M0222S 500 units
#M0222L 2,500 units



Description: *dam* Methyltransferase modifies the adenine residue (N^6) in the sequence to the left.

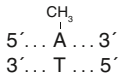
Reaction Conditions: 1X *dam* Methyltransferase Reaction Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

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 MboI restriction endonuclease.

Concentration: 8,000 units/ml

EcoGII Methyltransferase

#M0603S 200 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. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

Description: EcoGII Methyltransferase is a non-specific methyltransferase that modifies adenine residues (N^6) in any sequence context.

Reaction Conditions: 1X rCutSmart Buffer + SAM. Supplement with 160 µM S-adenosylmethionine (supplied). Incubate at 37°C. 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.

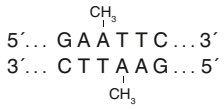
Concentration: 5,000 units/ml

Note: SAM is unstable at 37°C (pH 7.5) and should be replenished in reactions incubated > 4 hours. For use of methylation reaction SAM should be diluted 1:200 to a final concentration of 160 µM. EcoGII Methyltransferase is sensitive to salt. Make sure DNA solution is low in salt concentration or that it makes up only a small % of the final reaction volume.

This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

EcoRI Methyltransferase

#M0211S 10,000 units



Description: EcoRI Methyltransferase modifies the internal adenine residue (N^6) in the sequence to the left.

Reaction Conditions: 1X EcoRI Methyltransferase Reaction Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

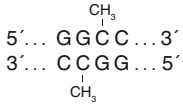
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by EcoRI restriction endonuclease.

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

#M0224S 500 units



Description: HaeIII Methyltransferase modifies the internal cytosine residue (C^5) in the sequence to the left.

Reaction Conditions: 1X HaeIII Methyltransferase Reaction Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

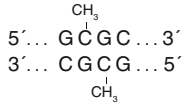
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by HaeIII restriction endonuclease.

Concentration: 10,000 units/ml

Note: HaeIII Methyltransferase protects DNA against cleavage by NotI.

HhaI Methyltransferase

#M0217S 1,000 units



Description: HhaI Methyltransferase modifies the internal cytosine residue (C^5) in the sequence to the left.

Reaction Conditions: 1X rCutSmart Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C.

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.

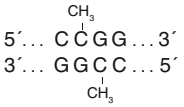
Concentration: 25,000 units/ml

Note: This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

HpaII Methyltransferase

rCutSmart RR SAM 37° 65°

#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: 1X rCutSmart Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C. 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.

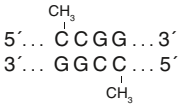
Concentration: 4,000 units/ml

Note: This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

MspI Methyltransferase

RR NEBU SAM 37° No

#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: 1X MspI Methyltransferase Reaction Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 37°C.

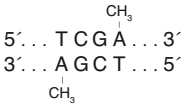
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by MspI restriction endonuclease.

Concentration: 5,000 units/ml

*Taq*I Methyltransferase

rCutSmart RR SAM 65° No

#M0219S 1,000 units



Description: *Taq*I Methyltransferase modifies the adenine residue (N^6) in the sequence to the left.

Reaction Conditions: 1X rCutSmart Buffer + SAM. Supplement with 80 µM S-adenosylmethionine (supplied). Incubate at 65°C.

Note: Activity at 37°C is 25%.

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: This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

Human DNA (cytosine-5) Methyltransferase (Dnmt1)

RR Epi NEBU SAM BSA 37° 65°

See page 286 for more information.

#M0230S 50 units
#M0230L 250 units

Dnmt1 methylates cytosine residues in hemimethylated DNA at 5′...CG...3′. Mammalian Dnmt1 is believed to be involved in carcinogenesis, embryonic development and several other biological functions. The bulk of the methylation takes place during DNA replication in the S-phase of the cell cycle.

For the past 4 years, Greg has worked as a Fermentation Scientist, helping to produce various enzymes and proteins. When not at work, he enjoys reading, traveling and attending sporting events.

RecA & RecA$_f$

RR NEBU 37° 65°

RecA
#M0249S 200 µg
#M0249L 1,000 µg

RecA$_f$
#M0355S 200 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 nucleoprotein filament binds the duplex DNA and searches for a homologous region, (iii) the strands are exchanged.

RecA$_f$ is a N-terminal 6X His tagged recombinant protein.

Reaction Conditions: 1X RecA Reaction Buffer. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Molecular Weight: RecA: 37,973 daltons. RecA$_f$: 38,907 daltons.

Concentration: 2 mg/ml

T4 Gene 32 Protein

RR NEB4 37° 65°

#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*

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: 1X NEBuffer 4. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: Sold by mass of pure protein as determined by OD_{280} (A_{280}=1.184 at 1 mg/ml, 1 cm)

Molecular Weight: 33,485 daltons.

Concentration: 10 mg/ml

ET SSB (Extreme Thermostable Single-Stranded Binding Protein)

RR No

#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 structure*

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} (A_{280} = 0.774 at 1 mg/ml, 1 cm).

Concentration: 500 µg/ml

Molecular Weight: 16 kDa

Usage Note: ET SSB is active in any polymerase buffer. Add 200 ng of ET SSB per 50 µl reaction.

Cloning Plasmids and DNAs

CLONING PLASMID/DNA	NEB #	FEATURES	CONCENTRATION	MW/SIZE	SIZE
pBR322 Vector	N3033S/L	• Commonly used cloning vectors • Amp resistance	1,000 µg/ml	2.83 x 10^6 Da/4,361 bp	50/250 µg
pUC19 Vector	N3041S/L		1,000 µg/ml	1.75 x 10^6 Da/2,686 bp	50/250 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/L	• Commonly used DNA substrate	500 µg/ml	31.5 x 10^6 Da/48,502 bp	250/1,250 µg
Lambda DNA (N^6-methyladenine-free)	N3013S/L		500 µg/ml	31.5 x 10^6 Da/48,502 bp	250/1,250 µg
ϕX174 RF I DNA	N3021S/L	• Commonly used DNA substrate • Covalently closed circular form of ϕX174	1,000 µg/ml	3.5 x 10^6 Da/5,386 bp	30/150 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/L	• Single-stranded viral DNA	1,000 µg/ml	1.7 x 10^6 Da/5,386 bp	50/250 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 of this catalog 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: 1.0 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 (see page 273).

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.

NEW

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.

Concentration: 1 µM

Note: Visit www.neb.com/M0665 for usage guidelines.

Competent Cell Selection Chart for Cloning

	NEB 5-alpha Competent *E. coli* (#C2987)	NEB Turbo Competent *E. coli* (#C2984)	NEB 5-alpha F´ I^q Competent *E. coli* (#C2992)	NEB 10-beta Competent *E. coli* (#C3019)	*dam*$^-$/*dcm*$^-$ Competent *E. coli* (#C2925)	NEB Stable Competent *E. coli* (#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

Need ultra HMW DNA? Check out our new kits for HMW DNA extraction, see page 136.

Monarch kits provide fast and reliable purification of high quality DNA and RNA from a variety of sources using best-in-class silica-column technology. DNA and RNA purified with Monarch kits is highly pure and suitable for use in a wide range of applications, including sequencing, cloning, PCR and other enzymatic manipulations. Monarch kits are all designed with sustainability in mind; wherever possible, they use less plastic and are packaged in responsibly-sourced, recyclable material. For convenience, all Monarch kit components are available separately. Learn more at **NEBmonarch.com**.

PRODUCT	NEB #	SIZE
Monarch Plasmid Miniprep Kit	T1010S/L	50/250 preps
Monarch DNA Gel Extraction Kit	T1020S/L	50/250 preps
Monarch PCR & DNA Cleanup Kit (5 µg)	T1030S/L	50/250 preps
Monarch Genomic DNA Purification Kit	T3010S/L	50/150 preps
Monarch Total RNA Miniprep Kit	T2010S	50 preps
Monarch RNA Cleanup Kit (10 µg)	T2030S/L	10/100 preps
Monarch RNA Cleanup Kit (50 µg)	T2040S/L	10/100 preps
Monarch RNA Cleanup Kit (500 µg)	T2050S/L	10/100 preps
COLUMNS AVAILABLE SEPARATELY		
Monarch Plasmid Miniprep Columns	T1017L	100 columns + tubes
Monarch DNA Cleanup Columns (5 µg)	T1034L	100 columns + tubes
Monarch RNA Purification Columns	T2007L	100 columns + tubes
Monarch gDNA Purification Columns	T3017L	100 columns + tubes
Monarch RNA Cleanup Columns (10 µg)	T2037L	100 columns + tubes
Monarch RNA Cleanup Columns (50 µg)	T2047L	100 columns + tubes
Monarch RNA Cleanup Columns (500 µg)	T2057L	100 columns + tubes


NEW ENGLAND
BioLabs® Inc.

Putting Recycling to Practice

New England Biolabs has always placed stewardship of the environment as one of our highest priorities. We continuously work to promote ecologically sound practices and environmental sustainability, in order to protect our natural resources. This was instilled in us by our Founder, Don Comb, who believed that all companies should share the responsibility of protecting the environment. Don had a passion for ecologically sustainable practices and encouraged employees to find ways to make a difference, both on the NEB campus and in their personal lives.

An undeniable burden that results from life science research is the extensive use of plastics. Its impact on the environment is increasingly at the forefront of the conscientious consumer's mind, particularly as the toxicity to both terrestrial and marine life becomes more evident. Since 1950, nine million tons of plastic have been produced, and it is predicted that only 9% of that colossal amount is recycled, while 12% is incinerated and 79% ends up in landfills. And while researchers are not solely responsible for the overuse of plastic, one cannot deny that we are a major contributor — the increase in precision, speed and convenience makes single-use microfuge and conical tubes, cell culture dishes, pipettes and gloves much more desirable over the alternatives.

Sustainable solutions focusing on reduction can be more effective than relying on the end-user to recycle or reuse. As one example, NEB carefully designed its Monarch® nucleic acid purification kits to reduce the amounts of plastic used. This was achieved by making the walls of the collection tubes thinner and by using appropriately-sized, lighter-weight bottles.

Cold chain shipping has long been a challenge from an environmental standpoint. Many life science reagents require shipment on ice or dry ice, and maintaining shipping temperature is critical. Expanded polystyrene (EPS), commonly referred to as Styrofoam®, has always been the gold standard for cold shipping — it is light, durable, and well-known for its insulative properties. That said, it is difficult to recycle and often ends up in landfills. For over 40 years, NEB has encouraged customers to use the free return label and send their shipping box back to NEB. More recently, we introduced a 100% recyclable ClimaCell® cooler as an alternative to EPS. The packaging liner is made of starch and paper and is designed with thousands of tiny air pockets that maintain the required temperature for the duration of product delivery.

There are other, smaller changes to business practices that can also help. In 1975, NEB printed its first catalog on recycled paper, and uses recycled, Forest Stewardship Council® (FSC®) certified paper for all its printing needs. In 2015, the NEB Catalog became the first carbon neutral-certified catalog produced in the U.S. At NEB headquarters, we manage an extensive recycling program for paper, plastics, EPS, glass, aluminum, batteries and electronics. These are just some examples of how NEB is working to minimize its impact.

In order to continue to address these types of challenges, we realize it is important to communicate and learn from each other. To that end, NEB supports Labconscious (Labconscious.com) — a community of biologists interested in sustainable laboratory practices that will protect nature and enhance lab productivity. Learn more at Labconscious.com.

NEB shipping boxes being prepared for use.
Credit: Mileidy Rodriguez, NEB

Explore the ClimaCell Cooler.

Nucleic Acid Purification

Time for change.

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 spent time with customers to better understand what could be done to improve upon current nucleic acid purification kits. This feedback helped us develop our line of Monarch® Nucleic Acid Purification kits, which have been optimized for maximum performance and minimal environmental impact.

Monarch kits are available for DNA and RNA extraction and cleanup, plasmid purification, and gel extraction. This year, we introduced a novel glass bead-based solution for extraction of high molecular weight DNA to support long read sequencing. Our other Monarch kits utilize unique column designs, which enable the isolation of highly-pure nucleic acids, free from contaminants and often in low volumes. 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.

Let's work together to clean up the world of nucleic acid purification, one prep at a time.

Featured Products

136 Monarch HMW DNA Extraction Kits

135 Monarch Genomic DNA Purification Kit

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Featured Tools & Resources

Visit NEBMonarch.com to access our full line of products for nucleic acid purification as well as associated protocols, videos, FAQs and troubleshooting guides

Make the right 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 technology and a novel glass-bead based workflow for HMW DNA extraction. 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.

Experience exceptional performance and streamlined workflows

- Efficient extraction of high quality DNA and RNA from a variety of samples
- Novel glass bead-based workflow for extraction of HMW DNA
- Simplified DNA and RNA cleanup in low elution volumes
- Enhanced column designs for improved performance
- Fast, user-friendly protocols
- Optimized buffer systems

Choose Monarch Kits for pure value

- Buffers and columns available separately
- No additional shipping or handling charges**
- No hazardous materials fees**
- Competitive pricing

Reduce your impact on the environment

- Less plastic used in product design
- Responsibly sourced and recyclable packaging
- Packaging and protocol cards are printed with water and soy-based inks
- Reusable kit boxes made from post-consumer content

*** In the US and select subsidiary locations. Contact your local distributor for shipping policies.*

Visit www.NEBMonarchPackaging.com for details.

To learn more, visit **NEBMonarch.com**

Monarch Genomic DNA Purification 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 gDNA Purification Columns
#T3017L 100 columns + tubes

Monarch Collection Tubes II
#T2018L 100 tubes

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

Proteinase K, Molecular Biology
#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*

The Monarch Genomic DNA Purification Kit is an excellent complement to the NEBNext Library Preparation products for NGS.

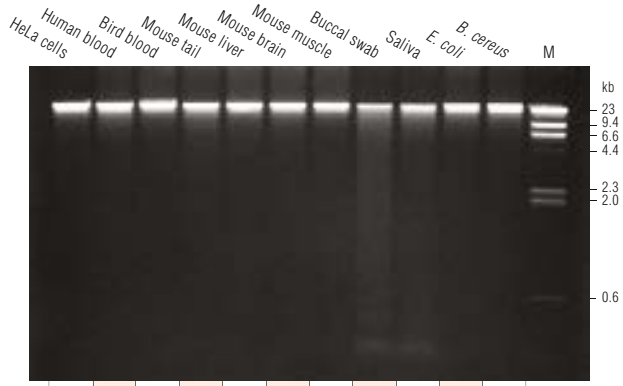
Description: The Monarch Genomic DNA Purification Kit is a comprehensive solution for cell lysis, RNA removal, and purification of intact genomic DNA (gDNA) from a wide variety of biological samples, including cultured cells, blood, and mammalian tissues. Additionally, bacteria and yeast can be processed 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 long-read sequencing platforms.

The Monarch gDNA Purification Kit Includes:

– gDNA Purification Columns
– Collection Tubes II
– gDNA Tissue Lysis Buffer
– gDNA Cell Lysis Buffer
– gDNA Blood Lysis Buffer
– gDNA Binding Buffer
– gDNA Wash Buffer
– gDNA Elution Buffer
– RNase A
– Proteinase K, Molecular Biology

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" (page 342) 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 Genomic DNA Purification 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.*

NEW

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 retainers

Monarch gDNA Nuclei Prep & Lysis Buffer Pack
#T3054L 1 pack

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

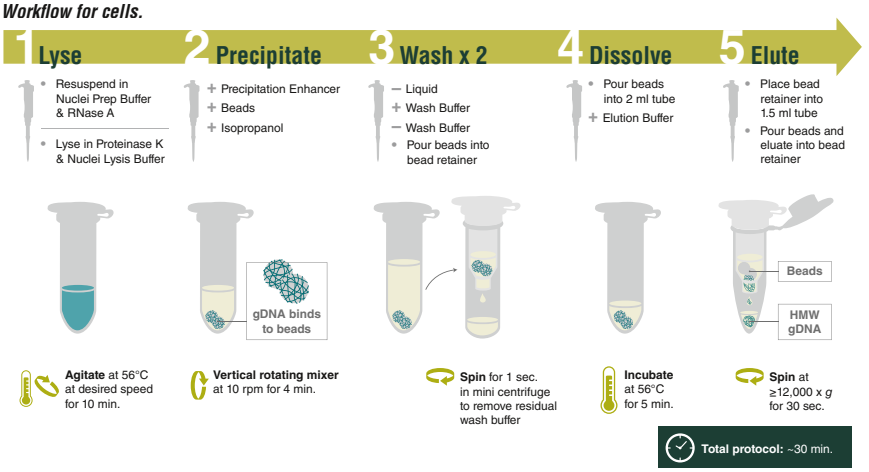
Monarch Precipitation Enhancer
#T3055L 10 ml

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®).

The Monarch HMW DNA Extraction Kits Include:

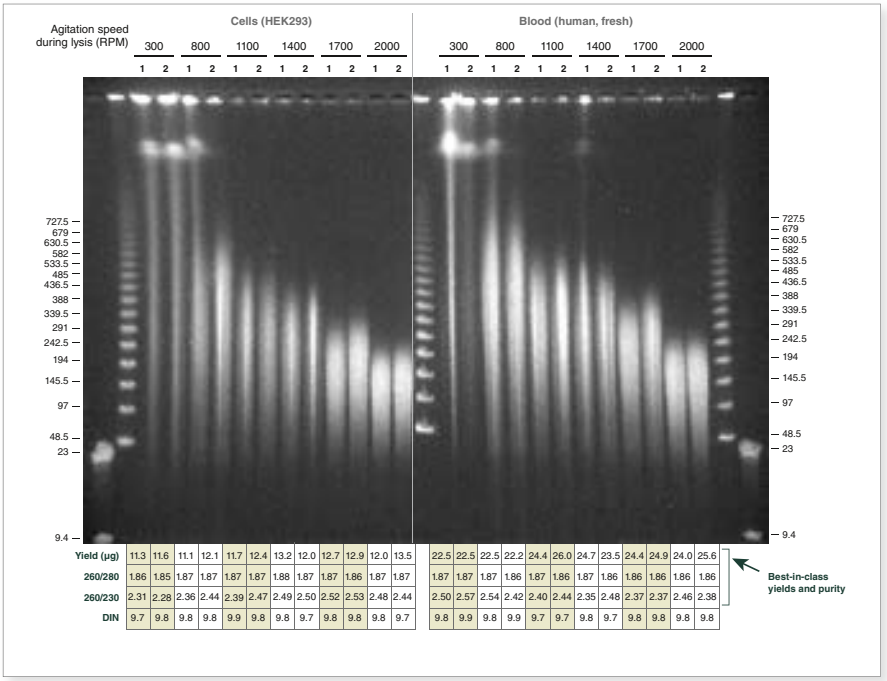
– DNA Capture Beads & Bead Retainers
– 2 ml Tubes & Collection Tubes II
– RNase A
– Proteinase K, Molecular Biology Grade
– RBC Lysis Buffer (NEB #T3050 only)
– gDNA Nuclei Prep & Nuclei Lysis Buffers (NEB #T3050 only)
– Precipitation Enhancer (NEB #T3050 only)
– Protein Separation Solution (NEB #T3060 only)
– Pestles & Pestle Tubes (NEB #T3060 only)
– HMW gDNA Tissue Lysis Buffer (NEB #T3060 only)
– gDNA Wash Buffer & Elution Buffer II

Workflow for cells.



- *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. See page 162 for details.



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 anpurity 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.*

Monarch Total RNA Miniprep Kit

#T2010S 50 preps

Companion Products:

Monarch RNA Purification Columns
#T2007L 100 columns + tubes

Monarch gDNA Removal Columns
#T2017L 100 columns + tubes

Monarch Collection Tubes II
#T2018L 100 tubes

Monarch DNA/RNA Protection Reagent
#T2011L 56 ml

Monarch RNA Lysis Buffer
#T2012L 100 ml

Monarch Total RNA Miniprep Enzyme Pack
#T2019L 1 pack

Monarch RNA Priming Buffer
#T2013L 56 ml

Monarch RNA Wash Buffer
#T2014L 50 ml

Nuclease-free Water
#B1500S 25 ml
#B1500L 100 ml

- *Use with a wide variety of sample types*
- *Purify RNA of all sizes, including miRNA & small RNAs > 20 nucleotides*
- *Includes DNase I, gDNA removal columns, Proteinase K, and a stabilization reagent*
- *Efficiently remove contaminating genomic DNA*
- *Compatible with Qiacube® and KingFisher™ Flex automation platforms*
- *Protocols available for RNA fractionation and RNA cleanup*
- *Save money with value pricing for an all-in-one kit*

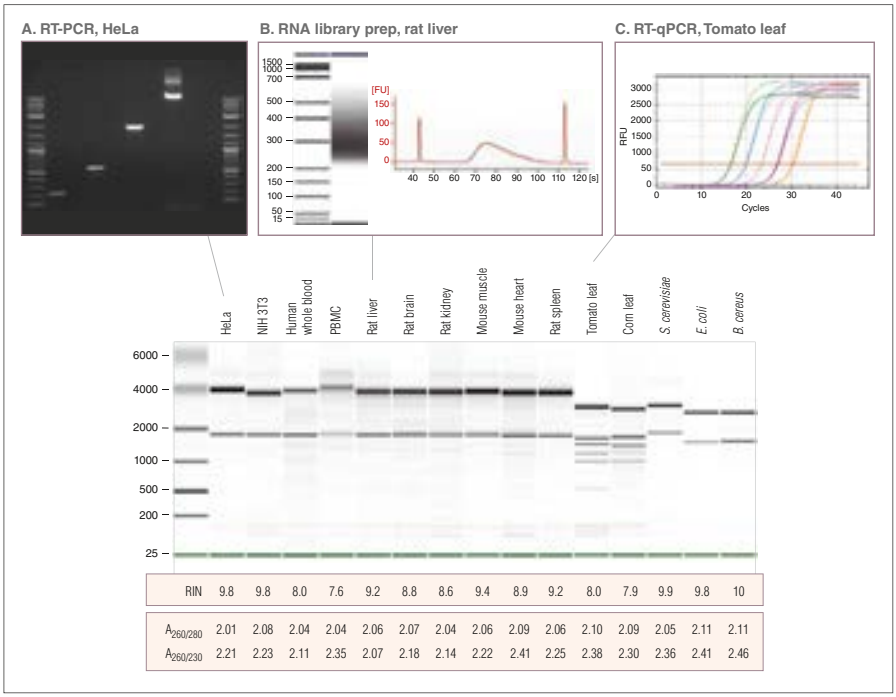
Description: The Monarch Total RNA Miniprep Kit is a comprehensive solution for sample preservation, cell lysis, gDNA removal, and purification of total RNA from a wide variety of biological samples, including cultured cells, blood, and mammalian tissues. Additionally, tough-to-lyse samples, such as bacteria, yeast, and plant, can be processed with additional steps that enhance lysis. Total RNA, including viral RNA, can also be extracted from clinically-relevant sample like saliva, and swabs (buccal, nasopharyngeal, etc.). Cleanup of enzymatic reactions or purification of RNA from TRIzol® -extracted samples is also possible using this kit. Purified RNA has high quality metrics, including $A_{260/280}$ and $A_{260/230}$ ratios > 1.8, high RIN scores, and minimal residual gDNA. Captured RNA ranges in size from full-length rRNAs down to intact miRNAs. Additionally, differential binding conditions allow selective capture or exclusion of the sub-200 nucleotide RNA pool that includes miRNA, 5S rRNA, and tRNA. Purified RNA is suitable for downstream applications, such as RT-qPCR, cDNA synthesis, RNA-seq, Northern blot analysis, etc.

The Monarch Total RNA Miniprep Kit Includes:

– gDNA Removal Columns
– RNA Purification Columns
– Collection Tubes II
– DNA/RNA Protection Reagent (2X)
– RNA Lysis Buffer
– Proteinase K
– Proteinase K Resuspension Buffer
– Proteinase K Reaction Buffer
– DNase I
– DNase I Reaction Buffer
– RNA Priming Buffer
– RNA Wash Buffer (5X)
– 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 10^7 cells or 50 mg tissue*
Elution Volume	30–100 µl
Yield	varies depending on sample type
Compatible downsteam applications	RNA Library prep for NGS, RT-PCR, RT-qPCR, Northern blots

*See "Guidelines for Choosing Sample Input Amounts" (page 344) or at **www.neb.com/MonarchRNAInputs**.



Monarch-purified RNA is high-quality and compatible with a wide variety of downstream applications. *Total RNA from a broad array of sample types was purified using the Monarch Total RNA Miniprep Kit. Aliquots were run on an Agilent Bioanalyzer® 2100 using the Nano 6000 RNA chip (S. cerevisiae RNA was run using a plant Nano assay). RIN values and O.D. ratios confirm the overall integrity and purity of the RNA. To demonstrate compatibility with downstream applications, samples were subsequently used for RT-PCR (+/– RT) (A) for detection of 4 different RNA species using Protoscript® II Reverse Transcriptase/LongAmp Taq DNA Polymerase, NGS library prep (B) using NEBNext® Ultra™ II RNA Library Prep Kit and RT-qPCR (C) using Luna® One-Step RT-qPCR Reagents.*

View tips for successful RNA purification using Monarch Total RNA Miniprep Kit.

Monarch RNA Cleanup Kits

Monarch RNA Cleanup Kit (10 µg)
#T2030S 10 preps
#T2030L 100 preps

Monarch RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

Monarch RNA Cleanup Kit (500 µg)
#T2050S 10 preps
#T2050L 100 preps

Companion Products:

Monarch RNA Cleanup Columns (10 µg)
#T2037L 100 columns + tubes

Monarch RNA Cleanup Columns (50 µg)
#T2047L 100 columns + tubes

Monarch RNA Cleanup Columns (500 µg)
#T2057L 100 columns + tubes

Monarch Collection Tubes II
#T2018L 100 tubes

Monarch RNA Cleanup Binding Buffer
#T2041S 80 ml

Monarch RNA Cleanup Wash Buffer
#T2042S 40 ml

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*
- *Compatible with Qiacube® and KingFisher™ Flex automation platforms*

Great for RNA cleanup following *in vitro* transcription with HiScribe™ Kits, see pages 185–187.

Description: The Monarch 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 swabs (buccal, nasopharyngeal, etc.). The Monarch 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).

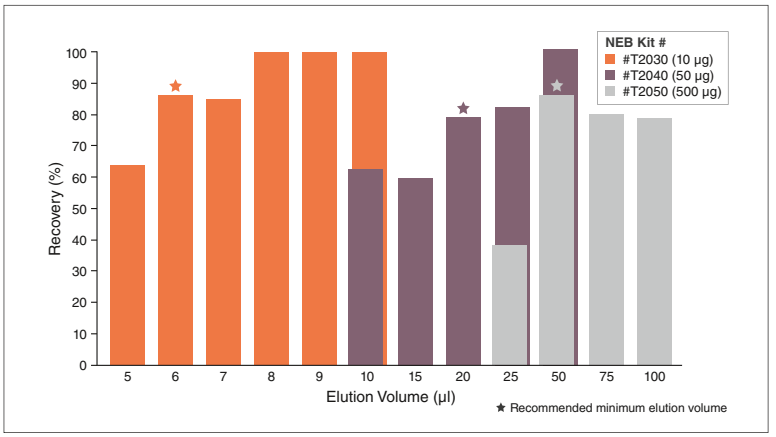
Applications:

- RNA cleanup and concentration (including from the TRIzol aqueous phase)
- Enzymatic reaction cleanup
- *In vitro* transcription cleanup
- Total RNA extraction from some samples
- RNA gel extraction
- RNA fractionation

The Monarch RNA Cleanup Kits Include:

– RNA Cleanup Columns (10, 50 or 500 µg)
– RNA Cleanup Binding Buffer
– RNA Cleanup Wash Buffer
– Collection Tubes II
– Nuclease-free Water

Monarch 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	6–20 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 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 A_{260} as measured using a Trinean® DropSense® 16. ~80% of RNA can be efficiently recovered in 6 µl from the Monarch RNA Cleanup Kit (10 µg, NEB #T2030), 20 µl from the Monarch RNA Cleanup Kit (50 µg, NEB #T2040), and 50 µl from the Monarch RNA Cleanup Kit (500 µg, NEB #T2050).*

View tips for successful purification using the Monarch RNA Cleanup Kits.

Monarch PCR & DNA Cleanup Kit (5 µg)

#T1030S 50 preps
#T1030L 250 preps

Companion Products:

Exo-CIP Rapid PCR Cleanup Kit
#E1050S 100 reactions
#E1050L 400 reactions

Q5 High-Fidelity DNA Polymerase
#M0491S 100 units
#M0491L 500 units

Monarch DNA Cleanup Columns (5 µg)
#T1034L 100 columns + tubes

Monarch DNA Cleanup Binding Buffer
#T1031L 175 ml

Monarch DNA Wash Buffer
#T1032L 25 ml

Monarch DNA Elution Buffer
#T1016L 25 ml

Monarch Plasmid Miniprep Kit
#T1010S 50 preps
#T1010L 250 preps

Monarch DNA Gel Extraction Kit
#T1020S 50 preps
#T1020L 250 preps

Monarch Genomic DNA Purification Kit
#T3010S 50 preps
#T3010L 150 preps

- *Elute in as little as 6 µl*
- *Prevent buffer retention and salt carry-over with optimized column design*
- *Purify small DNA and oligos with a slight protocol modification*
- *Save time with fast, user-friendly protocol*
- *Purchase optimized kit formats or buffers & columns separately for your convenience*

With the Monarch PCR & DNA Cleanup Kit, you can purify your DNA in as little as 5 minutes.

Prefer an enzymatic cleanup approach? Check out the Exo-CIP Rapid PCR Cleanup Kit (page 87).

SYBR® is a registered trademark of Molecular Probes, Inc.

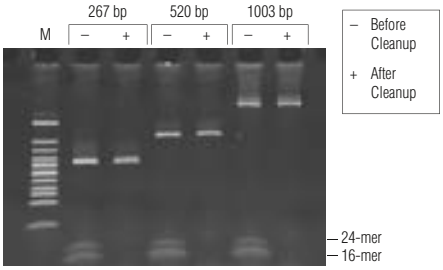
Description: The Monarch PCR & DNA Cleanup Kit (5 µg) is a rapid and reliable method for the purification and concentration of up to 5 µg of high-quality, double-stranded DNA from enzymatic reactions such as PCR, restriction digestion, ligation and reverse transcription. This method employs a bind/wash/elute workflow with minimal incubation and spin times, resulting in purification in less than 5 minutes. DNA Cleanup Binding Buffer is used to dilute the samples and ensure they are compatible for loading onto the proprietary silica matrix under high salt conditions. The DNA Wash Buffer ensures enzymes, short primers (≤ 40 nt), detergents and other low-molecular weight reaction components (e.g., nucleotides, DMSO, betaine) are removed, thereby allowing low-volume elution of concentrated, high-purity DNA. Eluted DNA is ready for use in restriction digests, DNA sequencing, ligation and other enzymatic manipulations. The unique column design ensures no buffer retention and no carryover of contaminants, allowing elution of sample in volumes as low as 6 µl. A slight protocol modification enables purification of small DNA and oligonucleotides.

Applications:

- PCR cleanup
- Enzymatic reaction cleanup
- cDNA cleanup
- Labeling cleanup
- Plasmid cleanup
- Oligonucleotide cleanup

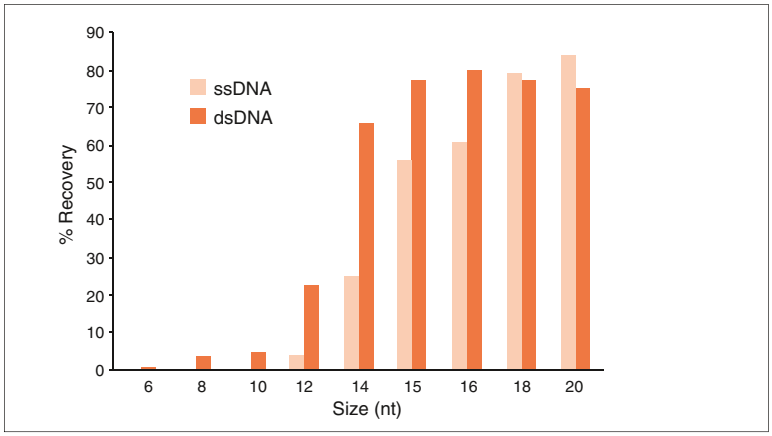
The Monarch PCR & DNA Cleanup Kit Includes:

– DNA Cleanup Columns (5 µg)
– DNA Cleanup Binding Buffer
– DNA Wash Buffer
– DNA Elution Buffer
– Collection Tubes



Monarch PCR & DNA Cleanup Kit (5 µg) removes low molecular weight primers from dsDNA samples. *Three independent amplicons (267 bp, 520 bp, 1003 bp) were spiked with two oligonucleotides (16-mer, 24-mer) to a final concentration of 1 µM. Half of each mix was purified with the Monarch PCR & DNA Cleanup Kit (5 µg) following the included protocol. Equivalent fractions of the original mixture and the eluted material were resolved on a 20% TBE acrylamide gel at 100V for one hour and stained with SYBR® Green II.*

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	≥ 6 µl
Typical Recovery	DNA 50 bp–10 kb 70–90% DNA 11–23 kb 50–70% ssDNA ≥ 18 nt and dsDNA ≥ 15 bp 70–85%
Protocol Time:	5 minutes



Recovery of ssDNA and dsDNA oligonucleotides (1 µg) using the Oligonucleotide Cleanup Protocol and the Monarch PCR & DNA Cleanup Kit. *Synthesized ssDNA and dsDNA oligonucleotides (1 µg in 50 µl H2O) of varying lengths (6-20 nt) were purified using the Oligonucleotide Cleanup Protocol and the Monarch PCR & DNA Cleanup Kit (NEB #T1030) and were eluted in 50 µl water. The average percent recovery (n=3) of the oligonucleotides was calculated from the resulting A260 as measured using a Trinean DropSense™ 16. Use of the Oligonucleotide Cleanup Protocol and the Monarch PCR & DNA Cleanup Kit (NEB #T1030) results in the efficient removal of small oligonucleotides (6-12 nt) and > 70% recovery and cleanup of oligonucleotides ≥ 15 bp (dsDNA) or ≥ 18 nt (ssDNA).*

View tips for using the Monarch PCR & DNA Cleanup Kit.

Monarch DNA Gel Extraction Kit

#T1020S 50 preps
#T1020L 250 preps

Companion Products:

β-Agarase I
#M0392S 100 units
#M0392L 500 units

Monarch DNA Cleanup Columns (5 µg)
#T1034L 100 columns + tubes

Monarch Gel Dissolving Buffer
#T1021L 235 ml

Monarch DNA Wash Buffer
#T1032L 25 ml

Monarch DNA Elution Buffer
#T1016L 25 ml

Monarch Plasmid Miniprep Kit
#T1010S 50 preps
#T1010L 250 preps

Monarch PCR & DNA Cleanup Kit (5 µg)
#T1030S 50 preps
#T1030L 250 preps

Monarch Genomic DNA Purification Kit
#T3010S 50 preps
#T3010L 150 preps

- *Elute in as little as 6 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*

With Monarch DNA Cleanup Columns, DNA can be eluted in as little as 6 µl.

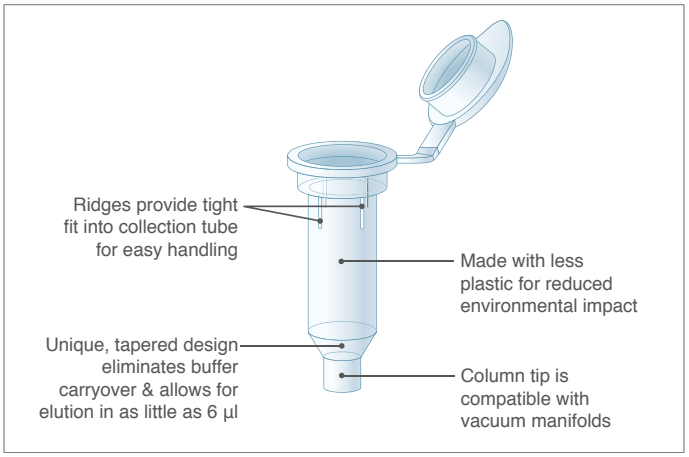
Description: The Monarch DNA Gel Extraction Kit rapidly and reliably purifies up to 5 µg of concentrated high-quality, double-stranded DNA from agarose gels. This method employs a bind/wash/elute workflow with minimal incubation and spin times, resulting in purification in less than 15 minutes. The Monarch Gel Dissolving Buffer is used to digest the agarose gel slice and ensure the sample is compatible for loading the DNA onto the proprietary silica matrix under high salt conditions. The wash buffer ensures trace amounts of DNA binding dyes, electrophoresis buffer salts and gel loading buffer components are removed. Low-volume elution produces concentrated, highly pure DNA ready for use in restriction digests, DNA sequencing, ligation, and other enzymatic manipulations. The unique column design ensures no buffer retention and no carryover of contaminants, allowing elution of sample in volumes as low as 6 µl.

The Monarch DNA Gel Extraction Kit Includes:

– DNA Cleanup Columns (5 µg)
– Gel Dissolving Buffer
– DNA Wash Buffer
– DNA Elution Buffer
– Collection Tubes

SPECIFICATIONS	
Binding Capacity	5 µg
DNA Size Range	50 bp–25 kb
Elution Volume	≥ 6 µl
Typical Recovery	DNA 50 bp–10 kb 70–90% DNA 11–25 kb 50–70%
Protocol Time:	~10 minutes

Monarch DNA Gel Extraction Kit reproducibly recovers DNA over a broad range of molecular weights. *A mixture of 7 DNA fragments ranging from 10 kb down to 0.5 kb was prepared and one-half of the mixture was resolved on a 1% gel. Each fragment was manually excised from the agarose gel and processed using the Monarch DNA Gel Extraction Kit. The entire elution of each fragment was resolved on a new gel with the remainder of the original mixture for comparison.*



Our optimized column design supplied with the Monarch DNA Gel Extraction and PCR & DNA Cleanup Kits enables elution in as little as 6 µl, and eliminates buffer retention

View tips for using the Monarch Gel Extraction Kit.

Monarch Plasmid Miniprep Kit

#T1010S 50 preps
#T1010L 250 preps

Companion Products:

Monarch Genomic DNA Purification Kit
#T3010S 50 preps
#T3010L 150 preps

Exonuclease V (RecBCD)
#M0345S 1,000 units
#M0345L 5,000 units

Monarch Plasmid Miniprep Columns
#T1017L 100 columns + tubes

Monarch Plasmid Resuspension Buffer (B1)
#T1011L 55 ml

Monarch Plasmid Lysis Buffer (B2)
#T1012L 2 x 27 ml

Monarch Plasmid Neutralization Buffer (B3)
#T1013L 110 ml

Monarch Plasmid Wash Buffer 1
#T1014L 2 x 27 ml

Monarch Plasmid Wash Buffer 2
#T1015L 30 ml

Monarch DNA Elution Buffer
#T1016L 25 ml

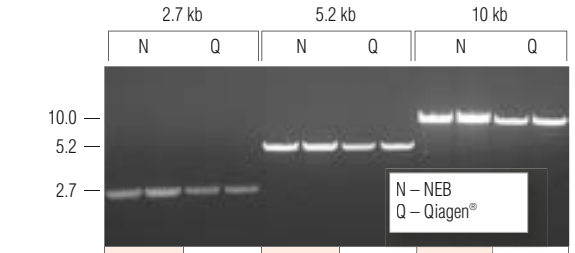
Monarch DNA Gel Extraction Kit
#T1020S 50 preps
#T1020L 250 preps

Monarch PCR & DNA Cleanup Kit (5 µg)
#T1030S 50 preps
#T1030L 250 preps

Description: The Monarch Plasmid Miniprep Kit is a rapid and reliable method for the purification of high-quality plasmid DNA. This method employs standard cell resuspension, alkaline lysis and neutralization steps, with the additional benefit of color indicators at certain steps to easily monitor completion. After clarification of the lysate by centrifugation, the DNA is bound to the proprietary silica matrix under high salt conditions. Unique wash buffers ensure salts, proteins, RNA and other cellular components (endotoxins) are removed, allowing low-volume elution of concentrated, highly pure DNA, ready for use in restriction digests, DNA sequencing, PCR and other enzymatic manipulations.

The Monarch Plasmid Miniprep Kit Includes:

– Plasmid Miniprep Columns
– Plasmid Resuspension Buffer (B1)
– Plasmid Lysis Buffer (B2)
– Plasmid Neutralization Buffer (B3)
– Plasmid Wash Buffer 1
– Plasmid Wash Buffer 2
– DNA Elution Buffer
– Collection Tubes



Conc. (ng/µl)	334.9	202.2	384.2	216.4	607.6	351.0
Total yield (µg)	10.0	10.1	11.5	10.8	18.2	17.6
$A_{260/280}$	1.9	1.9	1.9	1.9	1.9	1.9
$A_{260/230}$	2.4	2.3	2.4	2.4	2.3	2.4

Monarch Plasmid Miniprep Kits consistently yield more concentrated plasmid DNA with equivalent purity and functionality as the leading supplier. *Preps were performed according to recommended protocols using 1.5 ml aliquots of the same overnight culture. One microliter of each prep was digested with HindIII-HF (NEB #R3104) to linearize the vector and the digests were resolved on a 1% w/v agarose gel.*

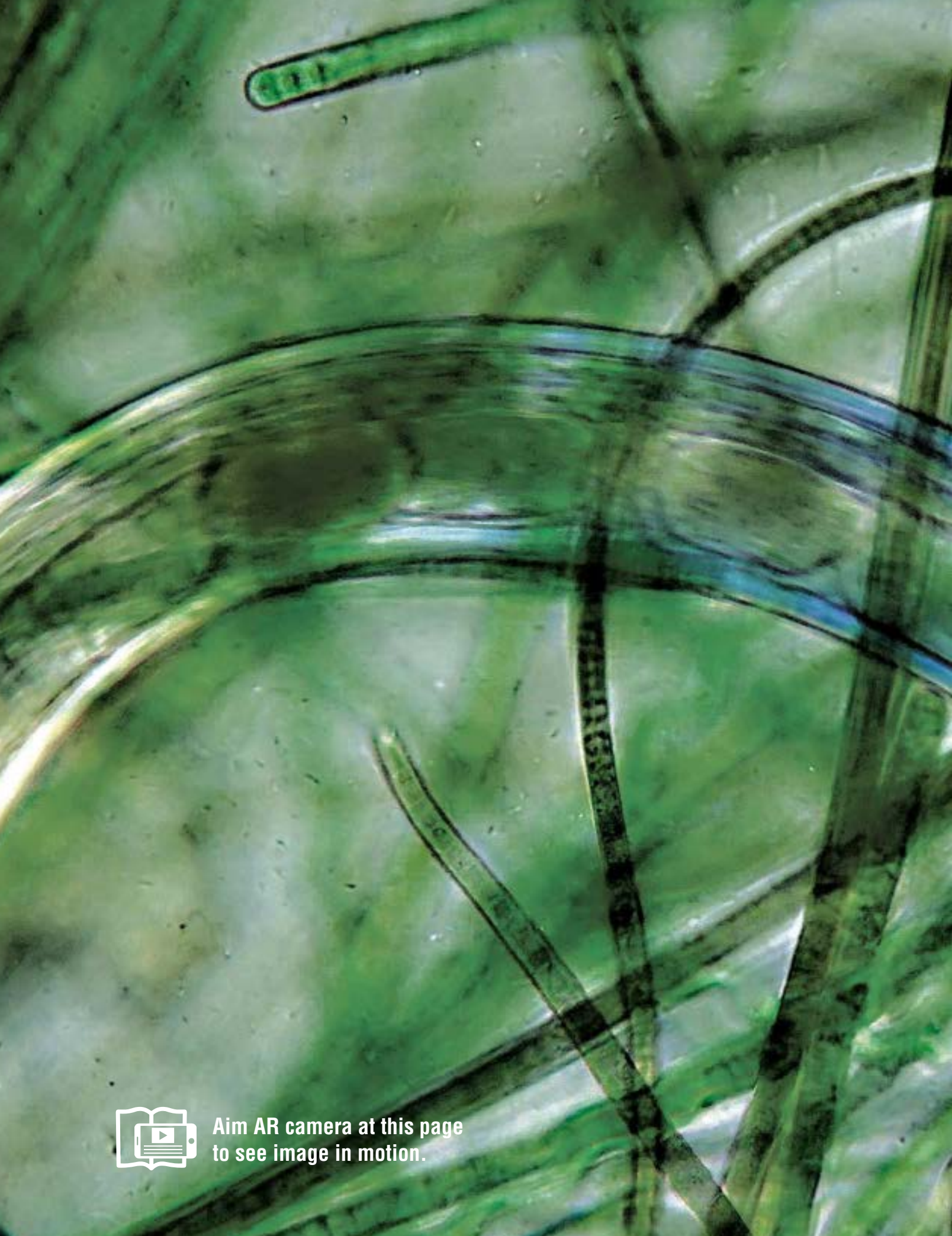
- *Prevent buffer retention and salt carry-over with optimized column design*
- *Monitor completeness of certain steps using colored buffer system*
- *No need to add RNase before starting*
- *Elute in low volumes*
- *Purchase optimized kit formats or buffers & columns separately for your convenience*
- *Easily label columns using tab and frosted surfaces*

Monarch Microfuge Tube EcoRack

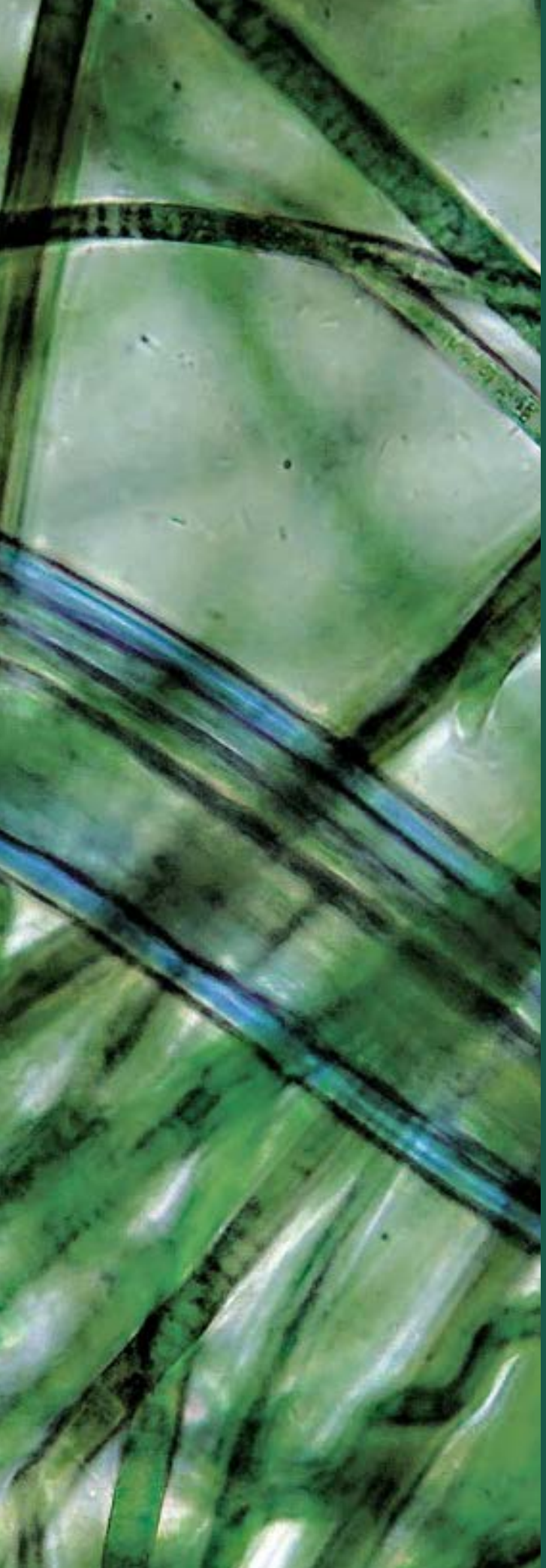
#T5020S 2 racks

Description: The Monarch Microfuge Tube EcoRack is a bench-top tube rack made from plastic recovered during the manufacture of Monarch Nucleic Acid Purification Columns. Plastic that would otherwise be discarded during the injection molding process is recovered and re-molded into this useful lab accessory that can hold up to 48 tubes each side. One side can accommodate tubes 1.5-2 mls and the other can accommodate 0.5 ml tubes.

View tips for using the Monarch Plasmid Miniprep Kit.



Aim AR camera at this page
to see image in motion.



Neglected Tropical Diseases

Neglected Tropical Diseases (NTDs) is the term given to 17 understudied diseases that are collectively the second leading cause of disability worldwide after mental health issues.

Among the NTDs, the diseases caused by filarial nematodes garner the least attention — they are the most understudied of the neglected diseases, yet they threaten the well-being of about 20% of the world's population. Insect vectors harbor eight species of these parasitic worms and transmit them to humans. They cause a range of devastating diseases such as lymphatic filariasis and onchocerciasis, commonly known as elephantiasis and river blindness, respectively. Because they are found in tropical regions and do not affect more developed countries, very little funding is dedicated to basic research or development of diagnostics, vaccines and drugs to ease suffering.

Over the years, Don Comb traveled extensively and saw firsthand the suffering caused by filariasis. Compelled to find a tangible way to make a difference in these burdened communities, he embarked on an altruistic research venture that has had a tremendous impact over the course of 40 years, and continues to forge new ground in an otherwise overlooked health crisis. Don used what he knew best to make a difference — science. He established the NEB parasitology research program in 1980, funding the initiative through profits from NEB's restriction enzyme sales.

NEB's parasitology group is uniquely positioned to identify the gaps needed to move the field forward. They have published hundreds of research papers shedding light on the biology of filarial parasites and their *Wolbachia* endosymbionts, describing advances in genomics, glycomics, vaccine and drug development, and developing diagnostics that can be used in low-resource settings.

There is a great need for simple tools that empower local agencies to perform diagnosis and surveillance, in an effort to control transmission of these devastating diseases. One example of NEB's contribution is the development of loop-mediated isothermal amplification (LAMP) tests for several filarial diseases. LAMP uses a simplified, single-temperature, robust nucleic acid amplification method that does not require sophisticated laboratory equipment. Successful amplification of filarial nucleic acid is determined by an easily visible, pH-based color change. It is suited for use in the field — no specialized lab equipment is required. Researchers in Africa are currently using LAMP-based tests for various human filarial infections to determine communities at risk, identify people who need treatment and provide information on the impact of the drugs used to control transmission. The NEB parasitology group also played a significant role in developing new antibiotic regimens, in use or in clinical trials, which target the *Wolbachia* endosymbiont of filarial parasites.

Don was a humanitarian at heart and wanted to see change happen where it is needed most. NEB's parasitology research program has bridged the gap between the bench and the field and has made significant contributions that continue to help make a difference to those suffering in neglected communities.

View parasitic nemotode in motion.

Parasitic nemotode in motion.
Credit: shoma81, Adobe Stock

NEBNext® Reagents for Next Generation Sequencing

Leading the way in sample preparation for next generation sequencing.

Library preparation is a critical part of the next generation sequencing workflow; successful sequencing requires the generation of high quality libraries of sufficient yield and quality.

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, for 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 sample preparation for DNA, RNA, ChIP, FFPE, small RNA, single cell and microbiome samples, for use with Illumina®, Oxford Nanopore Technologies®, 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 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 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 Custom RNA Depletion Design Tool** to obtain custom probe sequences.

Visit **www.NEBNext.com** to keep up to date on everything NEBNext.

REAGENTS FOR ILLUMINA SEQUENCING

Product Selection Charts

Ultra II DNA

Methylation Analysis

Ultra II RNA

Reagents for RNA Depletion

Customized RNA Depletion

Single Cell RNA

Small RNA

Modules & Enzymes

Adaptors & Primers

NEBNEXT ARTIC PRODUCTS FOR SARS-COV-2 SEQUENCING

REAGENTS FOR OXFORD NANOPORE TECHNOLOGIES SEQUENCING

Target Enrichment

Immune Sequencing Kits (Human & Mouse)

Library Quantitation

REAGENTS FOR ION TORRENT

REAGENTS SUITABLE FOR ANY PLATFORM

DNA Repair

DNA Separation

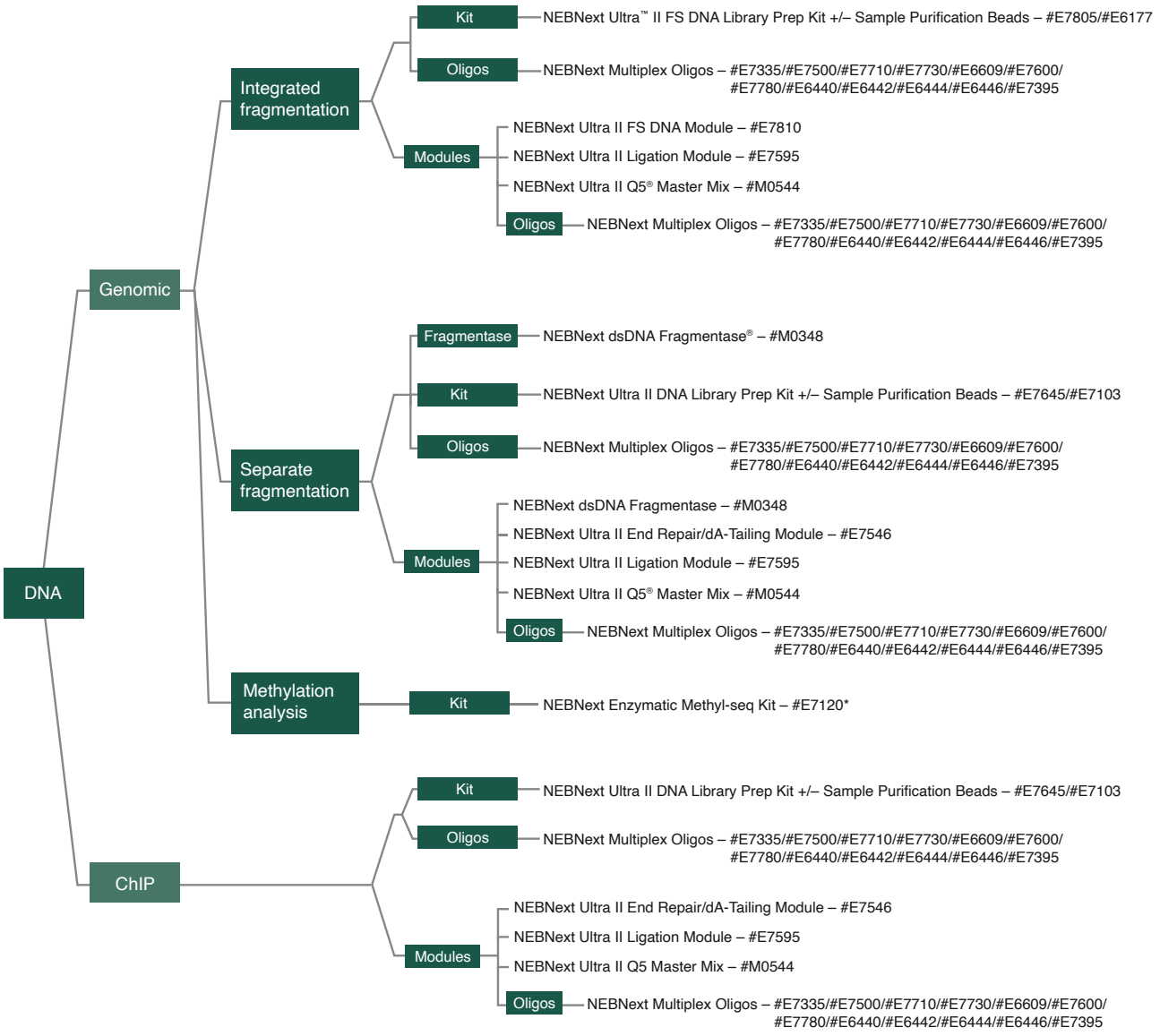
Modules & Enzymes

Magnetic Separation

Illumina® DNA Product Selection Chart

Use the following chart to determine the best NEBNext® products for your Illumina DNA library prep needs. For the most up-to-date product and pricing information, visit **NEBNext.com**.

For help selecting products, try our online product selection tool at **NEBNextSelector.neb.com**

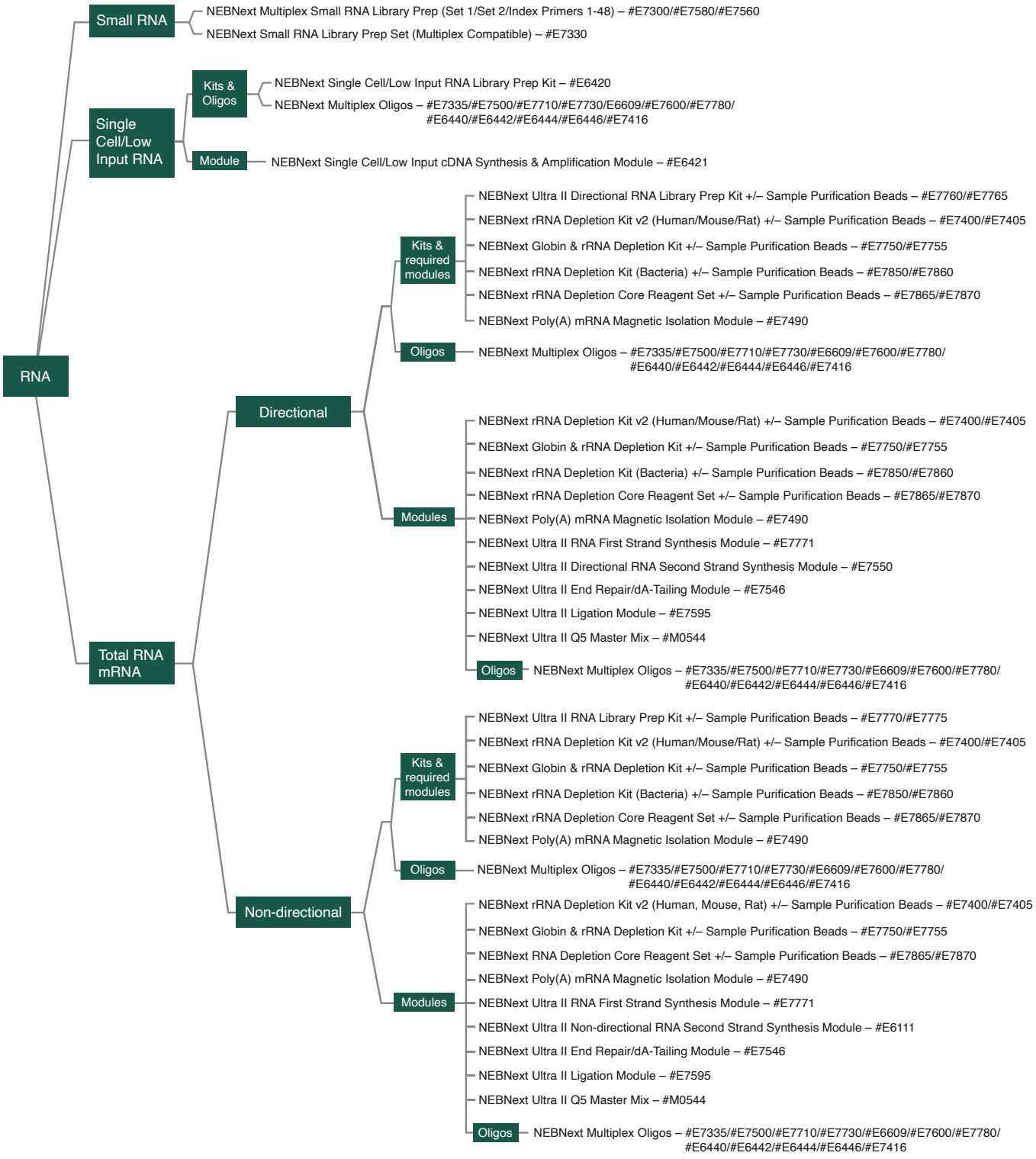


** Module and EM-seq Oligos also available.*
Reagents for original Ultra workflow and standard workflow are also available. See ordering information.

Illumina RNA Product Selection Chart

Use the following chart to determine the best NEBNext products for your Illumina RNA sequencing needs. For the most up-to-date product and pricing information, visit **NEBNext.com**.

For help selecting products, try our online product selection tool at **NEBNextSelector.neb.com**



Reagents for original Ultra workflow and standard workflow are also available. See ordering information.

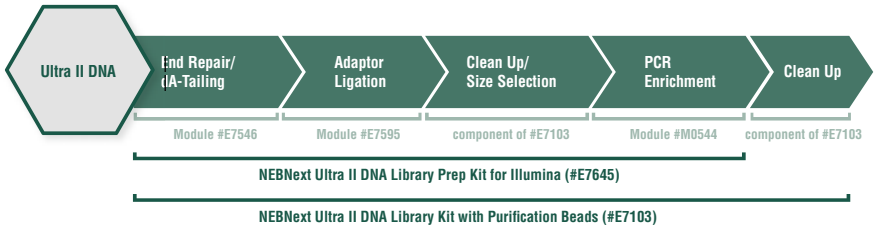
The heart of the matter – NEBNext® Ultra™ II Workflow

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.

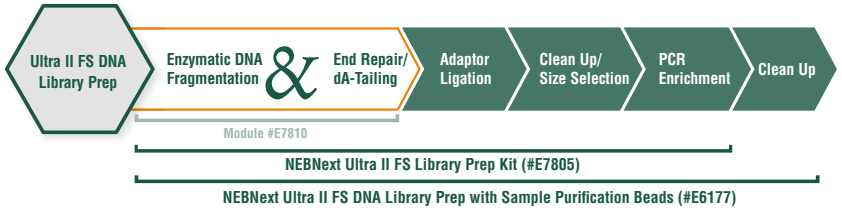
The NEBNext Ultra II workflow lies at the heart of NEB's portfolio for next generation sequencing library preparation. NEBNext Ultra II kits and modules for Illumina are the perfect combination of reagents, optimized formulations and simplified workflows, enabling you to generate DNA or RNA libraries 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:

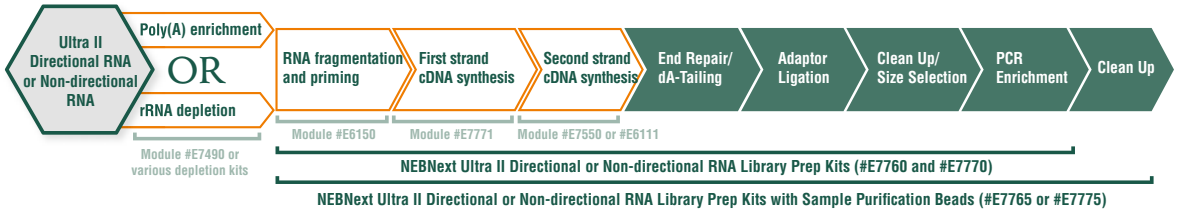


- *Hands on time: < 15 minutes*
- *Total time: ~ 2:30 – 3:00 hrs*



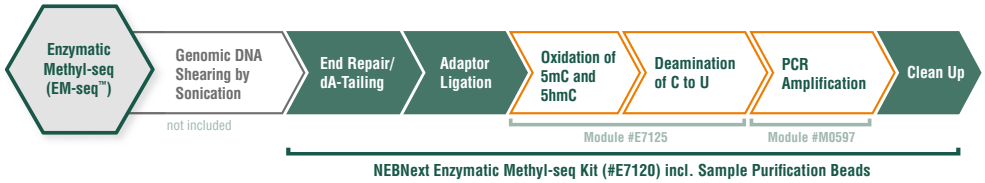
Ultra II DNA FS input amounts:
100 pg – 0.5 µg unsheared DNA

- *Hands on time: < 15 minutes*
- *Total time: ~ 2:30 hrs*



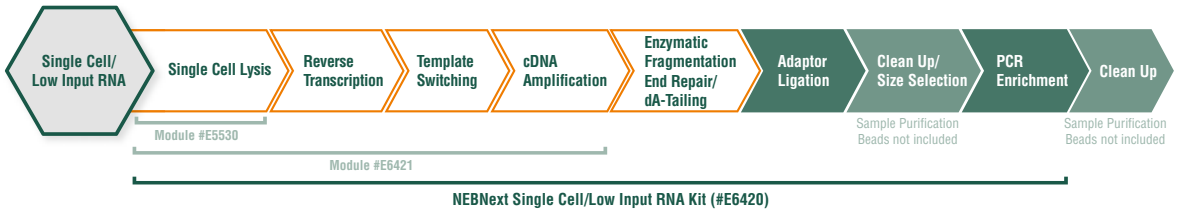
Ultra II RNA input amounts:
5 ng – 1 µg total RNA (rRNA depletion)
10 ng – 1µg total RNA (poly(A) mRNA)

- *Hands on time: < 30 minutes*
- *Total time: ~ 5:30 – 6:30 hrs*



EM-seq input amounts:
10 ng – 200 ng

- *Total time: 6 hrs – 7 hrs*



Single Cell/Low Input RNA input amounts:
2 pg – 200 ng total RNA

- *Hands on time: < 30 minutes*
- *Total time: 6 hrs – 7 hrs*

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 FS DNA Library 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

NEW
NEBNext Ultra II DNA PCR-Free Library Prep Kit for Illumina
#E7410S 24 reactions
#E7410L 96 reactions

NEW
NEBNext Ultra II DNA PCR-Free Library Prep with Sample Purification Beads
#E7415S 24 reactions
#E7415L 96 reactions

NEW
NEBNext Ultra II FS DNA PCR-Free Library Prep Kit for Illumina
#E7430S 24 reactions
#E7430L 96 reactions

NEW
NEBNext Ultra II FS DNA PCR-Free Library Prep with Sample Purification Beads
#E7435S 24 reactions
#E7435L 96 reactions

See ordering information for NEBNext Ultra II DNA modules.

- *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*

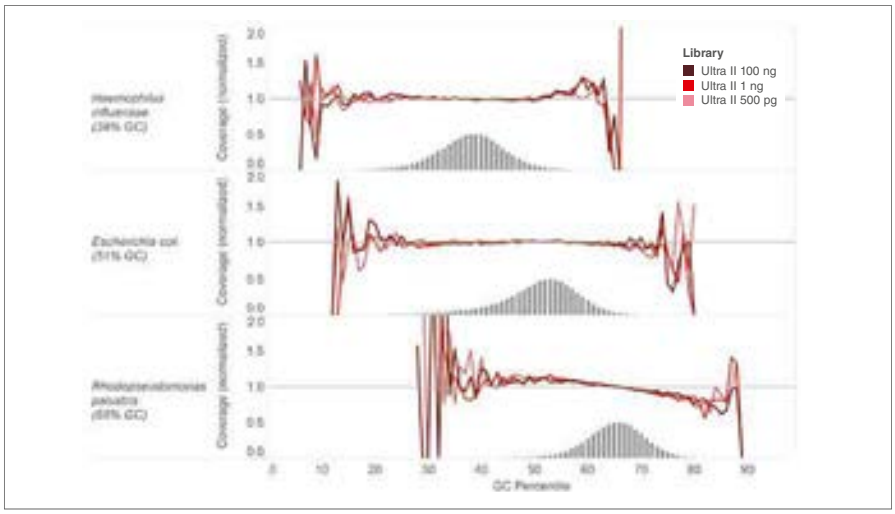
Visit **NEBNextUltraII.com** for more information, including our technical notes and protocol videos

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 500 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.

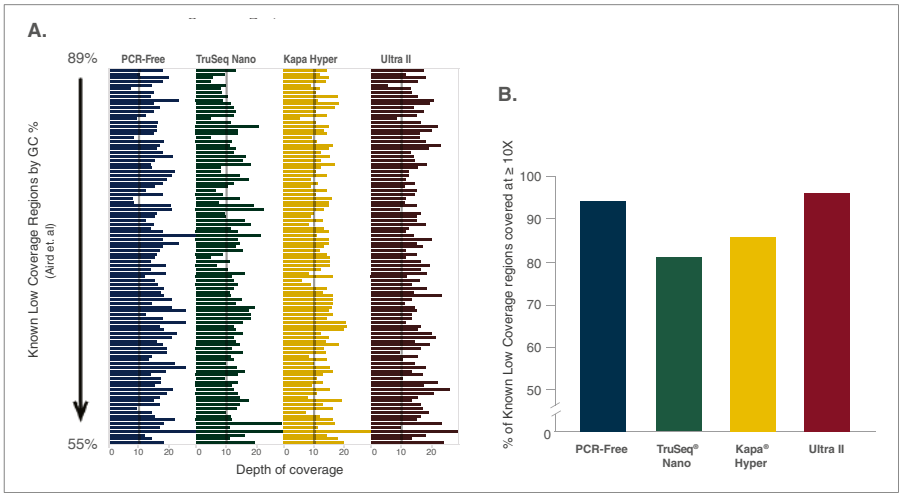
The Ultra II FS DNA Library Prep Kit combines robust enzymatic DNA fragmentation with end repair and dA-tailing, integrated into a streamlined library prep workflow.

PCR-free kits are now available for both the Ultra II DNA and Ultra II FS DNA workflows.

All Ultra II kits are available with or without SPRIselect® beads.



NEBNext Ultra II provides uniform GC coverage for microbial genomic DNA over a broad range of GC composition and input amounts. *Libraries were made using 500 pg, 1 ng and 100 ng of the genomic DNAs shown and the Ultra II DNA Library Prep Kit and sequenced on an Illumina MiSeq®. Reads were mapped using Bowtie 2.2.4 and 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 provides the highest and most uniform coverage of difficult sequence regions.
A: *Indexed libraries were prepared from 100 ng of Human NA19240 genomic DNA using a PCR-free workflow or the library prep kits shown, following manufacturers' recommendations. The PCR-free library was prepared using NEBNext Ultra II. Libraries were sequenced on the Illumina NextSeq® 500. 420 million reads were randomly extracted from each dataset, to produce 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. The number of reads overlapping distinct difficult, low-coverage regions of the human genome (1) are shown for each library. Ultra II provides the highest and most uniform coverage of these difficult regions, and provides the coverage closest to that obtained with a PCR-free protocol.*

B: *From the 420 million 75 bp reads randomly extracted from each dataset, 10X coverage was expected. The percentage of difficult regions covered at ≥ 10X is shown for each library prep kit and for the PCR-free workflow. Ultra II provides the highest percentage of reads at ≥ 10X coverage and also provides the coverage closest to that obtained with a PCR-free protocol. (1) Aird, D. et al. (2011). Analyzing and minimizing PCR amplification bias in Illumina sequencing libraries. Genome Biology 12(2), R18.*

NEBNext Ultra II DNA Reagents for Illumina Sequencing

NEBNext Ultra II Kits for DNA are available with or without integrated enzymatic DNA fragmentation. Note that adaptors and primers are supplied separately. 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.

Input Ultra II DNA Workflow: 500 pg – 1 µg (PCR); 250 ng – 1,000 ng (PCR-free) Ultra II FS DNA Workflow: 500 pg – 1 µg (PCR); 50 ng – 500 ng (PCR-free)

	Fragmentation	End Repair/dA-Tailing	Adaptor Ligation	Clean Up/ Size Selection	PCR Enrichment	Clean Up	Total Workflow
Ultra II DNA Library Prep Kits		**NEBNext Ultra II DNA Library Prep (NEB #E7645) – with Sample Purification Beads (NEB #E7103)**					**Hands-On** (not including fragmentation) 12 – 13 min **Total** 1.7 – 3.2 hrs
		• Ultra II End Prep Enzyme Mix • Ultra II End Prep Reaction Buffer (10X)	• Ultra II Ligation Master Mix • Ligation Enhancer	• Sample Purification Beads (SPRIselect) (NEB #E7103 only)	• NEBNext Ultra II Q5 Master Mix	• Sample Purification Beads (SPRIselect) (NEB #E7103 only)	
		NEBNext Ultra II DNA PCR-free Library Prep (NEB #E7410) – with Sample Purification Beads (NEB #E7415)					
		• Ultra II End Prep Enzyme Mix • Ultra II End Prep Reaction Buffer (10X)	• Ultra II Ligation Master Mix • Ligation Enhancer	• Sample Purification Beads (SPRIselect) (NEB #E7415 only)			
	NEBNext Ultra II FS DNA Library Prep (NEB #E7805) – with Sample Purification Beads (NEB #E6177)						**Hands-On** (including fragmentation) 12 – 13 min **Total** 1.4 – 3.2 hrs
	• Ultra II FS Enzyme Mix • Ultra II FS Reaction Buffer		• Ultra II Ligation Master Mix • Ligation Enhancer	• Sample Purification Beads (SPRIselect) (NEB #E6177 only)	• Ultra II Q5 Master Mix	• Sample Purification Beads (SPRIselect) (NEB #E6177 only)	
	NEBNext Ultra II FS DNA PCR-free Library Prep (NEB #E7430) – with Sample Purification Beads (NEB #E7435)						
	• Ultra II FS Enzyme Mix • Ultra II FS Reaction Buffer		• Ultra II Ligation Master Mix • Ligation Enhancer	• Sample Purification Beads (SPRIselect) (NEB #E7435 only)			
Ultra II DNA Modules	**NEBNext Ultra II FS DNA Module (NEB #E7810)**						
	• Ultra II FS Enzyme Mix • Ultra II FS Reaction Buffer						
	NEBNext dsDNA Fragmentase® (NEB #M0348)	**NEBNext Ultra II End Repair/dA-Tailing Module (NEB #E7546)**	**NEBNext Ultra II Ligation Module (NEB #E7595)**		**NEBNext Ultra II Q5 Master Mix (NEB #M0544)**		
	• dsDNA Fragmentase • Reaction Buffer v2 • Magnesium Chloride	• Ultra II End Prep Enzyme Mix • Ultra II End Prep Reaction Buffer (10X)	• Ultra II Ligation Master Mix • Ligation Enhancer		• Ultra II Q5 Master Mix		

NEBNext Enzymatic Methyl-seq

NEBNext Enzymatic Methyl-seq Kit
#E7120S 24 reactions
#E7120L 96 reactions

NEBNext Enzymatic Methyl-seq Conversion Module
#E7125S 24 reactions
#E7125L 96 reactions

NEBNext Q5U™ Master Mix
#M0597S 50 reactions
#M0597L 250 reactions

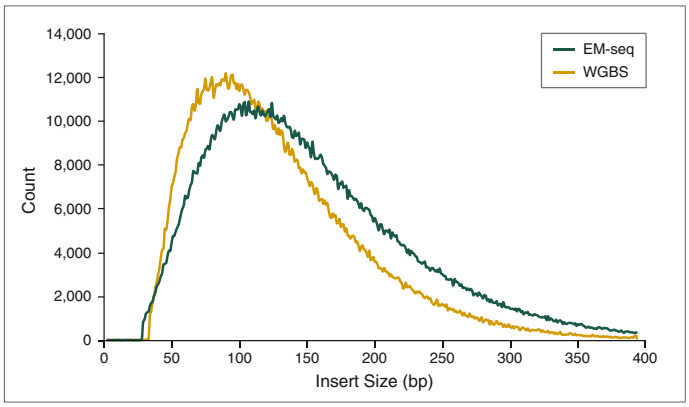
NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)
#E7140S 24 reactions
#E7140L 96 reactions

- *Superior sensitivity of detection of 5mC and 5hmC*
- *Larger library insert sizes*
- *More uniform GC coverage*
- *Greater mapping efficiency*
- *High-efficiency library preparation*

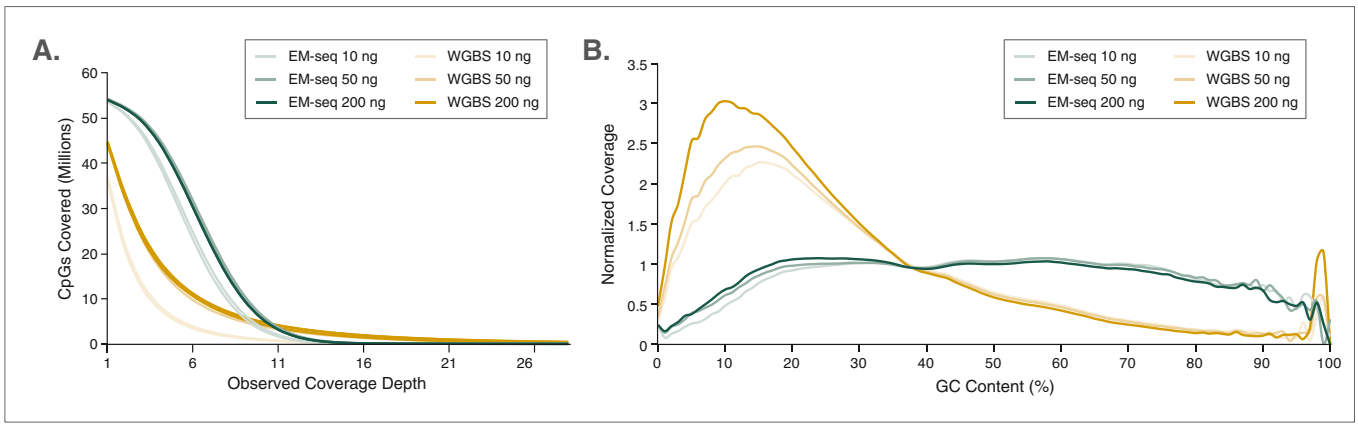
NEBNext Enzymatic Methyl-seq is an enzymatic alternative to bisulfite conversion with superior performance. For more information, including extensive performance data, visit **NEBNext.com**.

Description: While bisulfite sequencing has been the gold standard for the study of DNA methylation, this conversion treatment is damaging to DNA, resulting in DNA fragmentation, loss and GC bias. The NEBNext Enzymatic Methyl-seq Kit (EM-seq™) provides an enzymatic alternative to whole genome bisulfite sequencing (WGBS), combined with high-efficiency streamlined library preparation suitable for Illumina sequencing.

The highly effective EM-seq enzymatic conversion minimizes damage to DNA and, in combination with the supplied NEBNext Ultra II library preparation workflow reagents, results in high quality libraries that enable superior detection of 5mC and 5hmC from fewer sequencing reads.



NEBNext Enzymatic Methyl-seq libraries have larger insert sizes *50 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris® S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold™ kit for bisulfite conversion. Libraries were sequenced on an Illumina MiSeq (2 x 76 bases) and insert sizes were determined using Picard 2.18.14. The normalized frequency of each insert size was plotted, illustrating that library insert sizes are larger for EM-seq than for WGBS, and indicating that EM-seq does not damage DNA as bisulfite treatment does in WGBS.*



EM-seq identifies more CpGs than WGBS, at lower sequencing coverage depth with superior uniformity of GC coverage. *10, 50 and 200 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold Kit for bisulfite conversion. Libraries were sequenced on an Illumina NovaSeq® 6000 (2 x 100 bases). Reads were aligned to hg38 using bwa-meth 0.2.2.*

A: *Coverage of CpGs with EM-seq and WGBS libraries was analyzed using 324 million paired end reads, and each top and bottom strand CpGs were counted independently, yielding a maximum of 56 million possible CpG sites. EM-seq identifies more CpGs at lower depth of sequencing.*

B: *GC coverage was analyzed using Picard 2.17.2 and the distribution of normalized coverage across different GC contents of the genome (0-100%) was plotted. EM-seq libraries have significantly more uniform GC coverage, and lack the AT over-representation and GC under-representation typical of WGBS libraries.*

View EM-seq workflow.

NEBNext FFPE DNA Repair Mix

#M6630S	24 reactions
#M6630L	96 reactions

- *Construct high-quality NGS libraries from FFPE DNA samples*
- *Use upstream of library prep for any NGS platform*
- *No alteration of DNA sequence*
- *Rely on NEB's NGS validation process for FFPE DNA library prep*

Description: Archiving of clinical materials as Formalin-Fixed, Paraffin-Embedded (FFPE) samples is a common practice. However, the methods used for fixation and storage significantly damage and compromise the quality of nucleic acids from these samples. As a result, it can be challenging to obtain useful information, including high quality sequence data, especially when sample amounts are limited. The NEBNext FFPE DNA Repair Mix is a cocktail of enzymes formulated to repair DNA, and specifically optimized and validated for repair of FFPE DNA samples. The FFPE DNA Repair Mix increases library yield and overall library success rates, without introduction of bias.

Functional Validation: Each lot is functionally validated by repair of FFPE DNA followed by library construction and Illumina sequencing.

For the most up-to-date product and pricing information, visit **NEBNext.com**

FFPE DAMAGE TYPE	REPAIRED BY THE FFPE DNA ENZYME REPAIR MIX?
Deamination of cytosine to uracil	Yes
Nicks and gaps	Yes
Oxidized bases	Yes
Blocked 3´ ends	Yes
DNA fragmentation	No
DNA-protein crosslinks	No

Table 1: *Types of FFPE DNA damage and ability to be repaired by the NEBNext FFPE DNA Repair Mix.*

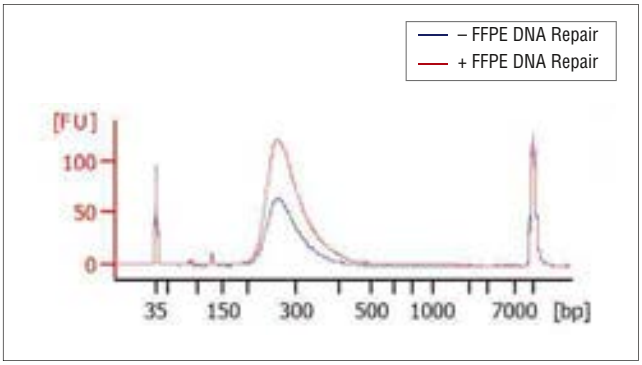


Figure 1: Effect of FFPE DNA Repair Mix on library yields. *An example of Agilent® Bioanalyzer® traces of libraries prepared from stomach tumor FFPE DNA that was treated with the FFPE DNA Repair Mix, or was untreated, before library construction. Yield improvements of 101% to 458% have been observed.*

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

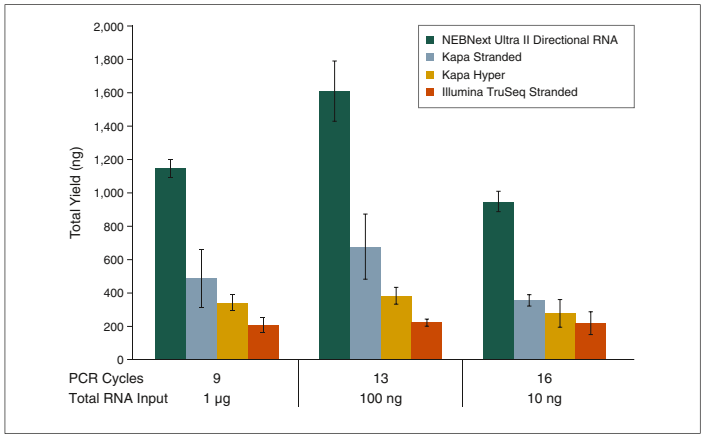
NEBNext Ultra II RNA Library Prep
with Sample Purification Beads
#E7775S 24 reactions
#E7775L 96 reactions

See ordering information for
NEBNext Ultra II RNA modules.

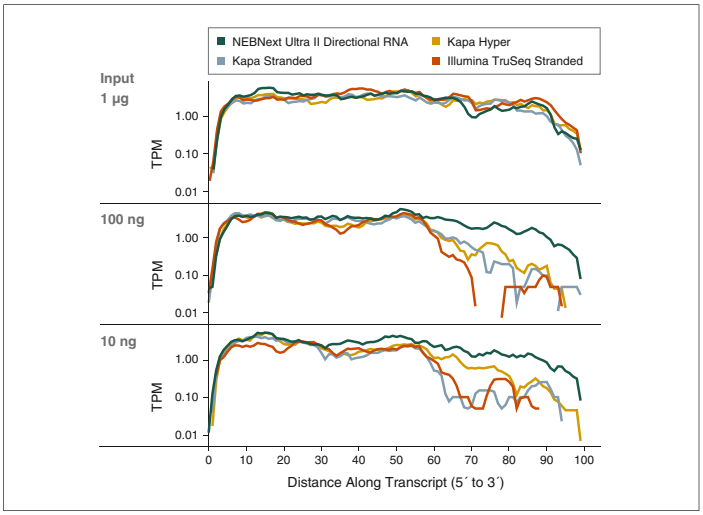
- *Get more of what you need, with the highest library yields*
- *Generate high quality libraries with limited amounts of RNA:*
  - *10 ng–1µg Total RNA (polyA mRNA workflow)*
  - *5 ng–1µg (rRNA depletion workflow)*
- *Minimize bias, with fewer PCR cycles required*
- *Maximize the flexibility to order 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*
  - *Adaptors and primers for multiplexing, 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*

Visit **UltraIIRNA.com** to learn more
and to view performance data

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 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.*



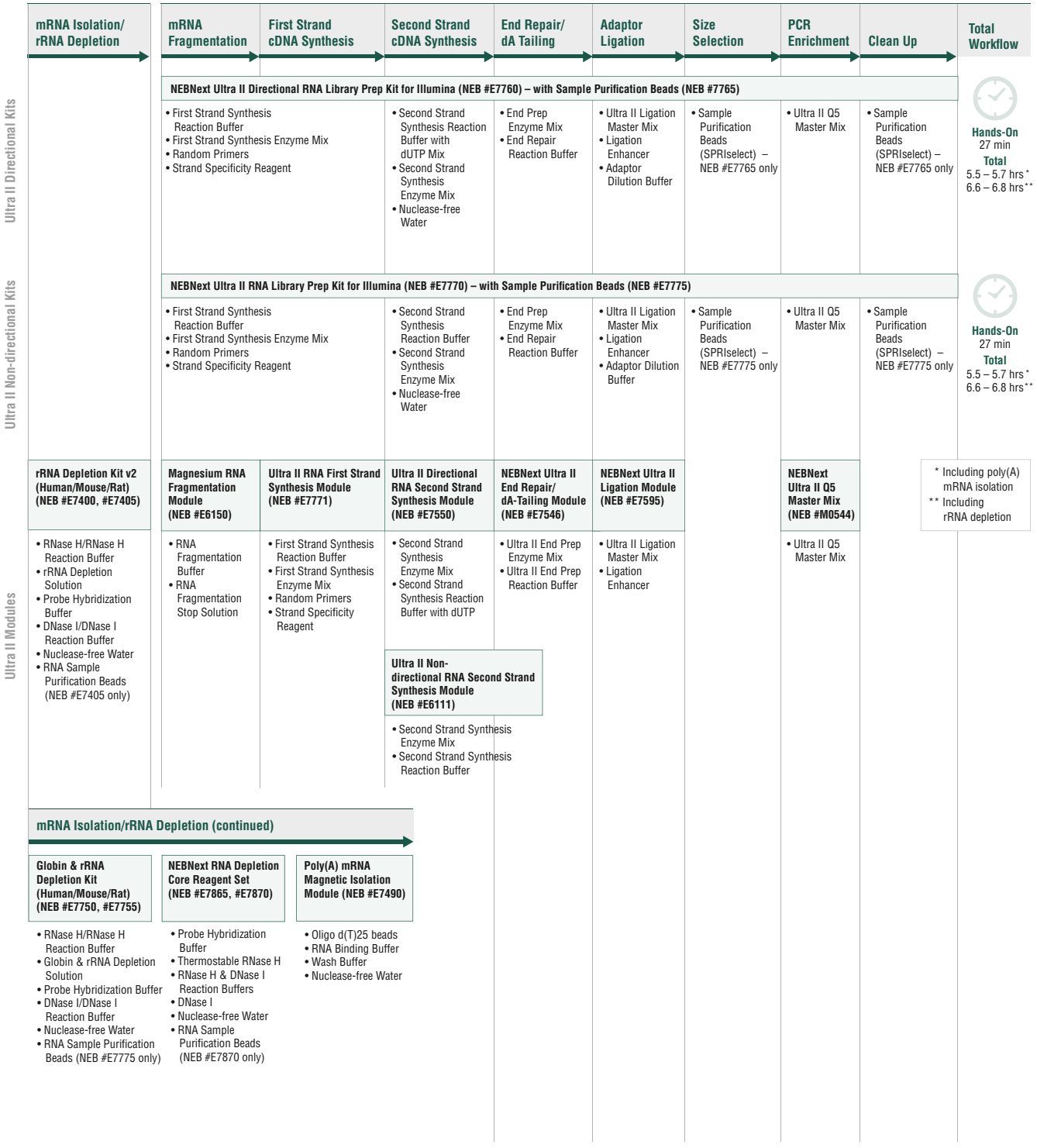
Uniformity of Coverage across the DAM1 transcript. *Poly(A)-containing mRNA was isolated from 10 ng, 100 ng and 1 µg of Universal Human Reference RNA (Agilent #740000) and libraries were made using the NEBNext Ultra II Directional RNA kit, Kapa Stranded mRNA-Seq kit, Kapa mRNA HyperPrep kit and Illumina TruSeq Stranded mRNA Kit. Coverage across transcript ENST00000369541.3 (DAM1) was assessed by mapping reads directly to the transcriptome (Hisat 2.0.3) and assessing coverage using bedtools cov in 100 bins along the transcript length. Libraries prepared using the NEBNext Ultra II Directional RNA Kit provided superior coverage across the transcript at 100 ng and 10 ng input amounts.*

View the NEBNext
Ultra II Directional
RNA Workflow.

NEBNext Ultra II RNA Reagents for Illumina Sequencing

NEBNext Ultra II 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 5 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.

Input Poly(A) mRNA Workflow: 10 ng – 1 µg rRNA Depletion Workflow: 5 ng – 1 µg



NEBNext rRNA Depletion Kits (Human/Mouse/Rat and Bacteria)

NEW
NEBNext rRNA Depletion Kit v2 (Human/Mouse/ Rat)

#E7400S	6 reactions
#E7400L	24 reactions
#E7400X	96 reactions

NEW
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads

#E7405S	6 reactions
#E7405L	24 reactions
#E7405X	96 reactions

NEW
NEBNext rRNA Depletion Kit (Bacteria)

#E7850S	6 reactions
#E7850L	24 reactions
#E7850X	96 reactions

NEW
NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads

#E7860S	6 reactions
#E7860L	24 reactions
#E7860X	96 reactions

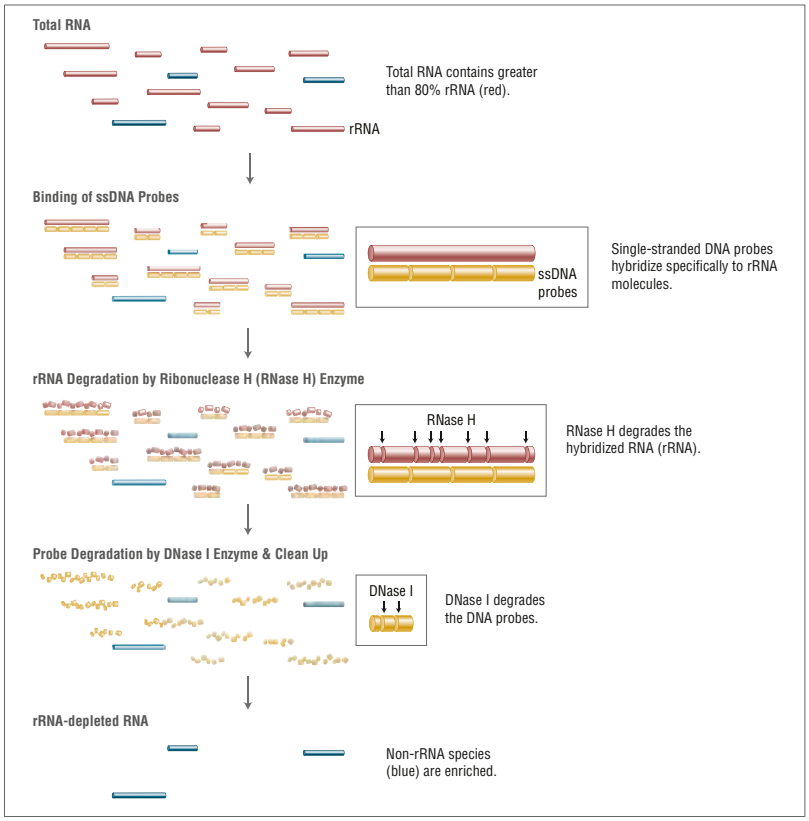
- *Suitable for low-quality (e.g., FFPE) and high-quality RNA*
- *Compatible with a broad range of input amounts: 10 ng–1 µg*
- *Superior depletion of abundant RNAs, with retention of RNAs of interest*
- *Fast workflow: 2 hours, with less than 10 minutes hands-on time*
- *Depleted RNA is suitable for 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.

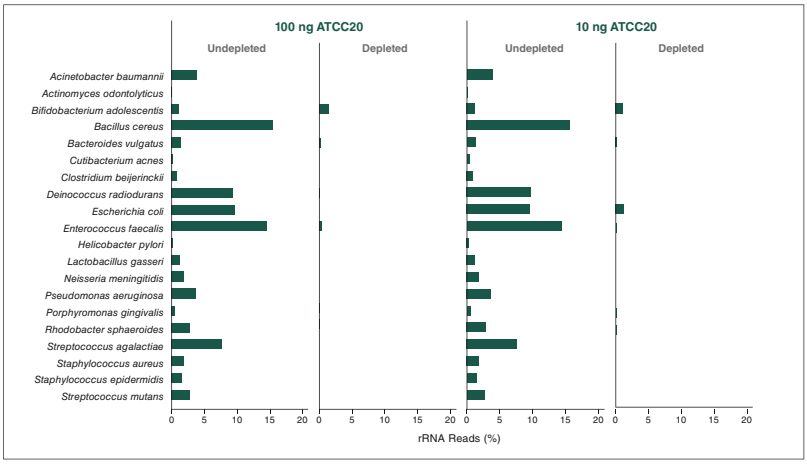
Specific enrichment of bacterial mRNAs is challenging due to their lack of poly(A) tails, precluding the use of oligo d(T)-based enrichment methods. For these samples, specific removal of bacterial rRNAs is an efficient way to enrich for RNAs of interest.

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–1 µg*
- *Superior deletion of abundant RNAs*
- *Fast workflow: 2 hours, with less than 10 minutes hands-on time*

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.

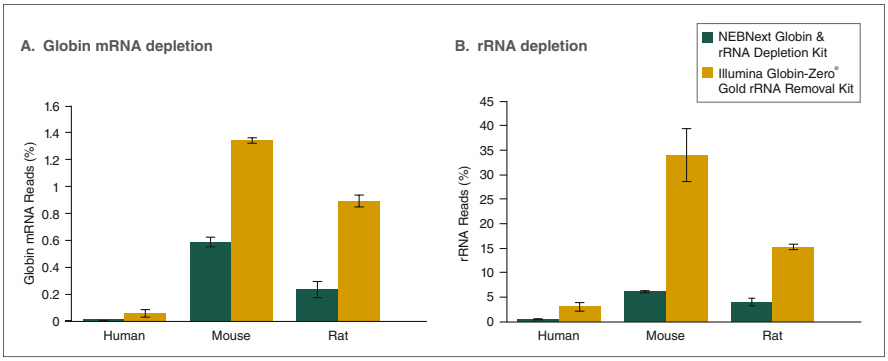
The NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) employs the NEBNext RNase H-based RNA depletion workflow to deplete the following:

- 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.*

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.

The mRNA Magnetic Isolation Module Includes:

– NEBNext Oligo $d(T)_{25}$ Beads
– NEBNext RNA Binding Buffer (2X)
– NEBNext Wash Buffer
– Nuclease-free Water
– NEBNext Tris Buffer

Customized Depletion of Unwanted RNA

NEW

NEBNext RNA Depletion Core Reagent Set

#E7865S	6 reactions
#E7865L	24 reactions
#E7865X	96 reactions

NEW

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*

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/

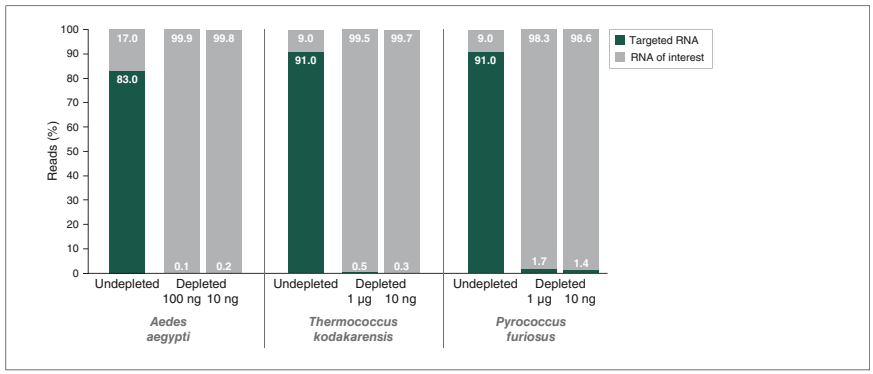
Description: In RNA-seq, highly expressed transcripts with minimal biological interest, such as ribosomal RNA (rRNA) can dominate readouts and mask detection of more informative low-abundance transcripts. This challenge is amplified when working with sample types for which pre-designed RNA depletion 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.

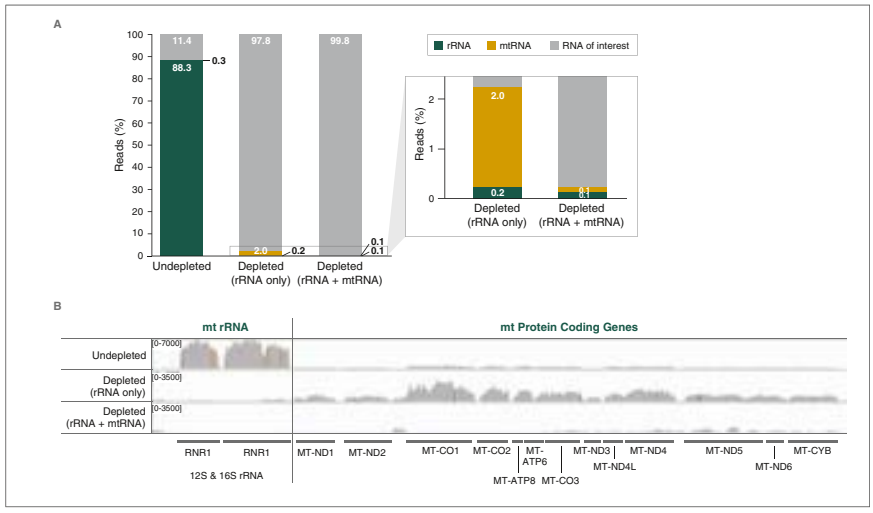
STEP 1: Use the online NEBNext Custom RNA Depletion Design Tool to obtain custom probe sequences, by entering the sequence of your target RNA.

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



NEBNext Custom RNA Depletion enriches for RNAs of interest by efficiently removing targeted RNA from total RNA across species and a wide range of inputs. *The NEBNext Custom RNA Depletion Design Tool was used to design probes against rRNA of the mosquito Aedes aegypti, and the archaeal species Thermococcus kodakarensis and Pyrococcus furiosus. Total RNA (1 µg, 100 ng or 10 ng) was used as input for rRNA depletion using the Core RNA Depletion Reagent Set with the designed probes. RNA-seq libraries were prepared using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina followed by paired-end sequencing (2 x 75 bp). 20 million reads were sampled (seqtk) from each library. Read pairs were identified as ribosomal using mirabait (6 or more shared 25-mers), and levels of rRNA remaining were calculated by dividing matched reads by the total number of reads passing instrument quality filtering. The data represents an average of 3 replicates. The method efficiently depletes targeted rRNA across species and a wide range of total RNA input amount (1 µg–10 ng).*



Combined probe pools efficiently deplete human rRNA and mitochondrial mRNA using NEBNext Custom RNA Depletion. *The NEBNext Custom RNA Depletion Design Tool was used to design probes against human mitochondrial mRNA. The probes were used in combination with the probe pool from the NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat). 1 µg of total Universal Human Reference RNA (Agilent®) was depleted of mitochondrial RNA and rRNA using the Core RNA Depletion Reagent Set. RNA-seq libraries were prepared using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina followed by paired-end sequencing (2 x 75 bp). 20 million reads were sampled (seqtk) from each library.*

A. Read pairs were identified as ribosomal and mitochondrial using mirabait (6 or more, 25-mers), and levels of rRNA and mtRNA remaining were calculated by dividing matched reads by the total number of reads passing instrument quality filtering. Both rRNA and mitochondrial RNA are efficiently depleted.

B. Integrative Genome Viewer (IGV) visualization of read coverage across the human mitochondrial genes.

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

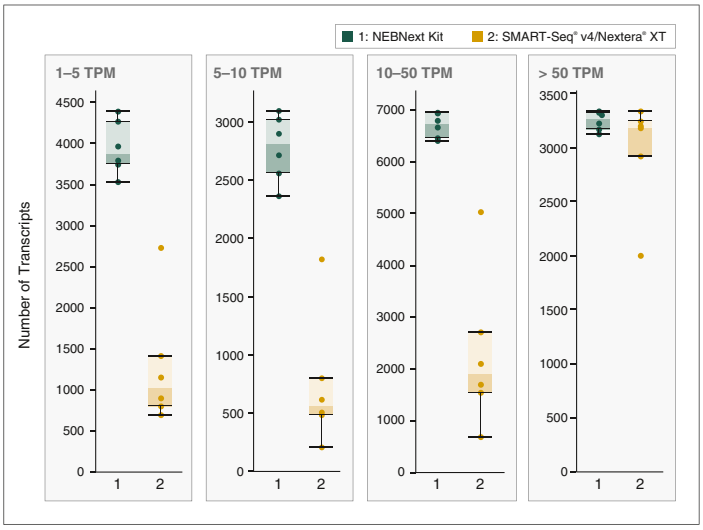
NEBNext Single Cell Lysis Module
#E5530S 96 reactions

- *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*
- *Rely on consistent transcript detection for a wide range of input amounts and sample types*
- *Obtain full-length, uniform transcript coverage, regardless of input amount or sample type*
- *Use with cultured or primary cells, or total RNA*
- *Save time with a fast, streamlined workflow, minimal handling steps and hands-on time*
  - *Single-tube protocol from cell lysis to cDNA*
  - *Enzymatic DNA fragmentation, end repair and dA-tailing reagents in a single enzyme mix, with a single protocol, regardless of GC content*
- *Available with or without library construction reagents*

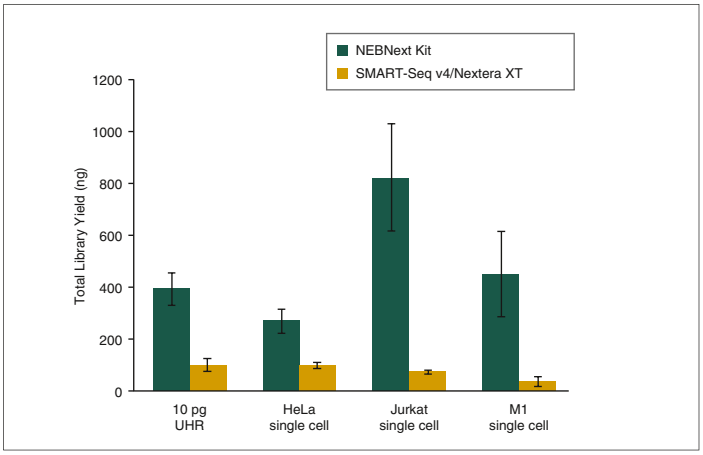
Description: The unique workflow of the NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina meets the demand for a highly sensitive, yet robust method that consistently generates high-quality, full-length transcript sequencing data from a single cell or ultra-low input RNA.

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

For adenylation of custom ssDNA adaptors, the 5´ DNA Adenylation Kit is available (NEB #E2610).

- *Minimized 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*

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)						
• 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	**Hands-On Time** 30 min **Total Time** 6 hrs
NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48) (NEB #E7560)						
• 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	**Hands-On Time** 30 min **Total Time** 6 hrs
NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible) (NEB #E7330)						
• 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	**Hands-On Time** 30 min **Total Time** 6 hrs

NEBNext Magnetic Separation Rack

#S1515S 24 tubes (0.2 ml)

- *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

NEW
NEBNext Multiplex Oligos for Illumina
(Unique Dual Index UMI Adaptors DNA Set 1)
#E7395S 96 rxns (96 indices)
#E7395L 384 rxns (96 indices)

NEW
NEBNext Multiplex Oligos for Illumina
(Unique Dual Index UMI Adaptors RNA Set 1)
#E7416S 96 rxns (96 indices)
#E7416L 384 rxns (96 indices)

NEBNext Multiplex Oligos for Illumina
(96 Unique Dual Index Primer Pairs)
#E6440S 96 rxns (96 indices)
#E6440L 384 rxns (96 indices)

NEW
NEBNext Multiplex Oligos for Illumina
(96 Unique Dual Index Primer Pairs Set 2)
#E6442S 96 rxns (96 indices)
#E6442L 384 rxns (96 indices)

NEW
NEBNext Multiplex Oligos for Illumina
(96 Unique Dual Index Primer Pairs Set 3)
#E6444S 96 rxns (96 indices)
#E6444L 384 rxns (96 indices)

NEW
NEBNext Multiplex Oligos for Illumina
(96 Unique Dual Index Primer Pairs Set 4)
#E6446S 96 rxns (96 indices)
#E6446L 384 rxns (96 indices)

- *Indexing strategies optimized by application*
  - *Index Primers: For NGS Library Prep workflows that include an amplification step*
  - *Index Adaptors: Enablement of 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*

NEBNext Multiplex Oligos for Enzymatic
Methyl-seq (Unique Dual Index Primer Pairs)
#E7140S 24 rxns
#E7140L 96 rxns

NEBNext Multiplex Oligos for Illumina
(Dual Index Primers Set 1)
#E7600S 96 rxns (8 x 12 indices)

NEBNext Multiplex Oligos for Illumina
(Dual Index Primers Set 2)
#E7780S 96 rxns (8 x 12 indices)

NEBNext Multiplex Oligos for Illumina
(Index Primers Set 1)
#E7335S 24 rxns (12 indices)
#E7335L 96 rxns (12 indices)

NEBNext Multiplex Oligos for Illumina
(Index Primers Set 2)
#E7500S 24 rxns (12 indices)
#E7500L 96 rxns (12 indices)

NEBNext Multiplex Oligos for Illumina
(Index Primers Set 3)
#E7710S 24 rxns (12 indices)
#E7710L 96 rxns (12 indices)

NEBNext Multiplex Oligos for Illumina
(Index Primers Set 4)
#E7730S 24 rxns (12 indices)
#E7730L 96 rxns (12 indices)

NEBNext Multiplex Oligos for Illumina
(96 Index Primers)
#E6609S 96 rxns (96 indices)
#E6609L 384 rxns (96 indices)

NEBNext Multiplex Oligos for Illumina
(Methylated Adaptor, Index Primers Set 1)
#E7535S 24 rxns (12 indices)
#E7535L 96 rxns (12 indices)

NEBNext Adaptor Dilution Buffer
#B1430S 1 x 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.

Multiplex Oligos Selection Chart

	 SINGLE INDEX	 DUAL INDEX	 UNIQUE DUAL INDEX	 UNIQUE DUAL INDEX UMIs
NEB PRODUCTS	NEB #E7335 NEB #E7500 NEB #E7710 NEB #E7730 NEB #E6609	NEB #E7600 NEB #E7780	NEB #E6440 NEB #E6442 NEB #E6444 NEB #E6446 NEB #E7140	NEB #E7395 NEB #E7416
Contains UMI	No	No	No	Yes
Addresses Index Hopping	No	No	Yes	Yes
Indexing Strategy	Index Primer	Index Primer	Index Primer	Index Adaptor
Applications	DNA-seq, RNA-seq (except small RNA)	DNA-seq, RNA-seq (except small RNA)	DNA-seq, RNA-seq (except small RNA)	PCR-free DNA-seq, RNA-seq (except small RNA)
Number of Indices for Multiplexing	up to 144	up to 384	up to 384	up to 96
Compatible with EM-seq™	Yes*	Yes*	Yes*	No
Compatible with EpiMark® Bisulfite Sequencing	Yes**	Yes**	Yes**	No
Number of Sets Available; Formats and 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 combinitorial mixing	Four; 96 indices in premixed, foil-sealed 96-well plates, including a version for EM-seq (up to 120 indices, either 96-well plate or 24 vial format)	One; 96 indices in premixed, foil-sealed 96-well plate (DNA-seq OR RNA-seq) and primers

* Requires the use of the EM-seq Adaptor; Single, dual and unique dual index are all compatible; NEB recommends using the Unique Dual Index Primers found in the NEBNext Enzymatic Methyl-seq Kit (NEB #E7120) or the NEBNext Multiplex Oligos for EM-seq (NEB #E7140), both supplied with the NEBNext EM-seq Adaptor; For higher levels of multiplexing, Unique Dual Index Primers Sets 3 and 4 (NEB #E6444 and #E6446) are also validated for EM-seq.

** Requires use of NEBNext EM-seq adaptor from NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs, #E7140S/L or NEBNext methylated adaptor from NEBNext Multiplex Oligos for Illumina® (Methylated Adaptor, Index Primers Set 1, #E7535S/L).

NEBNext ARTIC Products for SARS-CoV-2 Sequencing

NEW
NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina)
#E7658S 24 reactions
#E7658L 96 reactions

NEW
NEBNext ARTIC SARS-CoV-2 Library Prep Kit (Illumina)
#E7650S 24 reactions
#E7650L 96 reactions

NEW
NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies)
#E7660S 24 reactions
#E7660L 96 reactions

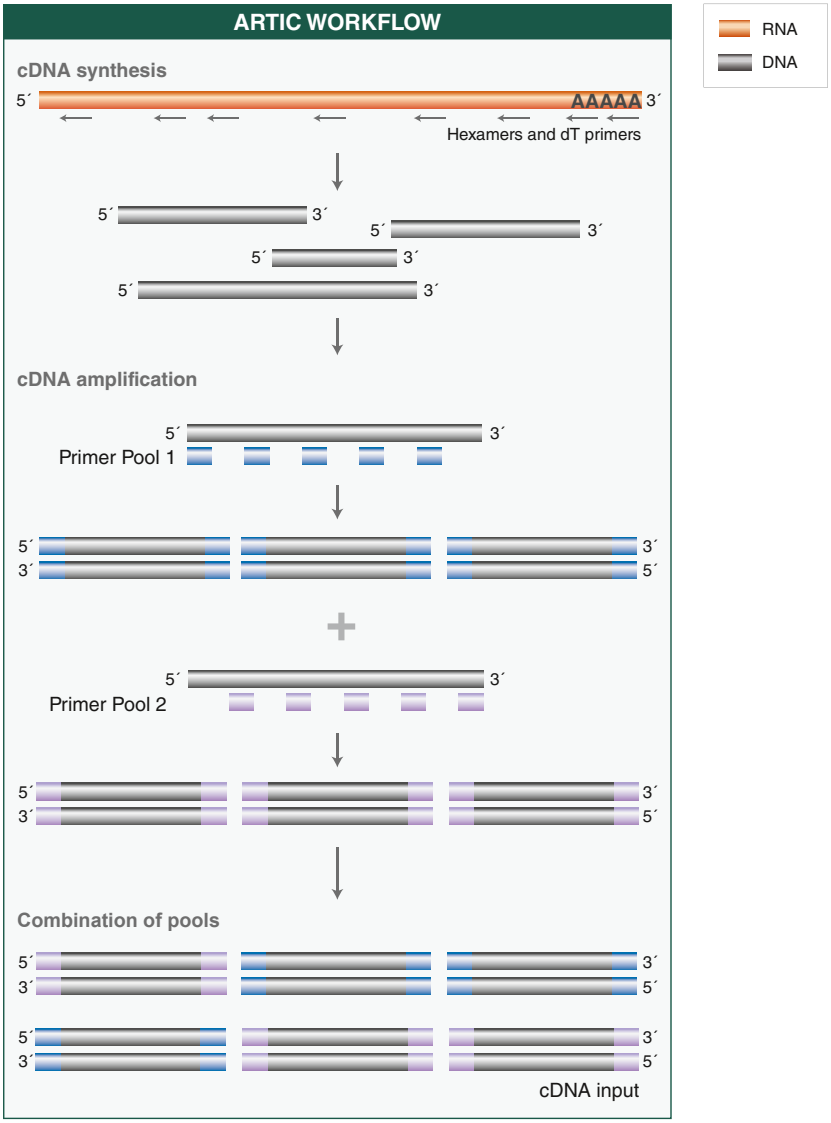
- *Streamlined, high-efficiency protocol*
- *Ample amplicon yields from a wide range of viral genome inputs*
- *Improved SARS-CoV-2 genome coverage depth with a more balanced primer pool*
- *Available for Illumina and Oxford Nanopore Technologies sequencing platforms*
- *No requirement for amplicon normalization prior to Illumina library preparation*

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. Two library prep options are available: 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. The NEBNext ARTIC SARS-CoV-2 Library Prep Kit (Illumina) does not include DNA fragmentation and library inserts are in the 400 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 for Oxford Nanopore Technologies Ligation Sequencing
#E7180S 24 reactions

NEW

NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies)
#E7660S 24 reactions
#E7660L 96 reactions

For adenylation of custom ssDNA adaptors, the 5´ DNA Adenylation Kit is available (NEB #E2610).

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 Genomic DNA Purification Kit
#T3010S 50 preps
#T3010L 150 preps

- *Component volumes tailored for use with many SQK-LSK109 and SQK-LSK110 workflows*
- *Simplified ordering and inventory management*
- *Compatible with all devices: MinION®, GridION®, PromethION™, Flongle®*
- *No unnecessary buffers or excess reagents*

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 for Oxford Nanopore Technologies Ligation Sequencing includes the NEBNext DNA repair, end repair and ligation reagents recommended in Oxford Nanopore Ligation library preparation. These are provided at volumes designed for use in several protocols alongside Oxford Nanopore Technologies SQK-LSK109 and SQK-LSK110.

The NEBNext Companion Module for Oxford Nanopore Technologies Ligation Sequencing Includes:

– NEBNext FFPE DNA Repair Mix (0.048 ml)
– NEBNext FFPE DNA Repair Buffer (0.084 ml)
– NEBNext Ultra II End Prep Enzyme Mix (0.072 ml)
– NEBNext Ultra II End Prep Reaction Buffer (0.084 ml)
– Quick T4 DNA Ligase (0.240 ml)

The NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies) is designed for sequencing of SARS-CoV-2 using the ARTIC protocol and the Oxford Nanopore Technologies platform. Optimized primers and reagents for RT-PCR deliver uniform, ample amplicon yields from gRNA across a wide copy number range.

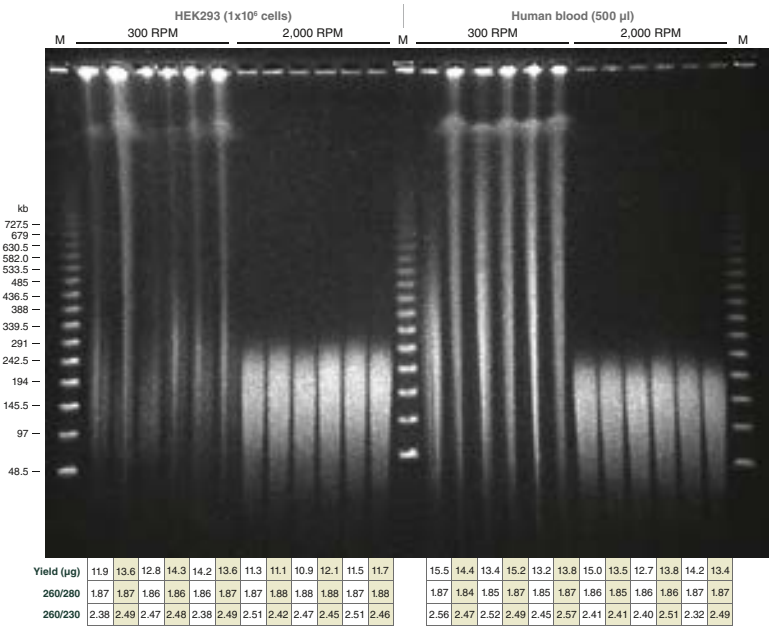
- Streamlined, high-efficiency protocol
- Ample amplicon yields from a wide range of viral genome inputs
- Improved SARS-CoV-2 genome coverage depth with a more balanced primer pool

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.

For more information see page 136.



Yield (µg)	11.9	13.6	12.8	14.3	14.2	13.6	11.3	11.1	10.9	12.1	11.5	11.7	15.5	14.4	13.4	15.2	13.2	13.8	15.0	13.5	12.7	13.8	14.2	13.4
260/280	1.87	1.87	1.86	1.86	1.86	1.87	1.87	1.88	1.88	1.88	1.87	1.88	1.87	1.84	1.85	1.87	1.85	1.87	1.86	1.85	1.86	1.86	1.87	1.87
260/230	2.38	2.49	2.47	2.48	2.38	2.49	2.51	2.42	2.47	2.45	2.51	2.46	2.56	2.47	2.52	2.49	2.45	2.57	2.41	2.41	2.40	2.51	2.32	2.49

Use of varying agitation speeds during lysis produces tunable fragment length of extracted HMW genomic DNA from cells and blood. *DNA extracted with Monarch HMW DNA Extraction Kit for Cells & Blood. 1 x 10⁵ fresh HEK293 cells and 500 µl fresh human blood were used as inputs and for preps performed according to the kit instructions using the agitation speed indicated above the gel lanes. 500 ng of DNA from the replicates was 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 (NEB #N0341) was used as molecular weight standard.*

Target Enrichment for NGS

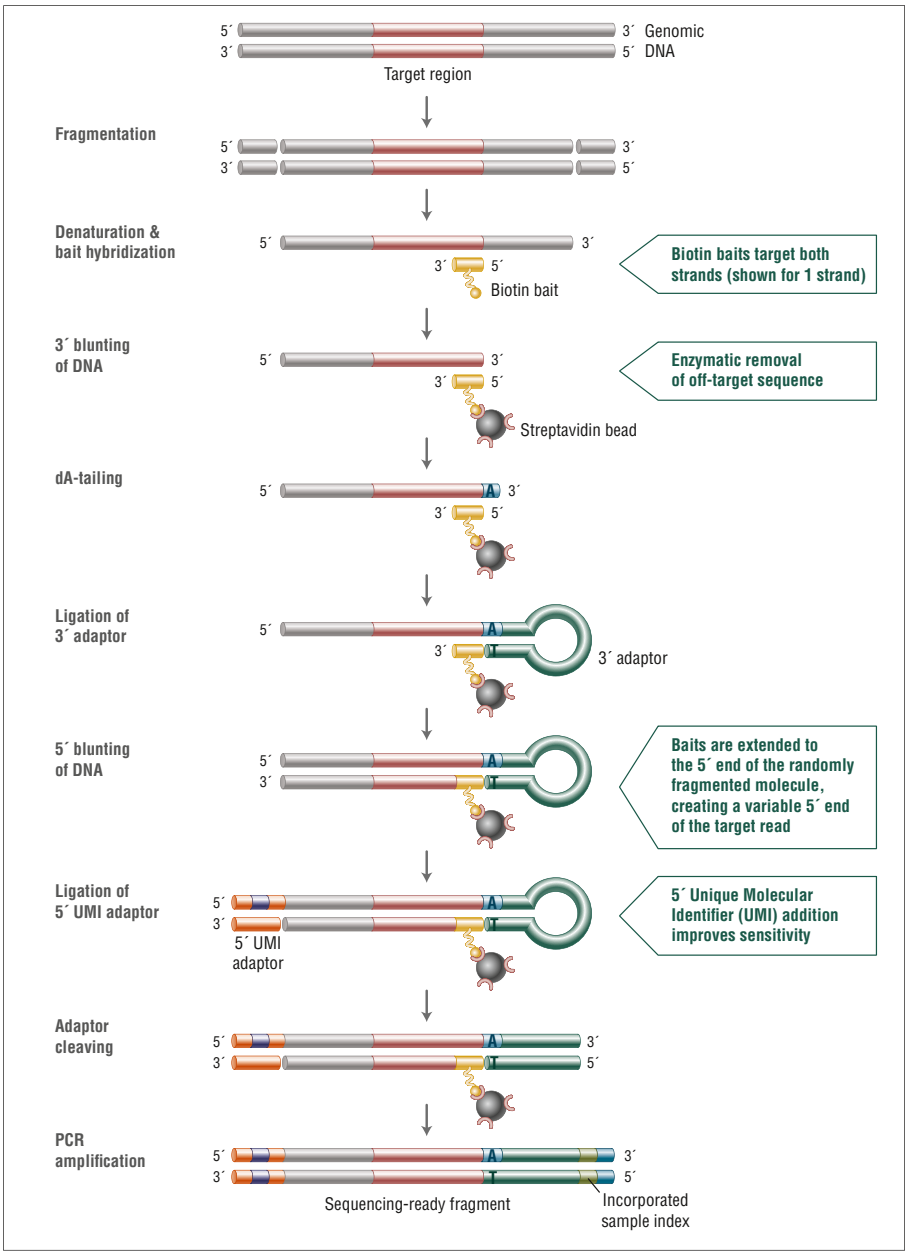
Target enrichment is used to describe a variety of strategies to selectively isolate specific genomic regions of interest for sequencing analysis.

NEBNext Direct® offers a novel approach to NGS Target Enrichment. Using a unique, capture-based protocol, NEBNext Direct offers a range of solutions for highly specific, efficient targeting of genomic loci of interest. Optimized, 1-day protocols allow for rapid profiling of targeted regions for a variety of applications, including germline and somatic variant calling for translational research and genotyping of genomic markers for applications in agricultural biology and sample identification.

- Define your own targeted sequencing panel by selecting only the genes you want from our expansive list of genes using NEBNext Direct Custom Ready Panels (workflow shown below).
- For genotyping applications, the NEBNext Genotyping Solution enables pre-capture pooling of up to 96 samples for cost-effective and scalable genotyping of up to 5,000 genomic markers.

The NEBNext Microbiome DNA Enrichment Kit facilitates enrichment of microbial DNA from samples containing methylated host DNA (including human), by selective binding and removal of the CpG-methylated host DNA. Importantly, microbial diversity remains intact after enrichment

With the NEBNext Immune Sequencing Kits, available for human and mouse, sequence the full-length immune gene repertoires of B cells and T cells. Profile somatic mutations across all relevant contexts with improved sequence accuracy.



NEBNext Direct Custom Ready Panels employ a fast hybridization-based workflow that couples capture with library preparation

View the NEBNext Direct Workflow.

NEBNext Direct Custom Ready Panels

#E6631S	8 reactions
#E6631L	24 reactions
#E6631X	96 reactions

Visit **www.neb.com/E6631** to learn more and request a quote.

- *Choose from a single gene to hundreds of genes.*
- *Experience unmatched specificity and coverage uniformity*
- *Eliminate synthesis and optimization steps, for a faster turnaround*
- *Improve sensitivity with our Unique Molecular Identifier (UMI)*
- *Generate results in one day with our automation-friendly workflow*

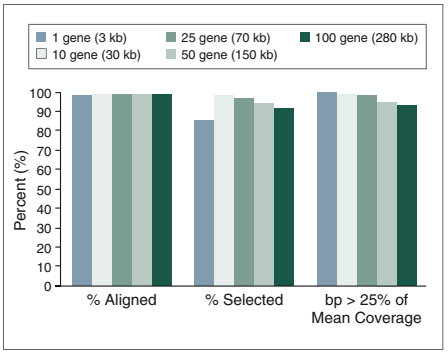
Panels can be designed and ordered by visiting **www.neb.com/CustomReadyPanelForm**

Description: Employing the unique NEBNext Direct hybridization-based enrichment method, NEBNext Direct Custom Ready Panels allow rapid customization of targeted gene panels for Illumina sequencing. Select from a list of human genes where baits have been carefully designed and optimized to provide complete coverage of the full coding (exon) regions. High quality panels can be designed by you and rapidly delivered, from any combination of genes.

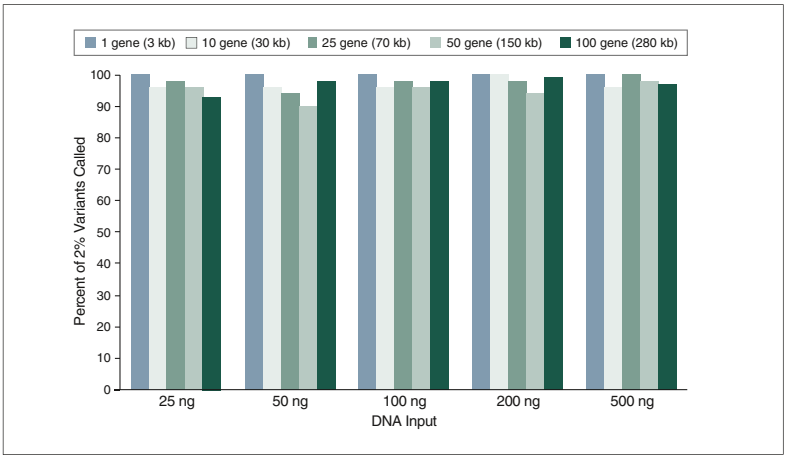
The genes available through the NEBNext Direct Custom Ready offering are continually updated, and currently include those associated with a variety of translational research areas, including cancer, neurological disorders, cardiological disease, autism, severe combined immunodeficiency, cystic fibrosis and the recommended genes for incidental findings by the American College of Medical Genetics. The full list of genes currently available can be found at www.neb.com/CustomReadyPanelForm.

NEBNext Direct Custom Ready Panels can include anywhere from a single specific gene up to 1.5 megabases of total target territory. There are no limitations on genes that can be combined together in a panel. Each panel is tested prior to shipment, and sequencing results are returned through a custom Performance Report.

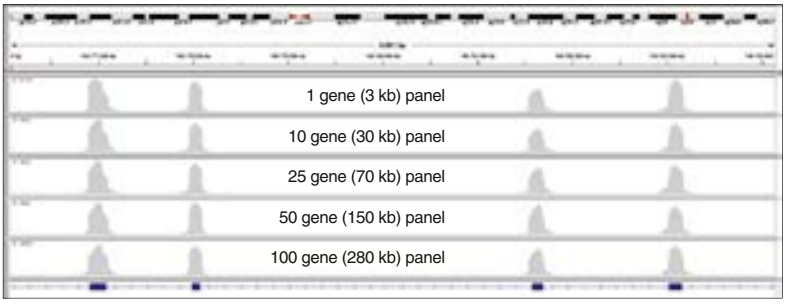
Bait sets for each gene included in the panel have undergone a rigorous development and optimization process to maximize specificity and target coverage uniformity.



NEBNext Direct Custom Ready Panels demonstrate optimum performance across a wide range of panel sizes. *Key target enrichment metrics demonstrate consistent performance across a range of panel sizes. 100 ng of DNA was tested against panels of 1, 10, 25, 50 and 100 genes, and sequenced using Illumina paired-end 150 bp sequencing. Larger panels included all genes present in smaller panels.*



Sensitivity in detection of variants across panel size and DNA input amount. *24 HapMap samples were blended to create a range of variant allele frequencies (VAF) down to 2%. 25, 50, 100, 200 and 500 ng of this blended DNA was enriched using NEBNext Direct Custom Ready Panels of 1, 10, 25, 50 and 100 genes. Larger panels were inclusive of the genes in smaller panels. Resulting libraries were sequenced using 2 x 150 bp Illumina sequencing and variants were called using Mutect and Vardict variant calling algorithms.*



NEBNext Direct Custom Ready Panels demonstrate retention of target behavior across panel sizes. *IGV image of coverage profile for 4 BRAF exons included in panels of 1, 10, 25, 50 and 100 genes, demonstrates consistent target behavior with the addition of gene targets. 100 ng of DNA was used as input for NEBNext enrichment using the 5 panels, including the BRAF gene. Libraries were sequenced on an Illumina 2 x 150 basepair sequencing.*

NEW

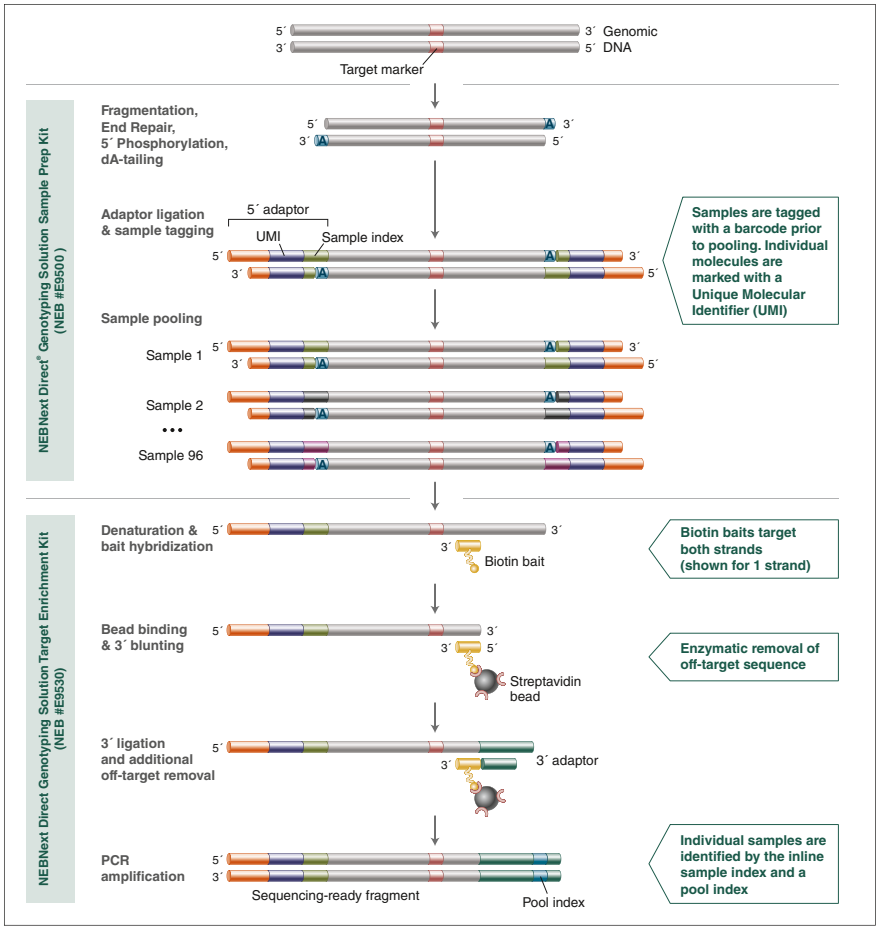
NEBNext Direct Genotyping Solution

NEBNext Direct Genotyping Solution
#E9530B-S 8 reactions
#E9500B-S 96 reactions

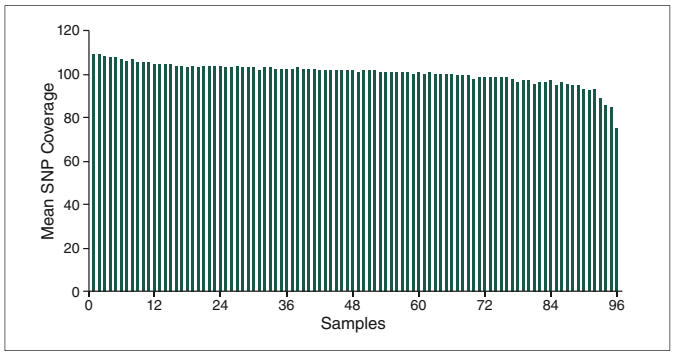
- *Ideal solution for genotyping hundreds to thousands of markers*
- *Reduce costs and streamline workflow through pre-capture pooling of up to 96 samples*
- *Maximize sequencer efficiency through dual barcode sample indexing plus Unique Molecular Identifier*
- *Unparalleled target coverage uniformity through unique capture-based enrichment*
- *Eliminate marker dropouts with finely tuned bait design*
- *Increase sample throughput using 1-day, automatable workflow*

Visit **www.neb.com/E9500** to learn more.

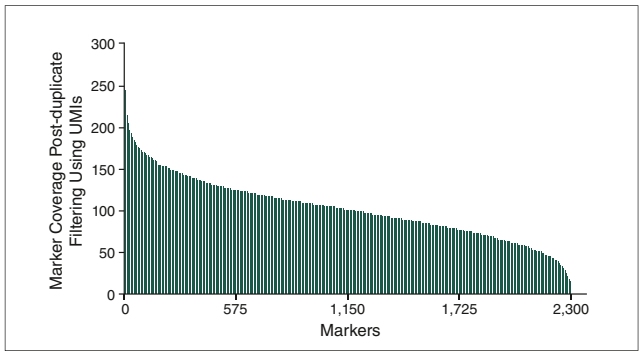
Description: The NEBNext Direct Genotyping Solution combines highly multiplexed, capture-based enrichment with maximum efficiency next-generation sequencing to deliver cost-effective, high-throughput, genotyping for a wide variety of applications. Applicable for marker ranges spanning 100-5,000 markers, 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.*

NEW

NEBNext Immune Sequencing Kits (Human & Mouse)

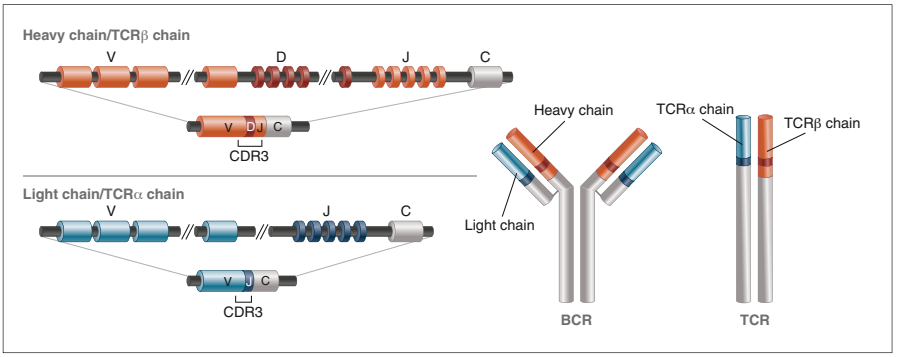
NEBNext Immune Sequencing Kit (Human)
#E6320S 24 reactions
#E6320L 96 reactions

NEBNext Immune Sequencing Kit (Mouse)
#E6330S 24 reactions
#E6330L 96 reactions

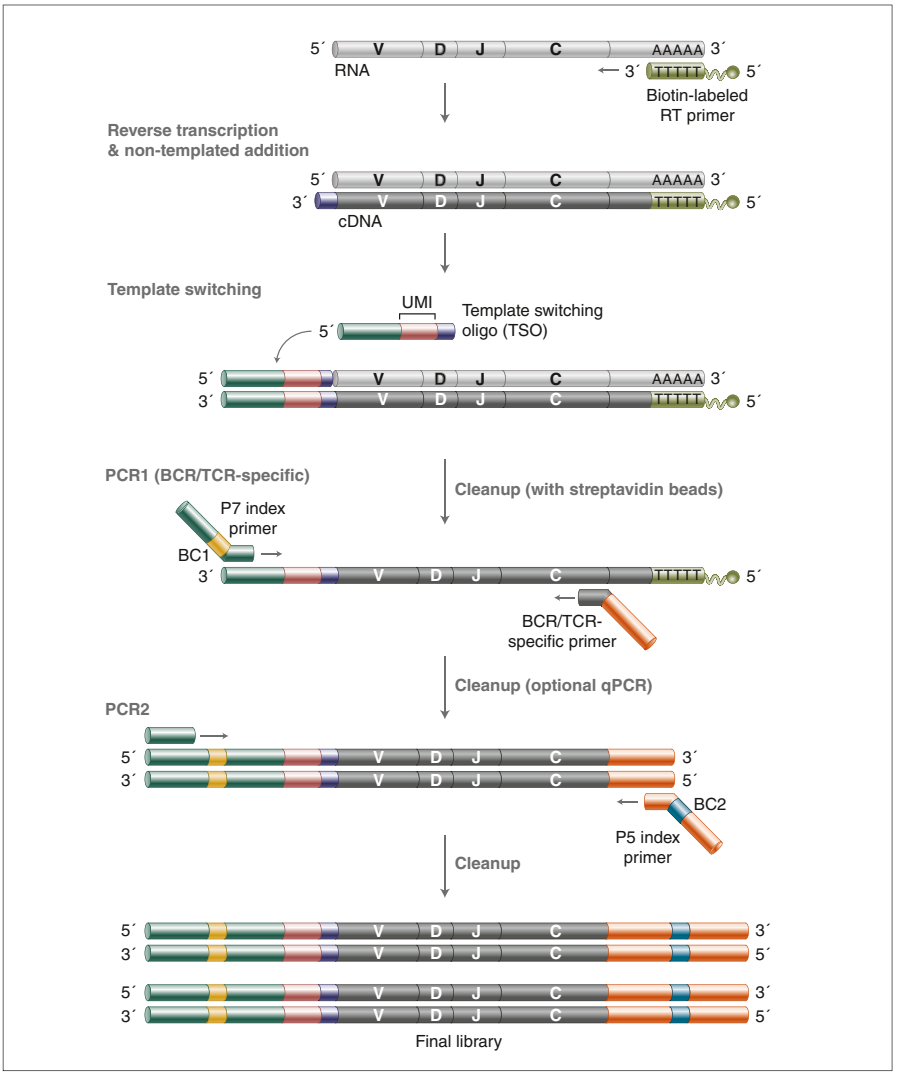
- *Generation of full-length variable sequences (including isotype information), allowing downstream antibody synthesis and functional characterization not possible with approaches sequencing only the CDR3 region.*
- *Eliminated use of variable region primers, reducing primer pool complexity and realizing 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.*

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, allowing for quantitative digital molecule counting.

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.



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

- *Effective separation of microbial DNA from contaminating host DNA*
- *Fast, simple protocol*
- *Compatible with downstream applications including next generation sequencing on all platforms, qPCR and end point PCR*
- *Suitable for a wide range of sample types*
- *No requirement for live cells*
- *Captured host DNA can also be eluted and retained*

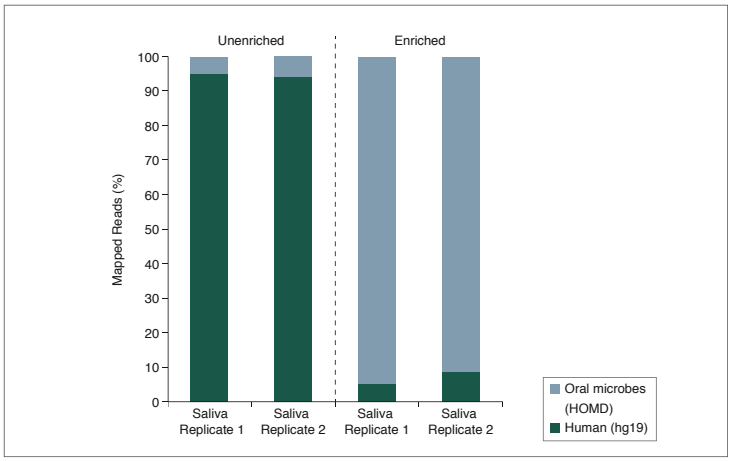
Description: The NEBNext Microbiome DNA Enrichment Kit facilitates separation of microbial DNA from methylated host DNA (including human) by selective binding and removal of the CpG methylated host DNA (1).

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.

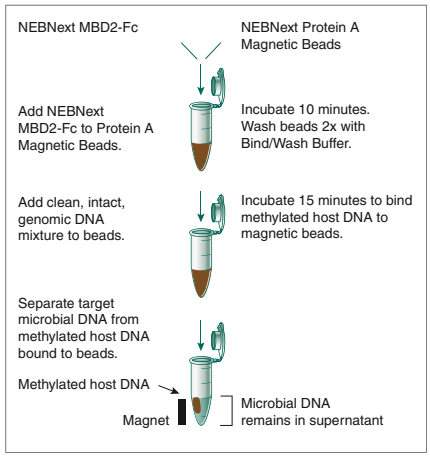
The Microbiome Enrichment Kit Includes:

– NEBNext MBD2-Fc Protein
– NEBNext Protein A Magnetic Beads
– NEBNext Bind/Wash Buffer (5X)
– 16S RNA Universal Bacteria Control Primers
– RPL30 Human DNA Control Primers

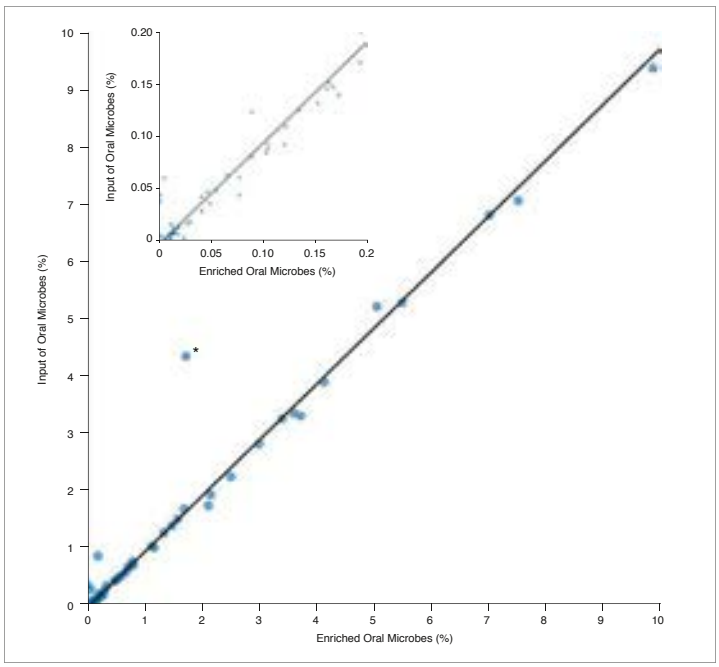
(1) Feehery, G.R. et al. (2013) *PLoS One*, 8: e76096.
(2) Chen, T., et al. (2010) *Database*, Vol. 2010, Article ID baq013, doi: 10.1093/database/baq013
(3) Langmead, B., et al. (2009) *Genome Biol.* 10:R25 doi:10.1186/gb-2009-10-3-r25



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.).* SOLiD™ 4 is a registered trademark of Life Technologies, Inc.



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 Niesseria species (N. mucosa, N. sicca and N. elognata) are represented, but do not exhibit this anomalous enrichment.*

NEBNext dsDNA Fragmentase®

37° 65°

#M0348S 50 reactions
#M0348L 250 reactions

Companion Product:

NEBNext dsDNA Fragmentase Reaction Buffer v2
#B0349S 6 x 1 ml

- *Generation of dsDNA fragments for sequencing on next generation sequencing platforms*
- *Generation of dsDNA fragments for libraries*

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.

Source: NEBNext dsDNA Fragmentase is composed of endonucleases isolated from two different *E. coli* sources: one construct expresses a fusion protein consisting of *E. coli* maltose binding protein and *Vibrio vulnificus* nuclease mutant protein; the other expresses a fusion protein consisting of maltose binding protein and T7 endonuclease mutant protein.

Reaction Conditions: 1X NEBNext dsDNA Fragmentase Reaction Buffer v2, supplemented with 100 µM $MgCl_2$, when required. Incubate at 37°C.

1X NEBNext dsDNA Fragmentase Reaction Buffer v2:
20 mM Tris-HCl
10 mM $MgCl_2$
50 mM NaCl
0.15% Triton X-100
pH 7.5 @ 25°C

Reagents Supplied with Enzyme:
10X NEBNext dsDNA Fragmentase Reaction Buffer v2
200 mM $MgCl_2$

Heat Inactivation: 65°C for 15 minutes in the presence of 50 mM DTT

MINELUTE® is a registered trademark of the Qiagen Group.

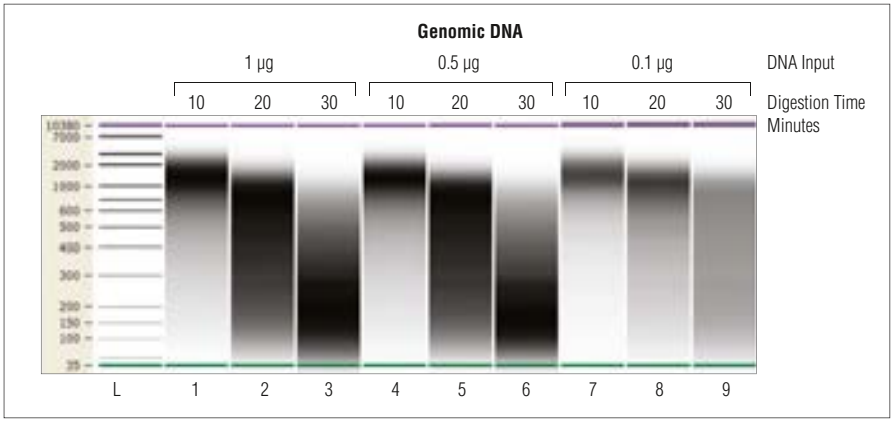


Figure 1: *Fragmentation of E. coli gDNA. E. coli gDNA was fragmented with NEBNext dsDNA Fragmentase for the indicated times and purified on MinElute® columns.*

Chrystel works at our subsidiary office in France and has been with the team for over 10 years. She spends her time working in Accounting and Human Resources, and also helps out with Customer Service. Chrystel also enjoys interior design and staging homes.

NEBNext Ultra II Q5® Master Mix

#M0544S	50 reactions
#M0544L	250 reactions

Additional 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 (50 µl)
#M0541L	250 reactions (50 µl)

- *Next generation sequencing library preparation*
- *High-fidelity amplification*
- *Uniform GC coverage*
- *Improves sequencing library coverage of known difficult regions of the human genome*

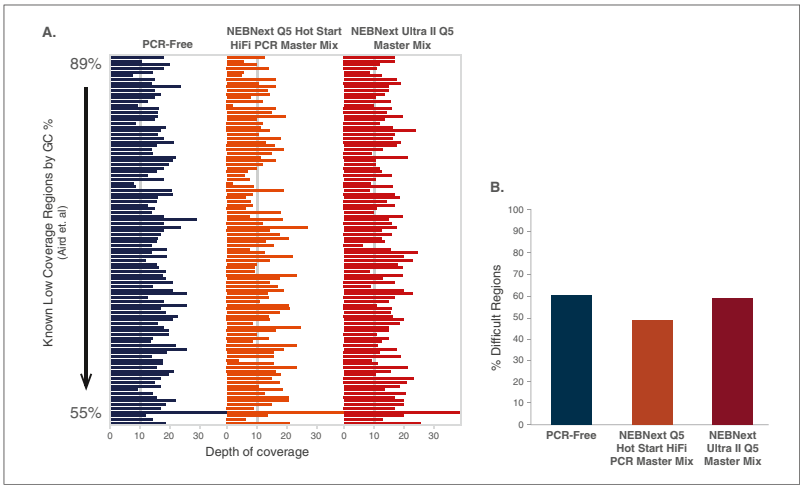
Description: The NEBNext Ultra II Q5 Master Mix is a new formulation of Q5 DNA Polymerase that has been optimized for robust, high-fidelity amplification of next-generation sequencing (NGS) libraries. This formulation further improves the uniformity of amplification of libraries, including superior performance 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.

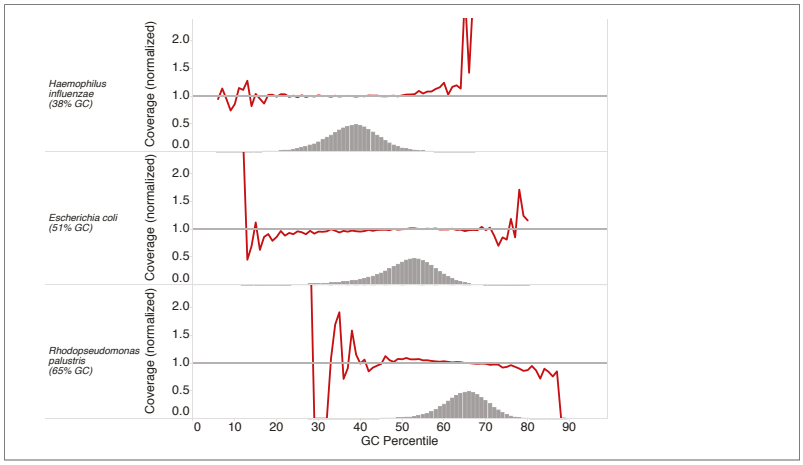
The NEBNext Ultra II Q5 Master Mix is an aptamer-based hot start formulation that allows convenient room temperature reaction set up. The convenient 2X master mix format contains dNTPs, Mg^{++} and a proprietary buffer, and requires only the addition of primers and DNA template for robust amplification. NEBNext Ultra II Q5 Master Mix is also included in the NEBNext Ultra II DNA Library Prep Kit for Illumina.

Source: An *E. coli* strain that carries the Q5 High-Fidelity DNA Polymerase gene.

Reaction Conditions: NEBNext Ultra II Q5 Master Mix, DNA template and 1 µM primers in a total reaction volume of 50 µl.



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 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 Library Quant Kit for Illumina

NEBNext Library Quant Kit for Illumina
#E7630S 100 reactions
#E7630L 500 reactions

Companion Product:

NEBNext Library Dilution Buffer
#B6118S 7.5 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*
- *Requires only 4 standards, allowing more libraries to be quantitated per kit*
- *Supplied with a convenient Library Dilution Buffer*
- *The NEBNext Library Quant Master Mix requires only the addition of primers*
- *Utilizes a single extension time for all libraries, regardless of insert size*
- *Library quant values can be easily calculated using NEB's online tool, at NEBioCalculator.neb.com*
- *ROX is included in the kit, for use with qPCR instruments that require a reference dye for normalization*

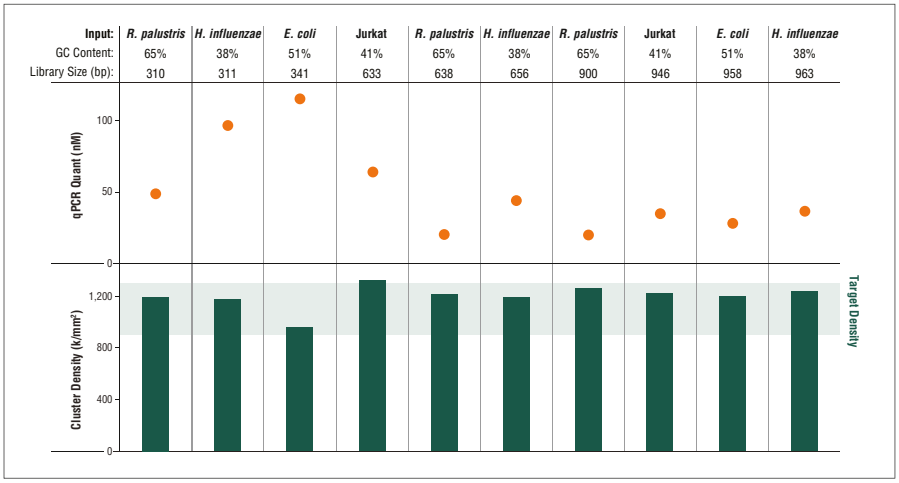
Description: Accurate quantitation of next-generation sequencing libraries is essential for maximizing data output and quality from each sequencing run. For Illumina sequencing specifically, accurate quantitation of libraries is critical to achieve optimal cluster densities, a requirement for optimal sequence performance. qPCR is considered to be the most accurate and effective method of library quantitation, providing considerably higher consistency and reproducibility than electrophoresis or spectrophotometry, which measure total nucleic acid concentration. Amplification-based methods quantitate only those molecules that contain both adaptor sequences, thereby providing a more accurate estimate of the concentration of the library molecules that can be sequenced.

The NEBNext Library Quant Kit delivers significant improvements to qPCR-based library quantitation for next gen sequencing. The NEBNext Library Quant Kit for Illumina contains components that are optimized for qPCR-based quantitation of libraries prepared for Illumina next-generation sequencing platforms. The NEBNext Library Quant Kit contains primers which target the P5 and P7 Illumina adaptor sequences and a set of high-quality, pre-diluted DNA standards to enable reliable quantitation of diluted DNA libraries between 150–1000 bp.

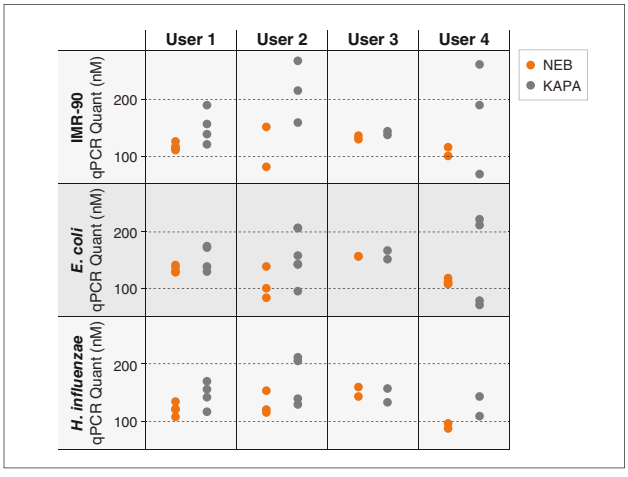
The Library Quant Kit Includes:

– NEBNext Library Quant Master Mix
– NEBNext Library Quant Primer Mix
– NEBNext Library Quant DNA Standards 1–4
– ROX (Low) and ROX (High)
– NEBNext Library Dilution Buffer

	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. 45 min.**



With NEBNext, optimal cluster density is achieved from quantitated libraries with a broad range of library size and GC content. Libraries of 310–963 bp from the indicated sources were quantitated using the NEBNext Library Quant Kit, then diluted to 8 pM and loaded onto a MiSeq (v2 chemistry; MCS v2.4.1.3). Library concentrations ranged from 7–120 nM, and resulting raw cluster density for all libraries was 965–1300 k/mm² (ave. =1199). Optimal cluster density was achieved using concentrations determined by the NEBNext Library Quant Kit for all library sizes.



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 (orange) versus those from the Kapa kit (gray).

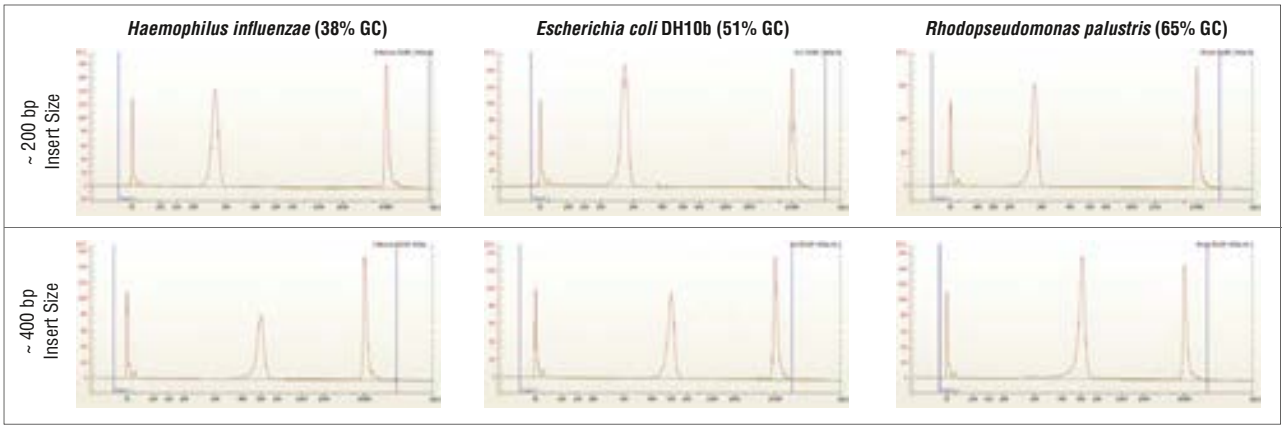
NEBNext Reagents for Ion Torrent™: DNA Library Preparation

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.*

NEBNext Reagents for DNA Library Preparation – Ordering Information

Illumina Platform:

KITS FOR ILLUMINA DNA LIBRARY PREPARATION		NEB #	SIZE
DNA & ChIP	NEBNext Ultra II DNA Library Prep Kit for Illumina	E7645S/L	24/96 rxns
	NEBNext Ultra II DNA Library Prep with Sample Purification Beads	E7103S/L	24/96 rxns
	NEBNext Ultra II FS DNA Library Prep Kit for Illumina	E7805S/L	24/96 rxns
	NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads	E6177S/L	24/96 rxns
	NEBNext Ultra II DNA PCR-free Library Prep Kit for Illumina	E7410S/L	24/96 rxns
	NEBNext Ultra II DNA PCR-free Library Prep with Sample Purification Beads	E7415S/L	24/96 rxns
	NEBNext Ultra II FS DNA PCR-free Library Prep Kit for Illumina	E7430S/L	24/96 rxns
	NEBNext Ultra II FS DNA PCR-free Library Prep with Sample Purification Beads	E7435S/L	24/96 rxns
	NEBNext Enzymatic Methyl-seq Kit	E7120S/L	24/96 rxns
	NEBNext Ultra DNA Library Prep Kit for Illumina	E7370S/L	24/96 rxns
MODULES & ENZYMES		**NEB #**	**SIZE**
DNA & ChIP	NEBNext Enzymatic Methyl-seq Conversion Module	E7125S/L	24/96 rxns
	NEBNext FFPE DNA Repair Mix	M6630S/L	24/96 rxns
	NEBNext Microbiome DNA Enrichment Kit	E2612S/L	6/24 rxns
	NEBNext Ultra II FS DNA Module	E7810S/L	24/96 rxns
	NEBNext Ultra II End Repair/dA-Tailing Module	E7546S/L	24/96 rxns
	NEBNext Ultra II Ligation Module	E7595S/L	24/96 rxns
	NEBNext Ultra II Q5 Master Mix	M0544S/L	50/250 rxns
	NEBNext Ultra End Repair/dA-Tailing Module	E7442S/L	24/96 rxns
	NEBNext Ultra Ligation Module	E7445S/L	24/96 rxns
	NEBNext dsDNA Fragmentase	M0348S/L	50/250 rxns
	NEBNext End Repair Module	E6050S/L	20/100 rxns
	NEBNext dA-Tailing Module	E6053S/L	20/100 rxns
	NEBNext Quick Ligation Module	E6056S/L	20/100 rxns
	NEBNext Q5 Hot Start HiFi PCR Master Mix	M0543S/L	50/250 rxns
	NEBNext High-Fidelity 2X PCR Master Mix	M0541S/L	50/250 rxns
	NEBNext Q5U Master Mix	M0597S/L	50/250 rxns
	NEBNext dsDNA Fragmentase Reaction Buffer v2	B0349S	6 ml
ADAPTORS & PRIMERS		**NEB #**	**SIZE**
	NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1)	E7395S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	E6440S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 2)	E6442S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 3)	E6444S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 4)	E6446S/L	96/384 rxns
	NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)	E7140S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 rxns
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2)	E7780S	96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	E7335S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	E7500S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)	E7710S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)	E7730S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (Methylated Adaptor, Index Primers Set 1)	E7535S/L	24/96 rxns
	NEBNext Adaptor Dilution Buffer	B1430S	1 x 9.6 ml
TARGET ENRICHMENT		**NEB #**	**SIZE**
	NEBNext Direct Custom Ready Panels	E6631S/L/X	8/24/96 rxns
	NEBNext Direct Genotyping Solution	E9500B-S	96 rxns
	NEBNext Direct Genotyping Solution	E9530B-S	8 rxns
	NEBNext Immune Sequencing Kit (Human)	E6320S/L	24/96 rxns
	NEBNext Immune Sequencing Kit (Mouse)	E6330S/L	24/96 rxns
LIBRARY QUANTITATION		**NEB #**	**SIZE**
	NEBNext Library Quant Kit for Illumina	E7630S/L	100/500 rxns
	NEBNext Library Dilution Buffer	B6118S	7.5 ml

Oxford Nanopore Technologies Platform:

PRODUCTS FOR DNA LIBRARY PREPARATION		NEB #	SIZE
DNA	NEBNext Companion Module for Oxford Nanopore Technologies Ligation Sequencing	E7180S	24 rxns

Ion Torrent Platform:

PRODUCTS FOR DNA LIBRARY PREPARATION		NEB #	SIZE
DNA	NEBNext Fast DNA Library Prep Set for Ion Torrent	E6270L	50 rxns
	NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent	E6285L	50 rxns

Suitable for Any Sequencing Platform:

DNA ENRICHMENT		NEB #	SIZE
DNA	NEBNext Microbiome DNA Enrichment Kit	E2612S/L	6/24 rxns
DNA REPAIR		**NEB #**	**SIZE**
DNA	NEBNext FFPE DNA Repair Mix	M6630S/L	24/96 rxns
MODULES & ENZYMES		**NEB #**	**SIZE**
DNA	NEBNext dsDNA Fragmentase	M0348S/L	50/250 rxns
	NEBNext Ultra II Q5 Master Mix	M0544S/L	50/250 rxns
	NEBNext Q5 Hot Start HiFi PCR Master Mix	M0543S/L	50/250 rxns
	NEBNext High-Fidelity 2X PCR Master Mix	M0541S/L	50/250 rxns
	NEBNext dsDNA Fragmentase Reaction Buffer v2	B0349S	6 ml
MAGNETIC SEPARATION		**NEB #**	**SIZE**
	NEBNext Magnetic Separation Rack	S1515S	24 tubes

Luis has been with NEB for 24 years and works in our Shipping Department. In addition to making sure NEB orders are packed quickly and efficiently, Luis enjoys snowboarding, saltwater fishing and camping.

Illumina Platform:

KITS FOR ILLUMINA RNA LIBRARY PREPARATION		NEB #	SIZE
Directional RNA	NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	E7760S/L	24/96 rxns
	NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	E7765S/L	24/96 rxns
	NEBNext Ultra Directional RNA Library Prep Kit for Illumina	E7420L	96 rxns
Non-directional RNA	NEBNext Ultra II RNA Library Prep Kit for Illumina	E7770S/L	24/96 rxns
	NEBNext Ultra II RNA Library Prep with Sample Purification Beads	E7775S/L	24/96 rxns
	NEBNext Ultra RNA Library Prep Kit for Illumina	E7530L	96 rxns
Small RNA	NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1)	E7300S/L	24/96 rxns
	NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2)	E7580S/L	24/96 rxns
	NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48)	E7560S	96 rxns
	NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible)	E7330S/L	24/96 rxns
Single Cell	NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina	E6420S/L	24/96 rxns
SARS-CoV-2	NEBNext ARTIC SARS-CoV-2 Library Prep Kit (Illumina)	E7650S/L	24/96 rxns
	NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina)	E7658S/L	24/96 rxns
MODULES & ENZYMES		**NEB #**	**SIZE**
RNA	NEBNext RNA Depletion Core Reagent Set	E7865S/L/X	6/24/96 rxns
	NEBNext RNA Depletion Core Reagent Set with RNA Sample Purification Beads	E7870S/L/X	6/24/96 rxns
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)	E7750S/L/X	6/24/96 rxns
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E7755S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)	E7400S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads	E7405S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Bacteria)	E7850S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads	E7860S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	E6310S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E6350S/L/X	6/24/96 rxns
	NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 rxns
	NEBNext Magnesium RNA Fragmentation Module	E6150S	200 rxns
	NEBNext Ultra II RNA First Strand Synthesis Module	E7771S/L	24/96 rxns
	NEBNext Ultra II Directional RNA Second Strand Synthesis Module	E7550S/L	24/96 rxns
	NEBNext Ultra II Non-directional RNA Second Strand Synthesis Module	E6111S/L	20/100 rxns
	NEBNext RNA First Strand Synthesis Module	E7525S/L	24/96 rxns
	NEBNext Single Cell/Low Input cDNA Synthesis and Amplification Module	E6421S/L	24/96 rxns
	NEBNext Single Cell Lysis Module	E5530S	96 rxns
DNA	NEBNext Ultra II End Repair/dA-Tailing Module	E7546S/L	24/96 rxns
	NEBNext Ultra II Ligation Module	E7595S/L	24/96 rxns
	NEBNext Ultra End Repair/dA-Tailing Module	E7442S/L	24/96 rxns
	NEBNext Ultra Ligation Module	E7445S/L	24/96 rxns
	NEBNext End Repair Module	E6050S/L	20/100 rxns
	NEBNext dA-Tailing Module	E6053S/L	20/100 rxns
	NEBNext Quick Ligation Module	E6056S/L	20/100 rxns
	NEBNext Ultra II Q5 Master Mix	M0544S/L	50/250 rxns
	NEBNext Q5 Hot Start HiFi PCR Master Mix	M0543S/L	50/250 rxns
	NEBNext High-Fidelity 2X PCR Master Mix	M0541S/L	50/250 rxns
ADAPTORS & PRIMERS		**NEB #**	**SIZE**
	NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors RNA Set 1)	E7416S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	E6440S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 2)	E6442S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 3)	E6444S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 4)	E6446S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 rxns
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2)	E7780S	96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	E7335S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 2)	E7500S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)	E7710S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)	E7730S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 rxns
	NEBNext Adaptor Dilution Buffer	B1430S	1 x 9.6 ml

Illumina Platform (continued):

LIBRARY QUANTITATION		NEB #	SIZE
	NEBNext Library Quant Kit for Illumina	E7630S/L	100/500 rxns
	NEBNext Library Dilution Buffer	B6118S	7.5 ml

Oxford Nanopore Technologies Platform:

PRODUCTS FOR DNA LIBRARY PREPARATION		NEB #	SIZE
RNA	NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies)	E7660S/L	24/96 rxns

Suitable for Any Sequencing Platform:

MODULES & ENZYMES		NEB #	SIZE
RNA	NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	E6310S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E6350S/L/X	6/24/96 rxns
	NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 rxns
	NEBNext Magnesium RNA Fragmentation Module	E6150S	200 rxns
	NEBNext Ultra II RNA First Strand Synthesis Module	E7771S/L	24/96 rxns
	NEBNext Ultra II Non-directional RNA Second Strand Synthesis Module	E6111S/L	20/100 rxns
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)	E7750S/L/X	6/24/96 rxns
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E7755S/L/X	6/24/96 rxns
MAGNETIC SEPARATION		**NEB #**	**SIZE**
	NEBNext Magnetic Separation Rack	S1515S	24 tubes

Featured Online Tools

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 **https://depletion-design.neb.com/**.

Be Adventurous

Nurturing a sense of adventure can take us out of our comfort zone, as we embrace new challenges and take risks. It is often easier to choose the familiar path, but in doing so we miss the opportunity to explore something new. It is our innate curiosity that pushes us to pursue new challenges, which can often lead to the most exciting and memorable experiences, and perhaps the opportunity to learn something new.

Don Comb was an adventurer who loved to be outdoors. Trout fishing was one of his most passionate pursuits, and entomology one of his greatest fascinations. As an adult, he enjoyed sailing with his children. They love to regale listeners with stories of their maritime escapades — sailing from St Barthélemy Island in the Caribbean to New England, for example, guided only by Don's celestial navigation skills and fearless determination. Don's family embraced his boundless curiosity and enthusiasm when visiting remote villages in Papua New Guinea on uncharted trekking expeditions, or traveling to the Bermuda Biological Station to snorkel and collect sea urchins for use in developmental research in his position at Harvard Medical School.

Don's wonderment of the world compelled him to question everything. He was always the scientist, even when on vacation, and he would often return with new questions and a sample or two, asking NEB researchers to investigate a specific novelty. A striped Bass that he caught in New England waters was in the process of digesting a lobster, so Don asked researchers at NEB to find the enzyme capable of breaking down the lobster shell. There are many stories like this, and while some of these projects may have seemed outlandish, they often resulted in techniques and findings that were subsequently applied to other research projects at NEB.

NEB researchers and staff are encouraged to bring their curiosity to work, where asking big questions is a vital part of the workplace culture.

In the early 1990s, Chris Taron, now a Scientific Director at NEB, recalls a time when Don was inspired to search for samples in the geothermal springs in Yellowstone National Park. This was the location where *Thermus aquaticus*, the temperature tolerant bacteria that produces *Taq* DNA Polymerase, was first isolated. Don reasoned that the *T. aquaticus* ecosystem might also harbor a bacteriophage that encodes a polymerase exhibiting equivalent performance to *Taq*. Chris, also an outdoor enthusiast, embraced the offer to hike, cross-country ski and snowmobile in the middle of winter to the Lower Geiser Basin. Using a golf ball retriever jerry-rigged with water sampling equipment, he collected samples and transported them back to NEB for analysis. The result — researchers identified the bacteriophage, which unfortunately did not possess the polymerase they had sought. But finding a marketable product was not Don's primary motivation, he just had a question that he wanted an answer to.

The drive behind Don's desire to be outdoors seeking adventure and his endless scientific questioning was the same — a voracious inquisitiveness. Scientific inquiry is driven by a desire to better understand the world around us, and to embrace the innovation that results from being guided by curiosity.

Exploring your adventurous side doesn't have to involve scaling rock walls or taking dangerous risks. Only you can define what adventure means to you. It can be anything that feels a little out of your routine — as long as it gets you excited!

Don maneuvers his sailboat while his son Michael watches for coral from the bow, Exuma Cays, Bahama Islands, 1987.
Credit: Dave Comb

Learn more about Don's adventures with his family.

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 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

182 **Quick-Load Purple 1 kb Plus DNA Ladder**

185 **Color Prestained Protein Standard, Broad Range (10–250 kDa)**

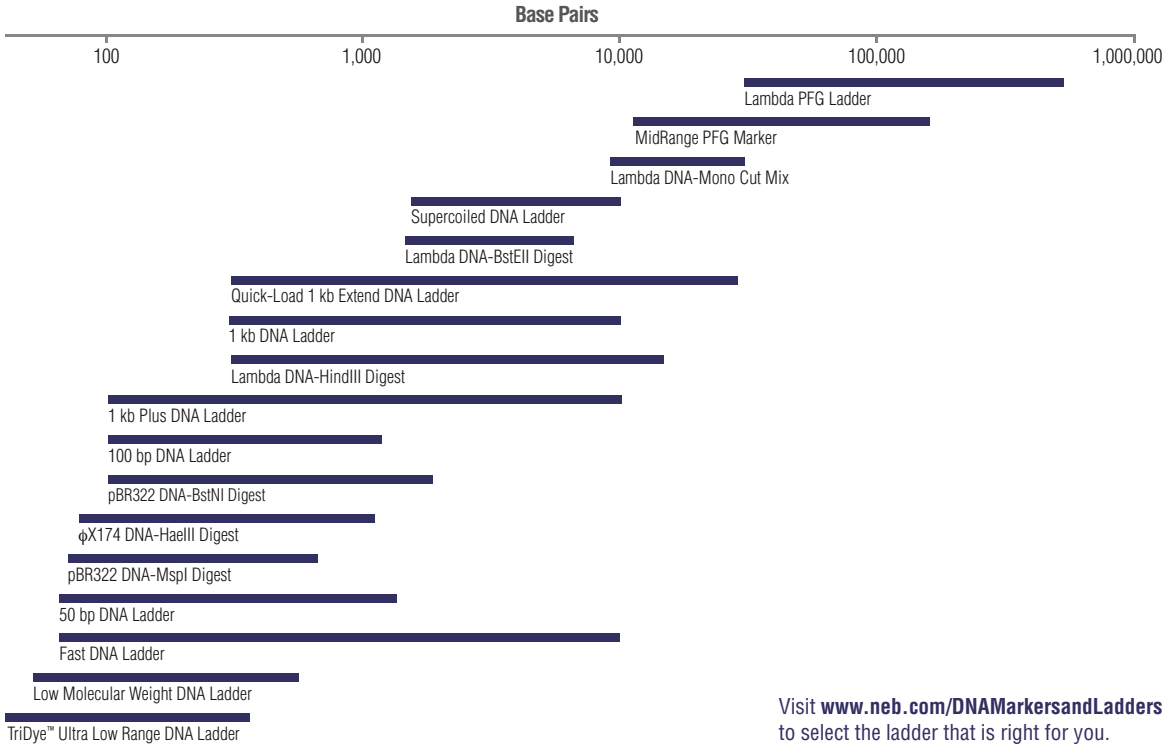
182 **1 kb Plus DNA Ladder for Safe Stains**

182 **TriDye Ultra Low Range DNA Ladder**

Featured Tools & Resources

Visit www.neb.com/DNAladders to find selection charts for NEB's DNA markers and ladders.

Size Ranges of DNA Ladders from NEB



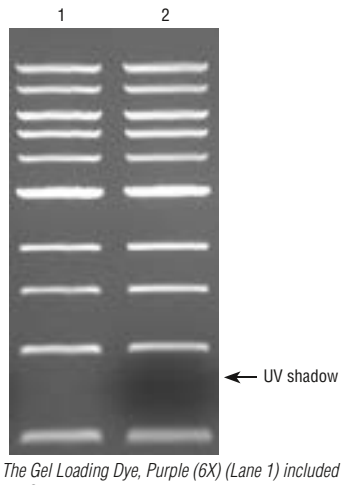
Visit **www.neb.com/DNAMarkersandLadders** to select the ladder that is right for you.

Purple Loading Dye

Gel Loading Dye, Purple (6X)
#B7024S 4.0 ml

Gel Loading Dye, Purple (6X), no SDS
#B7025S 4.0 ml

Our Gel Loading Dye, Purple (6X) (with and without SDS) is now 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 100-200 gel lanes
#N3200L 500-1,000 gel lanes

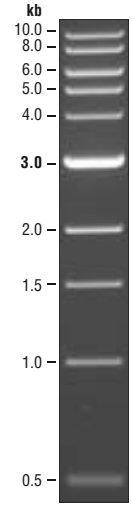
50 bp DNA Ladder
#N3236S 100-200 gel lanes
#N3236L 500-1,000 gel lanes

Low Molecular Weight DNA Ladder
#N3233S 100 gel lanes
#N3233L 500 gel lanes

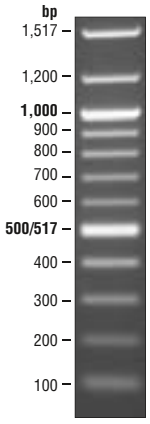
PCR Marker
#N3234S 100 gel lanes
#N3234L 500 gel lanes

NEB offers a variety of DNA Ladders with sizes ranging from 10 bp to 48.5 kb for use in agarose gel electrophoresis.

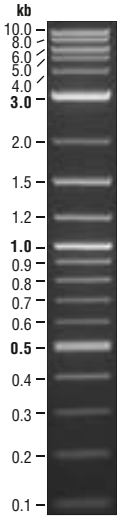
- Stable at room temperature
- Sharp, uniform bands
- Easy-to-identify reference bands
- Supplied with 1 vial of Gel Loading Dye, Purple (6X), no SDS
- Can be used for sample quantification (see www.neb.com for mass values)



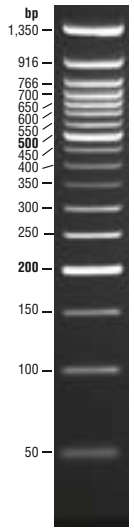
1 kb DNA Ladder
1 kb DNA Ladder visualized by ethidium bromide staining on a 0.8% TAE agarose gel.



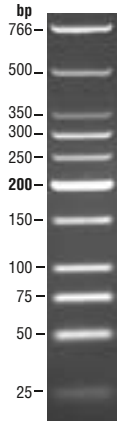
100 bp DNA Ladder
100 bp DNA Ladder visualized by ethidium bromide staining on a 1.3% TAE agarose gel.



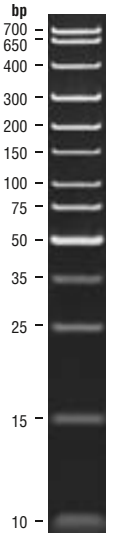
1 kb Plus DNA Ladder
1 kb Plus DNA Ladder visualized by ethidium bromide staining on a 1.0% TBE agarose gel.



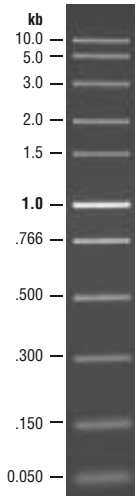
50 bp DNA Ladder
1.0 µg of 50 bp DNA Ladder visualized by ethidium bromide staining on a 3% TBE agarose gel.



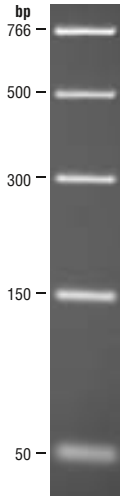
Low Molecular Weight DNA Ladder
0.5 µg of LMW DNA Ladder visualized by ethidium bromide staining on a 3% TBE agarose gel.



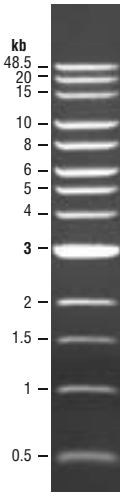
TriDye Ultra Low Range DNA Ladder
0.5 µg of Ultra Low Range DNA Ladder visualized by ethidium bromide staining on a 20% polyacrylamide gel.



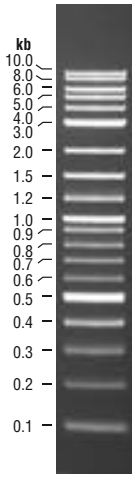
Fast DNA Ladder*
1.2% TBE agarose gel.



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% TBE agarose gel.



1 kb Plus DNA Ladder for Safe Stains**
1.0% TBE agarose gel.

Usage Notes: Dilution of these markers is recommended in TE or other buffer of minimal ionic strength. DNA may denature if diluted in dH_20.

Individual DNA fragments, as seen in the 1 kb DNA Ladder, are available on request.

Contact **info@neb.com** for more information.

* 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.

** 1 kb Plus DNA Ladder for Safe Stains, optimized for use with GelRed®, GelGreen® and SYBR® precast gels.

DNA Ladders in Convenient Pre-mixed Formats

Quick-Load® Purple Formats:

Quick-Load Purple 1 kb Plus DNA Ladder*
#N0550S 125-250 gel lanes
#N0550L 375-750 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 125-250 gel lanes

Quick-Load Purple Low Molecular Weight DNA Ladder*
#N0557S 125 gel lanes

* Supplied with 1 vial of Gel Loading Dye, Purple (6X), no SDS.

Other Ready-to-Load Formats:

NEW
1 kb Plus DNA Ladder for Safe Stains
#N0559S 125-250 gel lanes

Fast DNA Ladder
#N3238S 50-200 gel lanes

TriDye™ Formats:

TriDye 1 kb Plus DNA Ladder
#N3270S 125-250 gel lanes

TriDye 1 kb DNA Ladder
#N3272S 125 gel lanes

TriDye 100 bp DNA Ladder
#N3271S 125 gel lanes

NEW
TriDye Ultra Low Range DNA Ladder*
#N0558S 125-250 gel lanes

* TriDye Ultra Low Range DNA Ladder is suitable for use on both native polyacrylamide and agarose gels.

Quick-Load Formats:

Quick-Load 1 kb Plus DNA Ladder
#N0469S 125-250 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. Choose from the conventional ladder, the Quick-Load version using either non-fluorescing, purple dye or bromophenol blue as a tracking dye, or TriDye containing three dyes to facilitate monitoring of gel migration.

PFG Ladders

Lambda PFG Ladder
#N0341S 50 gel lanes

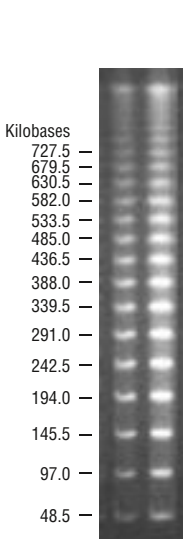
MidRange PFG Marker
#N0342S 50 gel lanes

Lambda DNA-Mono Cut Mix
#N3019S 100 gel lanes

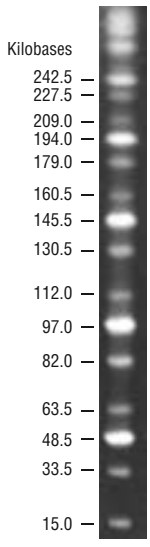
The Lambda PFG Ladder consists of one GelSyringe™ dispenser, sufficient for 50 gel lanes. Successively larger concatemers of lambda DNA (cI857 ind 1 Sam7) are embedded in 1% LMP agarose. Size range: 48.5–1,018 kb.

MidRange PFG Marker consists of concatemers of λ DNA isolated from the bacteriophage λ (cI857 ind1 Sam7) 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.

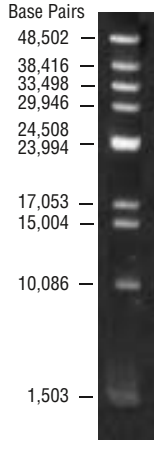
The Lambda DNA-Mono Cut Mix is best separated by pulsed field gel electrophoresis, but can be alternatively used with standard electrophoresis systems. It is supplied in a liquid format. Size range: 1.5–48.5 kb.



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

Lambda 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

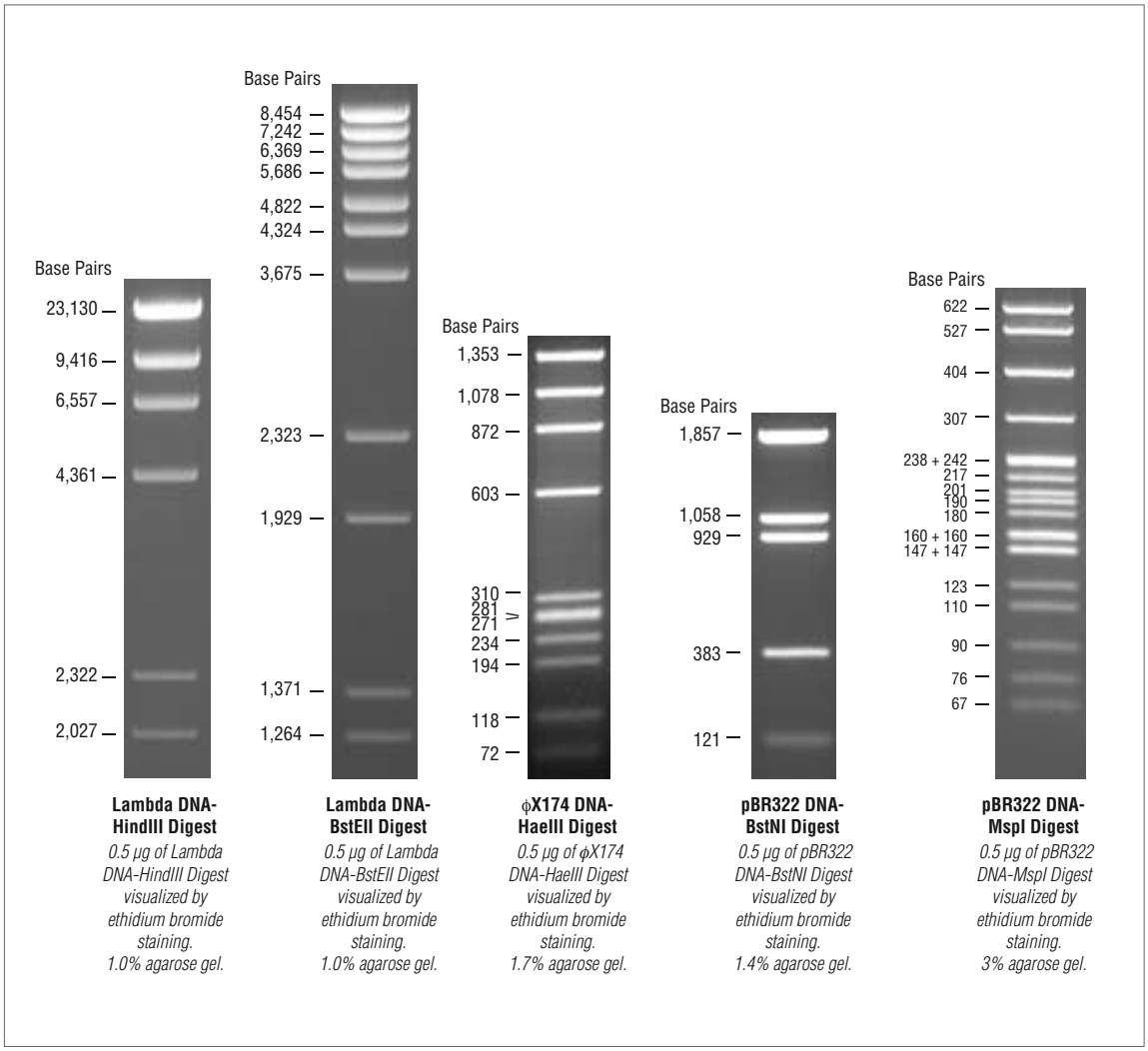
pBR322 DNA-MspI Digest
#N3032S 50 gel lanes

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.

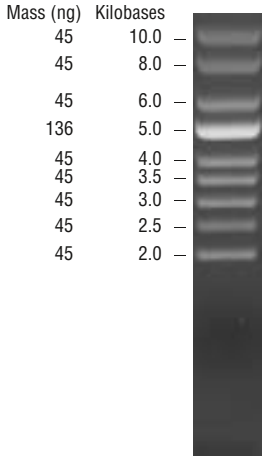
ϕX174 DNA-HaeIII Digest
0.5 µg of ϕX174 DNA-HaeIII Digest visualized by ethidium bromide staining. 1.7% agarose gel.

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
0.5 µg of pBR322 DNA-MspI Digest visualized by ethidium bromide staining. 3% agarose gel.

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.

Source: The 9 proprietary plasmids are purified, phenol extracted and equilibrated to 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA.

Concentration: 500 µg/ml.

Notes: 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.

Usage Recommendation: 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

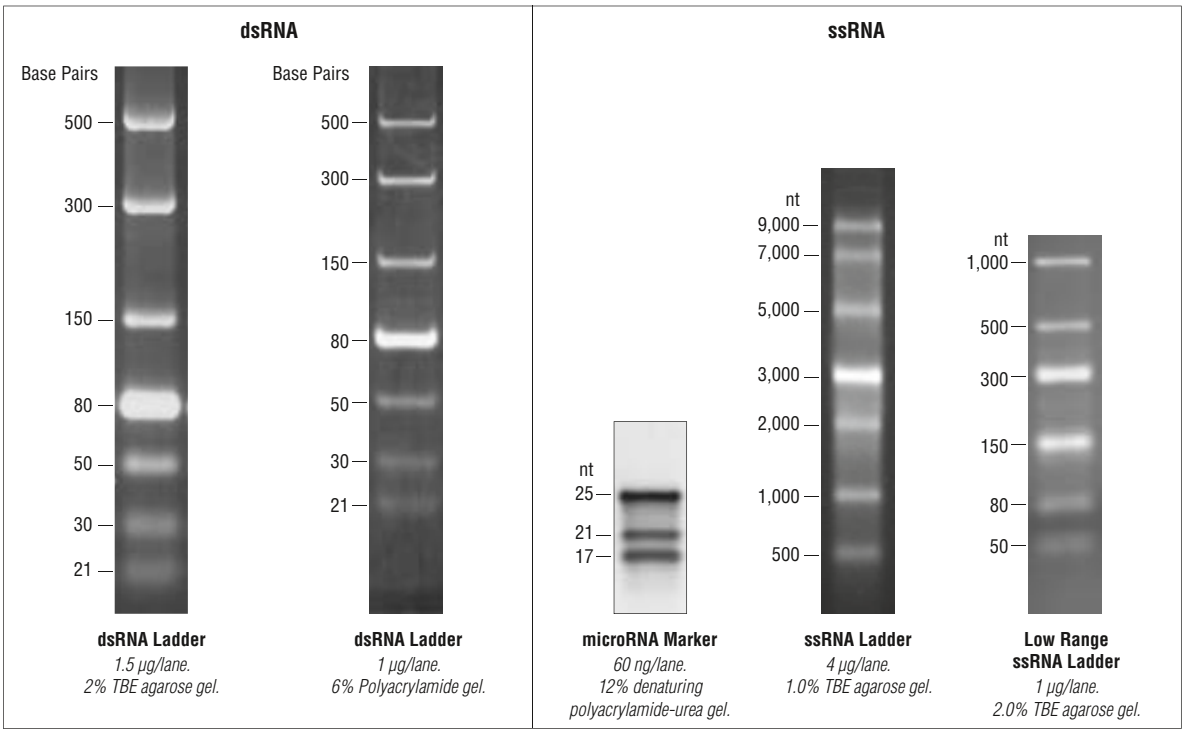
ssRNA Ladder
#N0362S 25 gel lanes

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 γ32-P-ATP and T4 PNK (NEB #M0201). The ds RNA Ladder is suitable for use as a size standard in dsRNA and RNAi analysis on both polyacrylamide and agarose gels.

Concentration: Low Range ssRNA Ladder and dsRNA Ladder are supplied at 500 µg/ml. ssRNA Ladder is supplied at 2,000 µg/ml. MicroRNA Marker is supplied at 12 ng/µl.

SYBR® is a registered trademark of Molecular Probes, Inc.



dsRNA Ladder
1.5 µg/lane.
2% TBE agarose gel.

dsRNA Ladder
1 µg/lane.
6% Polyacrylamide gel.

microRNA Marker
60 ng/lane.
12% denaturing polyacrylamide-urea gel.

ssRNA Ladder
4 µg/lane.
1.0% TBE agarose gel.

Low Range ssRNA Ladder
1 µg/lane.
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

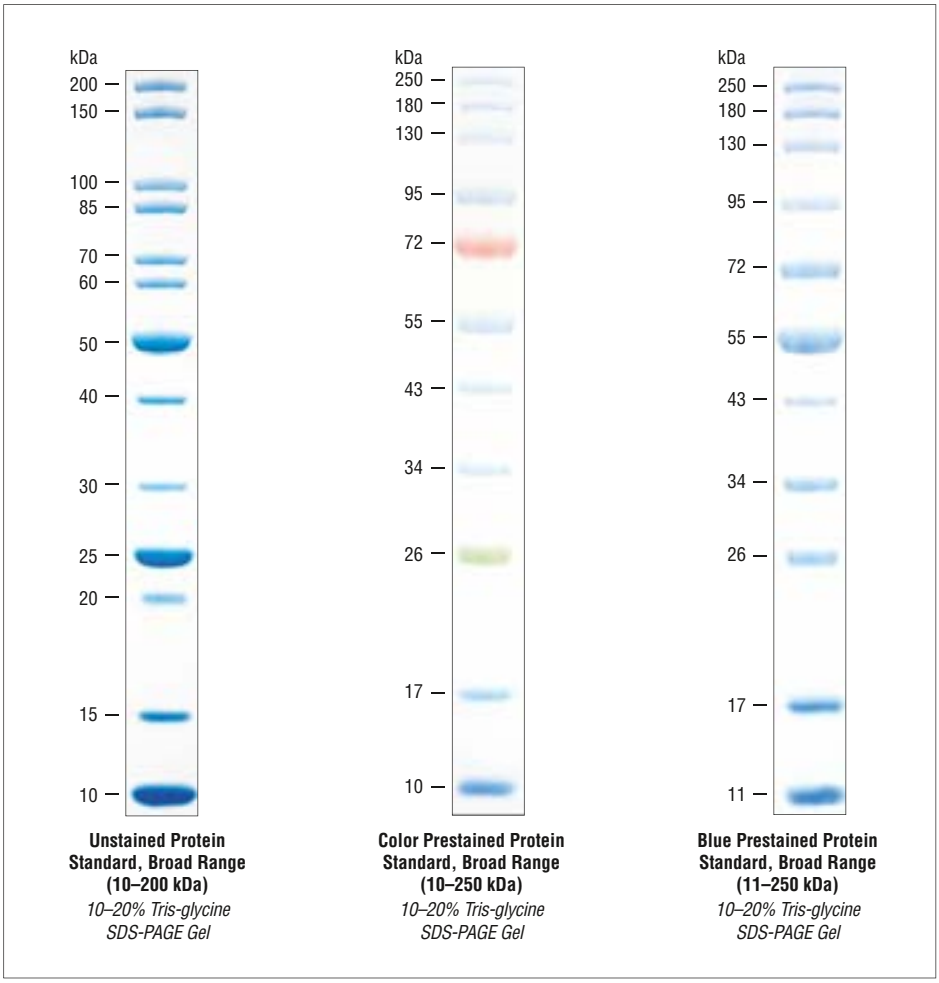
Companion Products:

Blue Protein Loading Dye
3X Blue Loading Buffer (8 ml)
30X Reducing Agent (1 ml)
#B7703S

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

Advancement of Science

Don Comb knew that the cultivation of scientific knowledge was critical to driving scientific breakthroughs. He took great joy in watching others learn and grow, and prioritized education and the sharing of knowledge and resources. While NEB has generously donated to numerous causes over the years, Don always felt that sharing science was the purest way to help communities and have the most significant impact.

Supporting the next generation of scientists, both locally and globally, has always been central to NEB's philosophy. This is done through hosting molecular biology courses, welcoming international students and scholars to the NEB campus, maintaining a rigorous postdoctoral program, and managing an active summer student intern program for college students. From the very beginning, NEB has donated reagents to support life science research in schools, community colleges, public and private universities, lab teaching programs, and synthetic biology competitions such as iGEM™ and BioBuilder®. From providing answers through technical support and the NEB Catalog to the sharing of online tools and educational content on our website, NEB has always made access to its scientific information a priority.

In the early days of NEB, Senior Scientist Rick Morgan's role was to find new enzymes in nature with exciting functionality to add to NEB's growing portfolio. In doing this, it occurred to both Don and Rick that the methods for screening the new samples involved relatively simple biology and could therefore be conducted in low resource countries. This, in turn, would help support research in communities that would otherwise not have that opportunity. Researchers from five developing countries came to NEB headquarters to learn molecular biology techniques that would allow them to analyze new samples and support their work. NEB also helped these researchers establish labs in their own countries — over a period of three decades these thriving labs discovered many new restriction endonucleases.

For over 35 years, NEB has collaborated with Smith College in Northampton, MA, to offer a two-week molecular biology program for scientists, lawyers, artists, graduate students, medical doctors, and the generally curious. In fact, anyone with an interest in molecular biology is welcome to participate. NEB provides sponsorships for teachers and people from developing countries. Often, the only way to learn is to roll up one's sleeves, and so the course is largely a practical laboratory experience, teaching foundational techniques and a big picture understanding of the molecular biology toolbox. This course has been attended by over 4,000 students to date.

More recently, "Kids Science Days" were introduced at NEB headquarters in Ipswich, MA. Local students (and teachers) are invited to participate in hands on experiments led by NEB scientists and staff. Seeing the curiosity in the eyes of the students, and being a part of that "aha" moment reminds us all why we are here.

Don's willingness to share what he had and what he knew has created a ripple effect of knowledge throughout research communities, including communities with fewer resources. He reminds us that we must all play a part in the advancement of science and supporting future generations of scientists.

Students participating in a demonstration at Ipswich High School, hosted by NEB. Credit: Bree Hall, NEB

Learn more about NEB's educational course support.

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

192 EnGen Mutation Detection Kit

193 EnGen sgRNA Synthesis Kit, *S. pyogenes*

190 EnGen Lba Cas12a (Cpf1)

190 EnGen Sau Cas9

Featured Tools & Resources

Visit www.neb.com/GenomeEditing for more information, including our feature article and latest brochure.

Learn more about genome editing.

FEATURED PRODUCTS SUPPORTING CRISPR WORKFLOWS

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	CRISPR/Cas9 APPLICATION	NEB #	SIZE
EnGen® Spy Cas9 NLS	*in vitro* cleavage of dsDNA. Genome engineering by direct introduction of active ribonucleoproteins. Recognizes 5´-NGG-3´ PAM.	M0646T/M	400/2,000 pmol
EnGen Mutation Detection Kit	Determination of the targeting efficiency of genome editing protocols	E3321S	25 rxns
EnGen sgRNA Synthesis Kit, *S. pyogenes*	Generation of microgram quantities of custom sgRNA. Recognizes 5´-NGG-3´ PAM.	E3322V/S	10/20 rxns
EnGen Spy Cas9 Nickase	*in vitro* nicking of dsDNA. Genome engineering by direct introduction of active nickase complexes. Recognizes 5´-NGG-3´ PAM.	M0650S/T	70/400 pmol
EnGen Spy dCas9 (SNAP-tag®)	Programmable binding of DNA. Compatible with SNAP-tag substrates for visualization and enrichment. Recognizes 5´-NGG-3´ PAM.	M0652S/T	70/400 pmol
EnGen Lba Cas12a (Cpf1)	*in vitro* cleavage of dsDNA. Genome engineering by direct introduction of active nuclease complexes. Signal generation for sequence detection assays. Recognizes 5´-TTTN PAM.	M0653S/T	70/2,000 pmol
EnGen Sau Cas9	*in vitro* cleavage of dsDNA. Genome engineering by direct introduction of active nuclease complexes. Recognizes 5´-NNGRRT-3´ PAM.	M0654S/T	70/400 pmol
Cas9 Nuclease, *S. pyogenes*	*in vitro* cleavage of dsDNA. Genome engineering by direct introduction of active ribonucleoproteins.	M0386S/T/M	70/400/ 2,000 pmol
Monarch® Total RNA Miniprep Kit	Purification of total RNA, with a binding capacity of up to 100 µg	T2010S	50 preps
Monarch RNA Cleanup Kit (50 µg)	Purification of sgRNA, with a capacity of up to 50 µg	T2040S/L	10/100 preps
Q5® Site-Directed Mutagenesis Kit (with or without competent cells)	Insertion of target sequence into a Cas9-sgRNA construct and modification of HDR templates	E0554S/E0552S	10 rxns
Q5 High-Fidelity DNA Polymerases	High-fidelity construct generation for use with CRISPR workflows and for sequencing	Multiple	Multiple
NEBuilder® HiFi DNA Assembly Master Mix	Single-tube, isothermal generation of the Cas9-sgRNA construct and HDR templates	E2621S/L/X	10/50/250 rxns
NEBuilder HiFi DNA Assembly Cloning Kit	Single-tube, isothermal generation of the Cas9-sgRNA construct and HDR templates	E5520S	10 rxns
HiScribe T7 ARCA mRNA Kit (with or without tailing)	Generation of Cas9 mRNA with ARCA cap	E2060S/E2065S	20 rxns
HiScribe T7 High Yield RNA Synthesis Kit	Generation of sgRNA and Cas9 mRNA	E2040S	50 rxns
HiScribe T7 Quick High Yield RNA Synthesis Kit	Generation of sgRNA and Cas9 mRNA	E2050S	50 rxns
T7 Endonuclease I	Determination of the editing efficiency of genome editing experiments	M0302S/L	250/1,250 units

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* and *S. aureus*, 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 #	FEATURES	SIZE
Cas9 Nuclease, *S. pyogenes*	M0386S/T/M	• 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	70/400/2,000 pmol
EnGen Spy Cas9 NLS	M0646T/M	• 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)	400/2,000 pmol
EnGen Spy Cas9 Nickase	M0650S/T	• 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)	70/400 pmol
EnGen Spy dCas9 (SNAP-tag)	M0652S/T	• 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)	70/400 pmol
EnGen Lba Cas12a (Cpf1)	M0653S/T	• 5´-TTTN-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 • *Lachnospiraceae* bacterium ND2006 (Lba) Cas12a maintains activity at lower temperatures than the *Acidaminococcus* orthologs, permitting editing in ectothermic organisms such as zebra fish and *xenopus* • High concentration enzyme can be used for microinjection, electroporation and lipofection	70/2,000 pmol
EnGen Sau Cas9	M0654S/T	• 5´-NNGRRT-3´ PAM • Dual NLS for improved transport to nucleus • High concentration enzyme can be used for microinjection electroporation and lipofection • Cleaves 3 bases upstream of PAM, blunt-ended cleavage	70/400 pmol
NEW **Tth Argonaute (TtAgo)**	M0665S	• Short 16-18 oligonucleotide 5´ phosphorylated ssDNA guides are cost effective and can be phosphorylated with T4 Polynucleotide Kinase • Guide target RNA sequence selection is not limited by the requirement of 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	50 pmol

EnGen Mutation Detection Kit

#E3321S 25 reactions

Companion Products:

Gel Loading Dye, Purple (6X), no SDS
#B7025S 4 ml

Quick-Load Purple 1 kb Plus DNA Ladder
#N0550S 250 gel lanes
#N0550L 750 gel lanes

Q5 Hot Start High-Fidelity 2X Master Mix
#M0494S 100 reactions
#M0494L 500 reactions
#M0494X 500 reactions

Monarch PCR & DNA Cleanup Kit (5 µg)
#T1030S 50 preps
#T1030L 250 preps

EnGen Spy Cas9 NLS
#M0646T 400 pmol
#M0646M 2,000 pmol

EnGen Spy Cas9 Nickase
#M0650S 70 pmol
for high (100X) concentrationn
#M0650T 400 pmol

EnGen Spy dCas9 (SNAP-tag)
#M0652S 70 pmol
for high (20X) concentration
#M0652T 400 pmol

Cas9 Nuclease, *S.pyogenes*
#M0386S 70 pmol
for high (20X) concentration
#M0386T 400 pmol
for high (20X) concentration
#M0386M 2,000 pmol

EnGen Lba Cas12a (Cpf1)
#M0653S 70 pmol
for high (100X) concentration
#M0653T 2,000 pmol

EnGen Sau Cas9
#M0654S 70 pmol
for high (20X) concentration
#M0654T 400 pmol

T7 Endonuclease I
#M0302S 250 reactions
#M0302L 1,250 reactions

- *T7 Endonuclease-based detection of genome editing events*

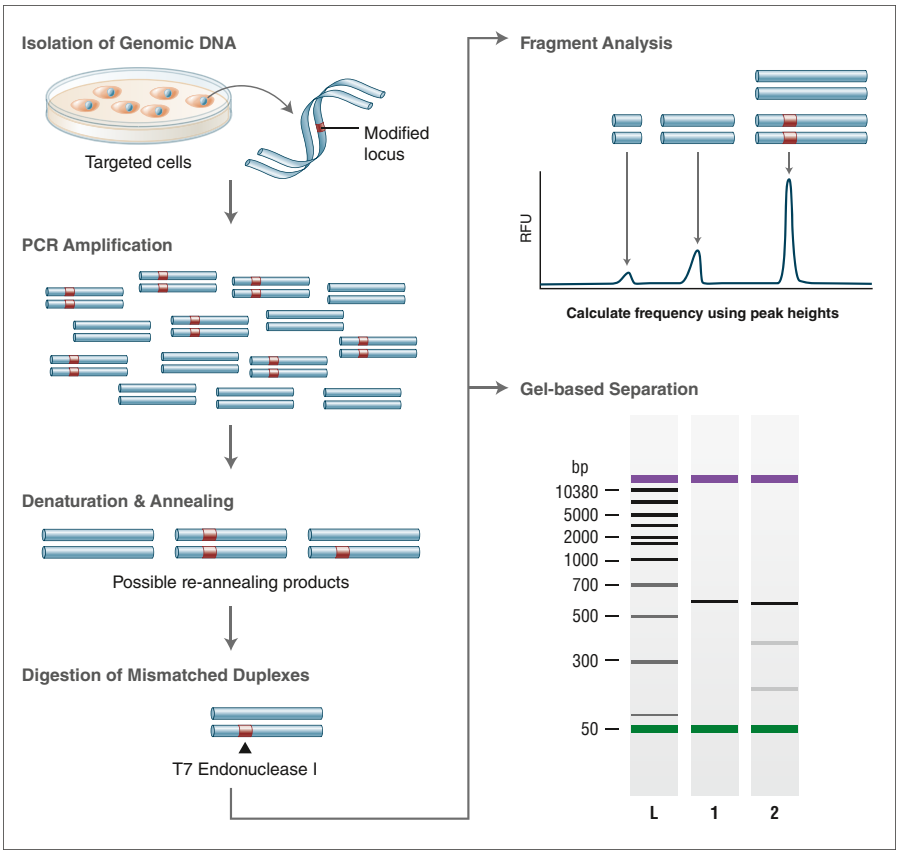
Description: The EnGen Mutation Detection Kit provides reagents for detection of on-target genome editing events. In the first step, regions targeted for genome editing (i.e. CRISPR/Cas9, TALENs, Zinc-finger Nucleases) are amplified using Q5 Hot Start High-Fidelity 2X Master Mix. Upon denaturation and 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.

The EnGen Mutation Kit Includes:

- Q5 Hot Start High-Fidelity 2X Master Mix
- NEBuffer 2
- EnGen T7 Endonuclease I
- 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



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*

EnGen sgRNA Synthesis Kit, *S. pyogenes*
#E3322V 10 reactions
#E3322S 20 reactions

Companion Products:

EnGen Spy Cas9 NLS
#M0646T 400 pmol
#M0646M 2,000 pmol

EnGen Spy Cas9 Nickase
#M0650S 70 pmol
for high (20X) concentration
#M0650T 400 pmol

EnGen Spy dCas9 (SNAP-tag)
#M0652S 70 pmol
for high (20X) concentration
#M0652T 400 pmol

Cas9 Nuclease, *S.pyogenes*
#M0386S 70 pmol
for high (20X) concentration
#M0386T 400 pmol
for high (20X) concentration
#M0386M 2,000 pmol

EnGen Mutation Detection Kit
#E3321S 25 rxns

RNA Loading Dye, (2X)
#B0363S 4 ml

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

Monarch RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

DNase I (RNase-free)
#M0303S 1,000 units
#M0303L 5,000 units

- *Rapid generation of microgram quantities of sgRNAs in less than one hour*

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**

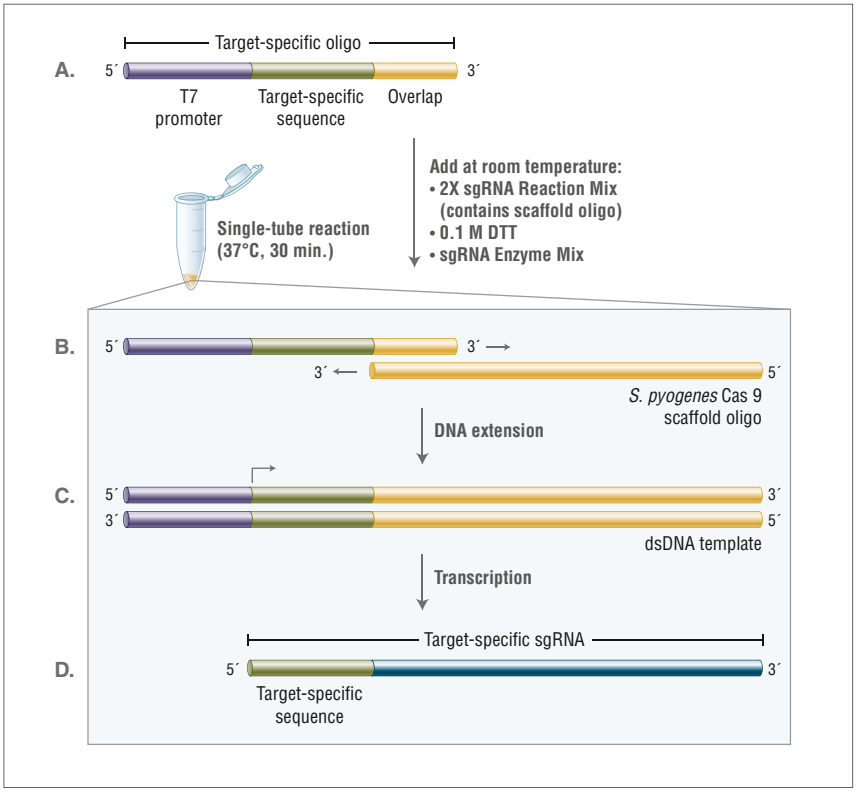
Description: The EnGen sgRNA Synthesis Kit, *S. pyogenes* provides a simple and quick method for transcribing high yields of sgRNA in a single 30 minute reaction, using the supplied reagents and target-specific DNA oligos designed by the user.

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.

The EnGen sgRNA Synthesis Kit, *S. pyogenes* Includes:

- EnGen sgRNA Enzyme Mix
- EnGen 2X sgRNA Reaction Mix, *S. pyogenes*
- DNase I (RNase-free)
- EnGen sgRNA Control Oligo, *S. pyogenes*
- DTT (0.1 M)



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.*

Recognizing Those Making a Difference

In 1974, Don Comb established New England Biolabs with the goal of providing research tools for the life science community. His devotion to the advancement of science, stewardship of the environment and altruistic philanthropy has been a priority since the beginning, and is reflected in NEB's three core values: passion, humility and being genuine. For over 45 years, these values have served as our inspiration, and they continue to guide us in both our science and our business practices.

In 2014, NEB was coming upon its 40th anniversary, and had been considering ways to recognize this milestone. It didn't feel right to celebrate ourselves — rather, we felt it was important to celebrate our customers who shared the values we so deeply embrace. This was the inspiration for our Passion in Science Awards®. We introduced these awards as a way to recognize members of the scientific community who are committed to making a difference through their science, service, stewardship or artistic endeavors. Based on NEB's foundational values, the Passion in Science Awards were offered in four categories: Scientific Mentorship and Advocacy, Humanitarian Duty, Environmental Stewardship, and Arts and Creativity.

The Passion in Science Awards were first hosted in 2014, and then again in 2016 and 2019 — with over 40 exceptional scientists being recognized to date. Award winners visited NEB's campus in Ipswich, MA for two days of engaging discussions, inspiring presentations, tours of NEB's facilities and an awards celebration. No one anticipated how memorable these events would be.

In 2014, awardee Paul McDonald shared insight into Army policy and practices that were resulting in high-risk behavior, including suicide. Also that year, Shelly Xie told stories of people affected by neglected tropical diseases through a touching sand art presentation. In 2016, Chris Martine described how he is sharing his passion for botany with the next generation of scientists, and Lisa Anderson taught us how laboratory gloves can be upcycled into new materials. In 2019, we learned about new ways science can be shared from several of our winners — Sarah Fankhauser established a peer-reviewed publication for middle- and high-school students, Sarah McAnulty founded Skype a Scientist, Garfield Kwan introduced us to SquidToons, scientifically-accurate comics designed to engage a broader audience, and Will Murta told the story of the discovery of DNA through a musical. And the list goes on….

These events have been most inspiring and humbling for the NEB community — hearing the stories of these "unsung heroes" have taught us how we can find ways, big and small, to give back to our communities and the world. The contributions of each winner aligned with the values that Don has inspired in us all, and helped to create a true celebration of the principles that guide New England Biolabs.

To learn more about our Passion in Science Winners, visit www.neb.com/PassionInScience.

Science and art come together in this sculpture displayed outside the NEB laboratory facility. Sculpture by Dale Rogers. Credit: Kari Goodwin, NEB.

Learn more about the Passion in Science Awards.

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 influences 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 analyses of gene function not previously possible.

Introducing 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

200 HiScribe™ T7 ARCA mRNA Kit (with tailing)

199 HiScribe T7 High Yield RNA Synthesis Kits

208 Template Switching RT Enzyme Mix

205 mRNA Decapping Enzyme

Featured Tools & Resources

198 Avoiding Ribonuclease Contamination

210 RNA Ligase Activity Chart

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.

RI Recombinant Enzyme

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 groups 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. These enzymes 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 in a typical laboratory include:

- Aqueous solutions, reagents used in experiments
- Exposure to RNase from environmental sources (lab surfaces, aerosols from pipetting, ungloved hands, etc.)
- Contaminated reagents

Laboratory Precautions (2,3):

New England Biolabs' enzymes certified RNase-free work have been purified to be free of ribonucleases. However, it is possible to reintroduce RNases during the course of experimentation from various sources. RNase contamination can be prevented by following 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 one 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 or 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, Murine, (NEB #M0314) is a recombinant protein RNase inhibitor of murine origin. Like human and porcine RNAse, it is a specific inhibitor of RNases A, B and C, but is more stable due to improved resistance to oxidation (4). The inhibitor requires low concentration of DTT (< 1 mM) to maintain activity, making it ideal for reactions where low DTT concentration is required (e.g., real-time RT-PCR).
- RNase Inhibitor, Human Placenta, (NEB #M0307), a recombinant protein of human placental origin, 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.).
- 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).

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., Fritsch, E.F. and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual,* (2nd ed.), (pp. 7.3–7.5). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
(4) Kim, B.M. et al. (1999) *Protein Science,* 8, 430–434.
(5) Berger, S.L. (1987) *Methods Enzymol.,* 152, 227–234.

Find tips for avoiding RNA contamination.

HiScribe™ T7 High Yield RNA Synthesis Kits

HiScribe T7 High Yield RNA Synthesis Kit
#E2040S 50 reactions

HiScribe T7 Quick High Yield RNA Synthesis Kit
#E2050S 50 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*

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 transcripts. 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 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

The HiScribe T7 Quick High Yield RNA Synthesis Kit Includes:
- T7 RNA Polymerase Mix
- NTP Buffer Mix
- FLuc Control Template
- DNase I (RNase-free)
- LiCl Solution

HiScribe SP6 RNA Synthesis Kit

#E2070S 50 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*

Use Monarch RNA Cleanup Kits to purify your synthesized RNA, see page 137–138.

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 high specific activity 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.

The HiScribe SP6 RNA Synthesis Kit Includes:
- SP6 Reaction Buffer (10X)
- ATP (Tris), GTP (Tris), UTP (Tris), CTP (Tris), (50 mM)
- SP6 Control Template
- SP6 RNA Polymerase Mix
- DNase I (RNase-free)
- LiCl Solution

Companion Products:

DNase I (RNase-Free)
#M0303S 1,000 units
#M0303L 5,000 units

RNA Loading Dye (2X)
#B0363S 4 ml

Vaccinia Capping System
#M2080S 400 units

Q5® Hot Start High-Fidelity DNA Polymerase
#M0493S 100 units
#M0493L 500 units

Monarch® PCR & DNA Cleanup Kit
#T1030S 50 preps
#T1030L 250 preps

Monarch RNA Cleanup Kit (10 µg)
#T2030S 10 preps
#T2030L 100 preps

Monarch RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

Monarch RNA Cleanup Kit (500 µg)
#T2050S 10 preps
#T2050L 100 preps

Low Range ssRNA Ladder
#N0364S 25 gel lanes

E. coli Poly(A) Polymerase
#M0276S 100 units
#M0276L 500 units

mRNA Cap 2´-O-Methyltransferase
#M0366S 2,000 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

HiScribe T7 ARCA mRNA 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

- *Synthesis of capped and tailed mRNA*
- *Incorporation of modified nucleotides*
- *Template removal and mRNA purification reagents included*

Description: Most eukaryotic mRNAs require a 7-methyl guanosine (m7G) 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 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

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

Advantages:
- Quick reaction setup and streamlined protocol
- Enables partial incorporation of 5mCTP, Pseudo-UTP and other modified CTP and UTP
- Ultra-high quality components ensure mRNA integrity

EnGen® sgRNA Synthesis Kit, *S. pyogenes*

See page 179 for more information.

#E3322V 10 reactions
#E3322S 20 reactions

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.

Bijoyita has been with NEB for four years. In her role as Senior Scientist, Bijoyita leads a research group focused on synthetic mRNA synthesis and modification. In her spare time, Bijoyita likes to travel and try new cuisines.

Recommended HiScribe RNA Synthesis Kits by Application

APPLICATION	T7 KITS				SP6 KITS
	HiScribe T7 High Yield RNA Synthesis Kit (#E2040)	HiScribe T7 Quick High Yield RNA Synthesis Kit (#E2050)	HiScribe T7 ARCA mRNA Kit (#E2065)	HiScribe T7 ARCA mRNA (with Tailing) (#E2060)	HiScribe SP6 RNA Synthesis Kit (#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 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, etc. • Post-transcriptional capping with Vaccinia mRNA Capping System	✔				✔
Partial substitution of NTPs: 5-mC, pseudouridine, 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

RR 37°

T3 RNA Polymerase
#M0378S 5,000 units

T7 RNA Polymerase
#M0251S 5,000 units
#M0251L 25,000 units

SP6 RNA Polymerase
#M0207S 2,000 units

NEW
T7 RNA Polymerase (High Concentration)
for high (20X) concentration
#M0460T 50,000 units

Hi-T7 RNA Polymerase
#M0658S 5,000 units

NEW
Hi-T7 RNA Polymerase (High Concentration)
for high (20X) 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*

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 acid-insoluble 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

RR 37°

#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.

Reaction Conditions: 1X Poly(A) Polymerase Reaction Buffer. Supplement with 1 mM ATP. Incubate at 37°C.

Reagents Supplied with Enzyme:
10X Poly(A) Polymerase Reaction Buffer
10 mM 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. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

Poly(U) Polymerase

RR 37° 65°

#M0337S 60 units

Companion Products:

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

Ribonucleotide Solution Set
#N0450S 10 µmol of each
#N0450L 50 µmol of each

- *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.

Reaction Conditions: 1X NEBuffer 2. Supplement with 0.5 mM UTP (not supplied). Incubate at 37°C. May heat inactivate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 2,000 units/ml

Notes: Poly(U) Polymerase in NEBuffer 2 will incorporate UMP or AMP from UTP or ATP into RNA. Tailing length of poly(U) varies with UTP and primer concentration. 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

NEW
NudC Pyrophosphatase
#M0607S 250 pmol

- *Increasing RNA yield in transcription reactions*
- *Enhancing DNA replication*

Thermostable Inorganic Pyrophosphatase retains 100% activity after incubation at 100°C for 4 hours.

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. Unit assay conditions can be found at www.neb.com.

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 Pyrosphatase: 10 µM

Note: This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

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. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

Ribonucleotides

Ribonucleotide Solution Set
#N0450S 10 µmol of each
#N0450L 50 µmol of each

Ribonucleotide Solution Mix
#N0466S 10 µmol of each
#N0466L 50 µmol of each

Description:
Ribonucleotide Solution Set:
The 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:
A buffered equimolar solution of ribonucleotide triphosphates: rATP, rCTP, rGTP and rUTP, pH 7.5, as sodium salts. Each nucleotide is supplied at a concentration of 25 mM (total rNTP concentration equals 100 mM).

Note: To ensure maximum activity upon long-term storage, aliquot and store at –80°C.

NEW Sce Pus1

#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: 1X NEBuffer r1.1. Incubate at 30°C.

Concentration: 100 pmol/µl

Note: This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

Sce PUS1 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.

Vaccinia Capping System

RR 37°

#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*

Description: Based on the Vaccinia Virus Capping Enzyme, the Vaccinia Capping System provides the necessary components to add 7-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.

Reaction Conditions: 1X Capping Buffer. Supplement with 0.5 mM GTP and 0.1 mM SAM. Incubate at 37°C.

Reagents Supplied:
Vaccinia Capping Enzyme
Capping Buffer (10X)
GTP Solution (10 mM)
SAM Solution (32 mM)

Unit Definition: One unit is defined as the amount of enzyme required to incorporate 10 pmol of (α-^{32}P) GTP into an 80 nucleotide transcript in 1 hour at 37°C.

Concentration: 10,000 units/ml

mRNA Cap 2´-*O*-Methyltransferase

Cloned at NEB RR 37°

#M0366S 2,000 units

- *Enhances translation of RNA*
- *Improving mRNA expression during microinjection and transfection*

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.

Reaction Conditions: 1X Capping Buffer. Supplement with 0.2 mM SAM (supplied). Incubate at 37°C.

Reagents Supplied:
Capping Buffer (10X)
SAM (32 mM)

Unit Definition: One unit is defined as the amount of enzyme required to methylate 10 pmol 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^7G 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	APPLICATION	SIZE
(ARCA) Anti-Reverse Cap Analog 3´-O-Me-m^7G(5´) ppp(5´)G (#S1411S/L)	• Produces 100% translatable capped transcripts • Co-transcriptional capping with T7 (NEB #M0251), SP6 (NEB #M0207) and T3 RNA polymerases • Synthesis of m^7G capped RNA for *in vitro* splicing assays • Synthesis of m^7G capped RNA for transfection or microinjection • Ensures incorporation in correct orientation	1/5 µmol
Standard Cap Analog m^7G(5´)ppp(5´)G (#S1404S/L)	• Co-transcriptional capping with T7, SP6 and T3 RNA polymerases • Synthesis of m^7G capped RNA for *in vitro* splicing assays • Synthesis of m^7G capped RNA for transfection or microinjection	1/5 µmol
Unmethylated Cap Analog G (5´)ppp(5´)G (#S1407S/L)	• Co-transcriptional capping with T7, SP6 and T3 RNA polymerases • Synthesis of unmethylated G capped RNA	1/5 µmol
Methylated Cap Analog for A +1 sites m^7G(5´)ppp(5´)A (#S1405S/L)	• Co-transcriptional capping with T7 RNA polymerase from the phi2.5 promoter that contains an A at the transcription initiation site • Synthesis of m^7G capped RNA for *in vitro* splicing assays • Synthesis of m^7G capped RNA for transfection or microinjection	1/5 µmol
Unmethylated Cap Analog for A +1 sites G(5´)ppp(5´)A (#S1406S/L)	• 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	1/5 µmol

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

RR NEBU 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^7G capped mRNA, leaving behind a diphosphorylated 5´ end and m^7GMP. yDcpS is capable of decapping full length mRNAs and the diphosphorylated 5´ end it leaves behind is suitable for recapping using Vaccinia Capping Enzyme.

Reaction Conditions: 1X yDcpS Reaction Buffer. Incubate at 37°C.

Concentration: 200,000 units/ml

NEW

mRNA Decapping Enzyme

RR e NEBU 37° No

#M0608S 2,000 units

- *Efficient replacement for Tobacco Acid Pyrophosphatase*
- *Cap0 and Cap1 are removed with equal efficiency*
- *Suitable for 5´ RLM-RACE and RNA-seq*

Description: mRNA Decapping Enzyme catalyzes the removal of 7-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 Cap0 and Cap1 structures with similar efficiency. mRNA Decapping Enzyme also converts 5´ triphosphate ends to 5´ monophosphate, albeit with reduced efficiency.

Reaction Conditions: 1X mRNA Decapping Enzyme Reaction Buffer. Incubate at 37°C.

Concentration: 100,000 units/ml

Sharon has been the Director of Information Technology for over 7 years, making sure that all of NEB's IT processes run smoothly, both internally and externally. When not at work, Sharon enjoys mountain hiking and creating fabric art.

cDNA Synthesis Selection Chart

cDNA SYNTHESIS	FEATURES	SIZE
KITS AND MIXES		
LunaScript® RT SuperMix Kit (NEB #E3010)	• Ideal for cDNA synthesis in a two-step RT-qPCR workflow • 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 • Primer-free master mix available (NEB #E3025)	25/100 rxns
ProtoScript® II First Strand cDNA Synthesis Kit (NEB #E6560)	• Generates cDNA at least 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	30/150 rxns
ProtoScript First Strand cDNA Synthesis Kit (NEB #E6300)	• 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	30/150 rxns
Template Switching RT Enzyme Mix (NEB #M0466)	• Incorporates a universal adaptor sequence at the 3´ end of cDNA during the RT reaction • Enzyme mix and buffer are optimzed 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	20/100 rxns
STANDALONE REAGENTS		
ProtoScript II Reverse Transcriptase (NEB #M0368) An alternative to SuperScript® II	• RNase H⁻ mutant of M-MuLV Reverse Transcriptase with increased thermostability and reduced RNase H activity • Increased reaction temperatures (37–50°C)	4,000/10,000/40,000 units
M-MuLV Reverse Transcriptase (NEB #M0253)	• Robust reverse transcriptase for a variety of templates • Standard reaction temperatures (37–45°C)	10,000/50,000 units
AMV Reverse Transcriptase (NEB #M0277)	• Robust reverse transcriptase for a broad temperature range (37–52°C) • Can be used for templates requiring higher reaction temperatures	200/1,000 units
WarmStart® RTx Reverse Transcriptase (NEB #M0380)	• Permits room temperature reaction setup • Increased reaction temperatures (50–65°C) • Optimized for RT-LAMP isothermal detection	50/250 rxns

For RT-PCR kits see page 81. *For RT-qPCR kits see page 74.*

ProtoScript® II Reverse Transcriptase

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 Total RNA Miniprep Kit

#T2010S	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⁻.

Reaction Conditions: 1X ProtoScript II Reverse Transcriptase Reaction Buffer, 10 mM DTT, 200 units M-MuLV (RNase H⁻), supplemented with 0.5 mM dNTPs (not included) and 5 µM $dT_{23}VN$ (not included). Incubate at 42°C for 50 minutes. If random primers are used, a 10 minute incubation at room temperature is recommended before transferring to 42°C. May heat inactivate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 200,000 units/ml

LunaScript® RT SuperMix Kit & LunaScript® RT Master Mix Kit (Primer-free)

See page 73 for more information.

LunaScript RT SuperMix Kit
#E3010S 25 reactions
#E3010L 100 reactions

NEW
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

#M0253S 10,000 units
#M0253L 50,000 units

Companion Product:
Monarch Total RNA Miniprep Kit
#T2010S 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.

Reaction Conditions: 1X M-MuLV Reverse Transcriptase Reaction Buffer. Supplement with dNTPs (not included). Incubate at 37–42°C. May heat inactivate at 65°C for 20 minutes.

Unit Definition: One unit is the amount of enzyme required to incorporate 1 nmol of dTTP into an acid-insoluble material in 10 minutes at 37°C using poly(rA)•oligo(dT) as template primer. Unit assay conditions can be found at www.neb.com.

Concentration: 200,000 units/ml

AMV Reverse Transcriptase

#M0277S 200 units
#M0277L 1,000 units

Companion Product:
Monarch Total RNA Miniprep Kit
#T2010S 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: Avian Myeloblastosis Virus (AMV)

Reaction Conditions: 1X AMV Reverse Transcriptase Reaction Buffer. Supplement with dNTPs (not included). Incubate at 37–42°C. May heat inactivate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 10,000 and 25,000 units/ml

Storage Note: 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

#M0380S 50 reactions
#M0380L 250 reactions

Companion Product:
Monarch Total RNA Miniprep Kit
#T2010S 50 preps

- *RT-LAMP*
- *cDNA Synthesis*
- *RT reactions requiring room temperature setup*

For more information on products for LAMP, see pages 76–79.

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 from 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 the consistency and specificity of amplification reactions. RTx contains intact RNase H activity.

Reaction Conditions: 1X Isothermal Amplification Buffer, 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 One-step RT-LAMP. May heat inactivate at 80°C for 10 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 20 minutes at 50°C using poly(rA)•oligo(dT)18 as template.

Concentration: 15,000 units/ml

Template Switching RT Enzyme Mix

#M0466S 20 reactions
#M0466L 100 reactions

Companion Products:

NEBNext High-Fidelity 2X PCR Master Mix
#M0541S 50 reactions
#M0541L 250 reactions

Q5 Hot Start High-Fidelity 2X Master Mix
#M0494S 100 rxns (2 x 1.25 ml)
#M0494L 500 rxns (10 x 1.25 ml)
#M0494X 500 rxns (1 x 12.5 ml)

NEB PCR Cloning Kit
#E1202S 20 reactions

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

LongAmp Hot Start *Taq* 2X Master Mix
#M0533S 100 reactions
#M0533L 500 reactions

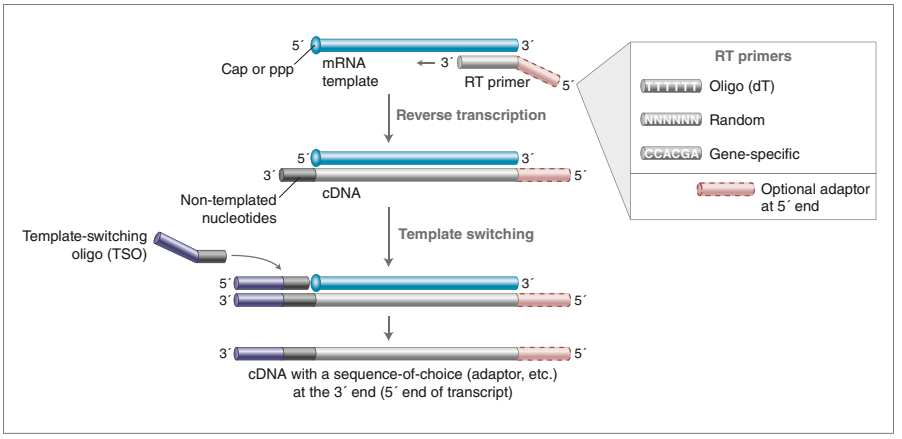
- Incorporates a universal adaptor sequence at the 3´ end of cDNA during the RT reaction
- High sensitivity for cDNA amplification – enables transcriptome analysis by RNA-seq from single cells or as low as 2 pg of human total RNA
- Robust and simple workflow for 5´-Rapid Amplification of cDNA Ends (RACE)
- 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 Enzyme Mix (10X)
Template Switching RT Buffer (4X)



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 (5´d(N$_6$)3´) ~14.6 nmol	S1230S	1.0 A_{260} unit
Random Primer 9 (5´d(N$_9$)3´) ~11.6 nmol	S1254S	1.0 A_{260} unit
Oligo d(T)$_{23}$ VN	S1327S	1.0 A_{260} unit
Random Primer Mix	S1330S	100 µl (60 µM)
Oligo d(T)$_{18}$ mRNA Primer	S1316S	5.0 A_{260} units

ProtoScript II First Strand cDNA Synthesis Kit

RR

#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

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

Random Primer Mix
#S1330S 100 µl (60 µM)

Oligo $d(T)_{23}VN$*
#S1327S 1.0 A_{260} unit

*Note: V = A, G or C, and N = A, G, C or T

- *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.

The ProtoScript II First Strand cDNA Synthesis Kit Includes:

– 10X ProtoScript II Enzyme Mix
– 2X ProtoScript II Reaction Mix
– Random Primer Mix (60 µM), Oligo $d(T)_{23}VN$ Primer (50 µM)** and Nuclease-free Water

**Oligo $d(T)_{23}$ VN and Random Primer Mix contain 1 mM dNTP

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

RR

#E6300S 30 reactions
#E6300L 150 reactions

Companion Products:

Magnetic mRNA Isolation Kit
#S1550S 25 isolations

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

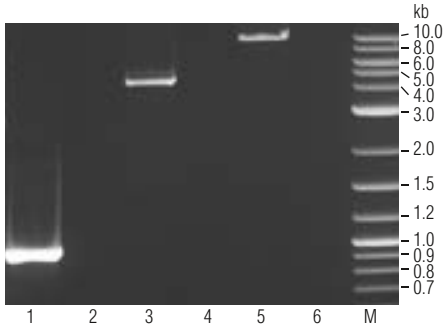
- *Enzyme and reaction mixes add flexibility to reaction setup*
- *Suitable for any PCR format*
- *Efficient reverse transcription from different starting RNA amounts*
- *Generates cDNA at least 5 kb*

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.

The ProtoScript First Strand cDNA Synthesis Kit Includes:

– 10X ProtoScript Enzyme Mix
– 2X ProtoScript Reaction Mix
– Random Primer Mix (60 µM), Oligo $d(T)_{23}VN$ Primer (50 µM)** and Nuclease-free Water

**Oligo $d(T)_{23}$ VN and Random Primer Mix contain 1 mM dNTP



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).*

RT-PCR & RT-qPCR Kits

RR

See pages 74–77 for more information.

NEW
Luna® Probe One-Step RT-qPCR Kit (No ROX)
#E3007E 2,500 reactions

NEW
Luna Probe One-Step RT-qPCR 4X Mix with UDG
#M3019S 200 reactions
#M3019L 500 reactions
#M3019X 1,000 reactions
#M3019E 2,000 reactions

NEW
Luna Cell Ready One-Step RT-qPCR Kit
#E3030S 100 reactions

NEW
Luna Cell Ready Probe One-Step RT-qPCR Kit
#E3031S 100 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

One*Taq*® One-Step RT-PCR Kit
#E5315S 30 reactions

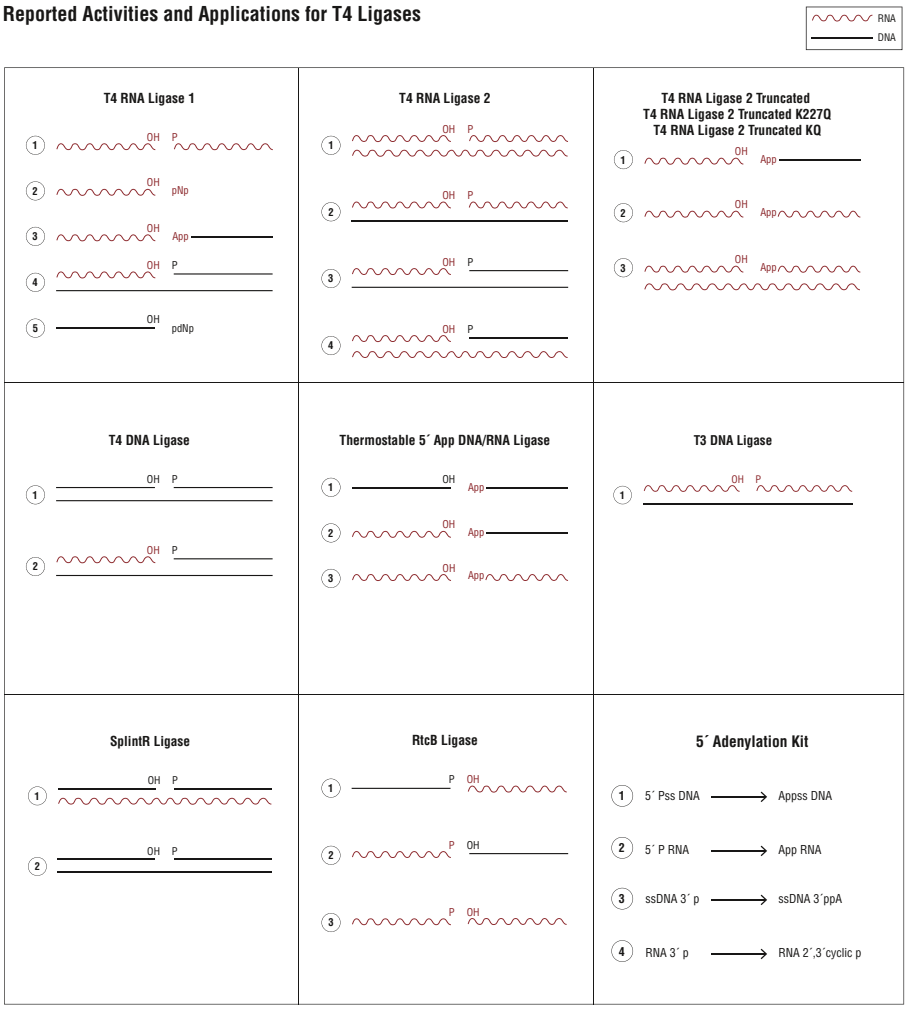
One*Taq* RT-PCR Kit
#E5310S 30 reactions

NEW
Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit
#E3019S 96 reactions
#E3019L 480 reactions

RNA Ligase Activity Chart

NEB offers a variety of ligases for DNA and RNA research. Many of these enzymes are recombinant and all offer the quality and value you have come to expect from our products. The chart below highlights reported activities of our T4 ligases ranked by application. A substrate-based selection chart for DNA ligases can be found on page 100.

Reported Activities and Applications for T4 Ligases



The ligation activities depicted have been reported, but may require optimized reaction conditions.

RNA Ligase Selection Chart

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´ App DNA/RNA Ligase	5´ Adeny-lation Kit	SplintR® Ligase	RtcB Ligase
RNA APPLICATIONS									
Ligation of 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	✔	✔	✔	✔	✔	✔	✔	✔	✔

★★★ 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

Michael is a Development Scientist in our Organic Synthesis Division, developing and producing nucleotides, oligonucleotides and beaded resins. When he is not in the lab, Michael enjoys the outdoors, fitness, reading, and music.

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´-[32P] pCp*
- *Inter- and intramolecular joining of RNA and DNA molecules*
- *Synthesis of ss-oligodeoxyribonucleotides*
- *Incorporation of unnatural amino acids into proteins*

Description: Catalyzes ligation of a 5´ phosphoryl-terminated 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.

Reaction Conditions: 1X T4 RNA Ligase Reaction Buffer. Supplement with 1 mM ATP. Incubate at 25°C. May heat inactivate at 65°C for 15 minutes or boiling for 2 minutes.

Notes on Use: Addition of DMSO to 10% (v/v) is required for pCp ligation.

Reagents Supplied with Enzyme:
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´-[32P] rA16 into a phosphate resistant form in 30 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

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.

Reaction Conditions: 1X T4 RNA Ligase 2 Reaction Buffer. Incubate at 37°C. May heat inactivate at 80°C for 5 minutes.

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. Unit assay conditions can be found at www.neb.com.

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*
- *Join a single-stranded adenylated primer to RNA for strand-specific cDNA library construction*

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.

Reaction Conditions: 1X T4 RNA Ligase Reaction Buffer. Incubate at 25°C. May heat inactivate at 65°C for 20 minutes.

Reagents Supplied with Enzyme:
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. Unit assay conditions can be found at www.neb.com.

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*
- *Join a single stranded adenylated primer to RNA for strand-specific cDNA library construction*

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 pre-adenylated 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, 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. Incubate at 25°C. May heat inactivate at 65°C for 20 minutes.

Reagents Supplied with Enzyme:
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. Unit assay conditions can be found at www.neb.com.

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*

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.

Description: RtcB Ligase from *E. coli* joins single stranded RNA with a 3´-phospate 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 His-tagged fusion in *E. coli*.

Reaction Conditions: 1X RtcB Reaction Buffer. Supplement with 0.1 mM GTP and 1 mM $MnCl_2$ (supplied). Incubate at 37°C.

Reagents Supplied with Enzyme:
10X RtcB Reaction Buffer
$MnCl_2$ (10 mM)
GTP (10 mM)

Concentration: 15 µM

Thermostable 5´ AppDNA/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 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.

Reaction Conditions: 1X NEBuffer 1. Incubate at 65°C.

Reagents Supplied with Enzyme:
10X NEBuffer 1
10X $MnCl_2$

Concentration: 20 µM

Usage Note: For optimal ligation of ssDNA to preadenylated linkers, we recommend using NEBuffer 1 supplemented with manganese (supplied).

5´ DNA Adenylation Kit

RR 65°

#E2610S 10 reactions
#E2610L 50 reactions

- *Enzymatic 5´ adenylation of ss-DNA 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.

The 5´ DNA Adenylation Kit Includes:
– *Mth* RNA Ligase (Recombinant)
– 5´ DNA Adenylation Reaction Buffer (10X)
– 1 mM ATP

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

RR 25° 65°

#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: 1X SplintR Ligase Reaction Buffer. Incubate at 25°C. May heat inactivate at 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. Unit assay conditions can be found at www.neb.com.

Concentration: 25,000 units/ml

RNA 5´ Pyrophosphohydrolase (RppH)

RR 37°

#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.

Reaction Conditions: 1X NEBuffer 2. Incubate at 37°C.

Reagents Supplied with Enzyme:
10X NEBuffer 2

Unit Definition: One unit is defined as the amount of enzyme that converts 1 µg 300-mer RNA transcript into an XRN-1 digestible RNA in 30 minutes at 37°C.

Concentration: 5,000 units/ml

5´ Deadenylase

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.

Reaction Conditions: 1X NEBuffer 1 and 5–50 pmol adenylated DNA (AMP-DNA) in 20 µl. Incubate at 30°C. May heat inactivate at 70°C for 20 minutes.

Unit Definition: One unit is defined as the amount of 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

RNase I_f

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.

Reaction Conditions: 1X NEBuffer 3. Incubate at 37°C. May heat inactivate at 70°C for 20 minutes.

Notes on Use: RNase I_f will not degrade DNA. It has a strong preference for single-stranded RNA over double-stranded RNA.

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 (40:1 Bis) stained with SYBR® Gold. Unit assay conditions can be found at www.neb.com.

Concentration: 50,000 units/ml

SYBR® is a registered trademark of Molecular Probes, Inc.

RNase H

RR 37° 65°

#M0297S 250 units
#M0297L 1,250 units

- *Removal of poly(A) tails of mRNA hybridized to poly(dT)*
- *Removal of mRNA during second strand cDNA synthesis*

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.

Reaction Conditions: 1X RNase H Reaction Buffer. Incubate at 37°C. May heat inactivate at 65°C for 20 minutes.

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. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

Thermostable RNase H

RR 50° No

#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.

Reaction Conditions: 1X RNase H Reaction Buffer. Incubate at 50°C.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of ribonucleotides from 40 picomoles of a fluorescently labelled 25 base pair RNA-DNA hybrid in a total reaction volume of 50 µl in 20 minutes at 50°C. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

RNase HII

RR 37° No

#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: 1X ThermoPol Reaction Buffer. Incubate at 37°C.

Unit Definition: One unit is defined as the amount of enzyme required to yield a fluorescence signal consistent with the nicking of 100 pmol 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 Reaction Buffer. Unit assay conditions can be found at www.neb.com.

Concentration: 5,000 units/ml

Note: Incubation with 0.1% SDS is sufficient to inactivate RNase HII.

See pages 104–106 for more information.

NEW

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

ShortCut® RNase III

RRI 37° No

#M0245S	200 units
#M0245L	1,000 units

- *Generates siRNAs for RNA interference studies*
- *Gene silencing*
- *Target validation*
- *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. 1.5 units (1 µl) of ShortCut RNase III is sufficient to convert 1 µg of dsRNA into siRNA.

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).

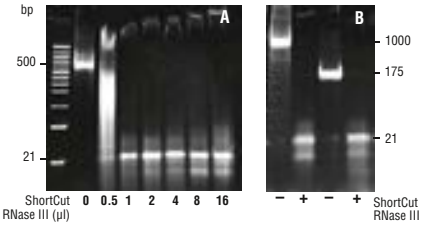
Reaction Conditions: 1X ShortCut RNase III Reaction Buffer. Supplement with 20 mM $MnCl_2$ (supplied). Incubate at 37°C.

Unit Definition: One unit is the amount of enzyme required to digest 1 µg of dsRNA to siRNA in a total reaction volume of 50 µl in 20 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 2,000 units/ml

Advantages of the ShortCut RNase III:

- Make effective siRNAs against any gene target
- Heterogeneous population of siRNA ensures silencing of target gene
- From DNA template to transfection in just 1 day
- Eliminates trial and error approach of synthetic siRNA



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.*

XRN-1

RRI 37° 70°

#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.

Reaction Conditions: 1X NEBuffer 3. Incubate at 37°C. May heat inactivate at 70°C for 10 minutes.

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

#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 Exo T by Factor Xa cleavage and then purified. When 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: 1X NEBuffer 4. Incubate at 25°C. May heat inactivate at 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 0.1 nmol of TCA soluble nucleotides from 1 nmol of [^{3}H]-labeled polythymidine in a total reaction volume of 100 µl in 30 minutes at 25°C in 1X NEBuffer 4 with 1 nmol [^{3}H]-labeled polythymidine DNA.

Concentration: 5,000 units/ml

Usage Note: Exo T is has different activity on RNA vs. DNA. For RNA, 1 unit of Exo T is required to completely digest 1.0 pmol of rA20 under standard reaction condition as measured by gel electrophoresis.

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: 1X Nucleoside Digestion Mix Reaction Buffer. Incubate at 37°C. May heat inactivate at 65°C for 20 minutes.

DNase I (RNase-free)

#M0303S 1,000 units
#M0303L 5,000 units

- *Degradation of DNA template in transcription reactions*
- *Removal of contaminating genomic DNA from RNA samples*
- *DNase I footprinting*
- *Nick Translation*

Description: DNase I (RNase-free) is an endonuclease that nonspecifically cleaves DNA to release di-, tri- and oligonucleotide products with 5´-phosphorylated and 3´-hydroxylated ends. DNase I acts on single- and double-stranded DNA, chromatin and RNA:DNA hybrids.

Reaction Conditions: 1X DNase I Reaction Buffer. Incubate at 37°C. May heat inactivate at 75°C for 10 minutes.

Unit Definition: 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.

Complete degradation is defined as the reduction of the majority of DNA fragments to tetranucleotides or smaller.

Concentration: 2,000 units/ml

Note: EDTA should be added to a final concentration of 5 mM to protect RNA from being degraded during enzyme inactivation.

RNase Inhibitor, Murine

#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*
- *Enzymatic RNA labeling reaction*

RNase Inhibitor, Murine has significantly improved resistance to oxidation compared to human & porcine RNase inhibitors.

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 1, 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 reactions where high concentration DTT is adverse to the reaction (e.g., RT-qPCR).

Unit Definition: One unit is defined as the amount of RNase Inhibitor, Murine 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 1, 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}.

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 (200 mM)

Ribonucleoside Vanadyl Complex is an equimolar mixture of all four ribonucleosides, complexed with oxovanadium IV by a modification of the procedures by Berger (1). Each lot of the complex is assayed for oxovanadium V content and inhibition of ribonuclease activity.

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.

Reference:

(1) Berger, S.L. and Birkenmeier, C.S. (1979) *Biochemistry* 18, 5143–5149.

NEBNext® Reagents for RNA Library Preparation

NEBNext Ultra II Directional RNA Library Prep Kit for Illumina
#E7760S 24 rxns
#E7760L 96 rxns

NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads
#E7765S 24 rxns
#E7765L 96 rxns

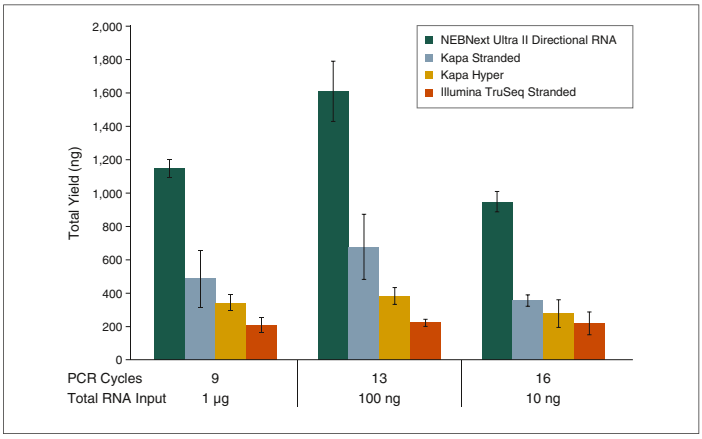
NEBNext Ultra II RNA Library Prep Kit for Illumina
#E7770S 24 rxns
#E7770L 96 rxns

NEBNext Ultra II RNA Library Prep with Sample Purification Beads
#E7775S 24 rxns
#E7775L 96 rxns

NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina
#E6420S 24 rxns
#E6420L 96 rxns

NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module
#E6421S 24 rxns
#E6421L 96 rxns

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. Our NEBNext RNA products also include kits for Small RNA Library Prep, and multiple options for removal of abundant RNAs as well as a growing range of adaptors and primers.



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®.*

Kits for rRNA depletion from a variety of samples, including human, mouse, rat and bacteria are available, as well as a customizable option for any sample type. Kits for Small RNA library construction are also available. More information on NEBNext reagents for library preparation can be found on pages 144–175.

Magnetic mRNA Isolation Kit

#S1550S 25 isolations

Component Sold Separately:

Oligo $d(T)_{25}$ Magnetic Beads
#S1419S 25 mg

Companion Products:

6-tube Magnetic Separation Rack
#S1506S 6 tubes (1.5 ml)

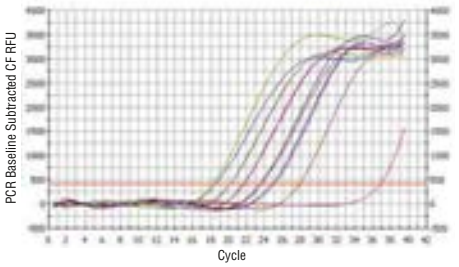
12-tube Magnetic Separation Rack
#S1509S 12 tubes (1.5 ml)

- *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.

The Magnetic mRNA Isolation Kit Includes:

– Oligo $d(T)_{25}$ Magnetic Beads
– Lysis/Binding Buffer
– Wash Buffer I, II, III, Elution Buffer



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.

Oligo $d(T)_{25}$ Magnetic Beads

#S1419S 25 mg

Companion Product:

96-well Microtiter Plate Magnetic Separation Rack
#S1511S 96 well

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 precipation 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.

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 (20 mg)

Companion Products:

6-Tube Magnetic Separation Rack
#S1506S 6 tubes (1.5 ml)

96-well Microtiter Plate Magnetic Separation Rack
#S1511S 96 well

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.

Support Matrix: 1 µm non-porous 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

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.

The EpiMark N6-Methyladenosine Enrichment Kit Includes:

– N6-Methyladenosine Antibody
– m6A Control RNA (100 nM)
– Unmodified Control RNA (100 nM)

N6-Methyladenosine Antibody is produced by Cell Signaling Technology, Inc. and sold by New England Biolabs, Inc.

Monarch Kits for Cleanup & Isolation

See pages 137–138 for more information.

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

Monarch RNA Cleanup Kit (10 µg)
#T2030S 10 preps
#T2030L 100 preps

Monarch RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

Monarch RNA Cleanup Kit (500 µg)
#T2050S 10 preps
#T2050L 100 preps

The Monarch Total RNA Miniprep Kit is a comprehensive solution for sample preservation, cell lysis, gDNA removal, and purification of total RNA from a wide variety of biological samples, including cultured cells, blood, and mammalian tissues. Additionally, tough-to-lyse samples, such as bacteria, yeast, and plant, can be processed with additional steps that enhance lysis. Total RNA, including viral RNA, can also be extracted from clinically-relevant samples like saliva, buccal swabs and nasopharyngeal swabs. Cleanup of enzymatic reactions or purification of RNA from TRIzol® -extracted samples is also possible using this kit. Purified RNA has high quality metrics, including $A_{260/280}$ and $A_{260/230}$ ratios ≥ 1.8, high RIN scores, and minimal residual gDNA. Captured RNA ranges in size from full-length rRNAs down to intact miRNAs. Additionally, differential binding conditions allow selective capture or exclusion of the sub-200 nucleotide RNA pool that includes miRNA, 5S rRNA, and tRNA. Purified RNA is suitable for downstream applications, such as RT-qPCR, cDNA synthesis, RNA-seq, Northern blot analysis, etc.

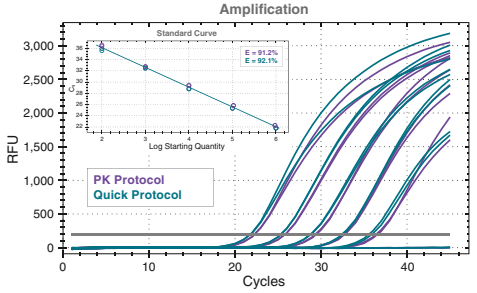
The Monarch RNA Cleanup Kits provide a fast and simple silica spin column-based solution for RNA cleanup and concentration after any enzymatic reaction (including *in vitro* transcription, DNase I treatment, capping and labeling) and after other purification methods such as phenol/chloroform extraction. These kits can also be used to extract total RNA from cells, saliva and buccal/nasopharyngeal swabs. The Monarch 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 Total RNA Miniprep Kit Includes:

– gDNA Removal Columns
– RNA Purification Columns
– Collection Tubes II
– DNA/RNA Protection Reagent (2X)
– RNA Lysis Buffer
– Proteinase K and associated buffers
– DNase I & associated reaction buffer
– RNA Priming Buffer
– RNA Wash Buffer (5X)
– Nuclease-free Water

The Monarch RNA Cleanup Kits Include:

– RNA Cleanup Columns (10, 50 or 500 µg)
– RNA Cleanup Binding Buffer
– RNA Cleanup Wash Buffer (5X)
– Collection Tubes II
– Nuclease-free Water



The Monarch Total RNA Miniprep Kit successfully purifies synthetic SARS-CoV-2 viral RNA from saliva samples: *The Monarch Total RNA Miniprep Kit Proteinase K and Quick Protocols were used to isolate total RNA from saliva samples containing 10-fold serial dilutions of synthetic SARS-CoV-2 N-gene RNA. Purified RNA was eluted in 100 µl nuclease-free water to yield 50 to 500,000 copies of viral RNA/µl. Using the Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006), titers as low as 50 copies (the lowest input tested) were detected and linear, quantitative recovery of the SARS-CoV-2 N-gene was observed over a 5-Log range.*

RNA Markers & Ladders

dsRNA Ladder
#N0363S 25 gel lanes

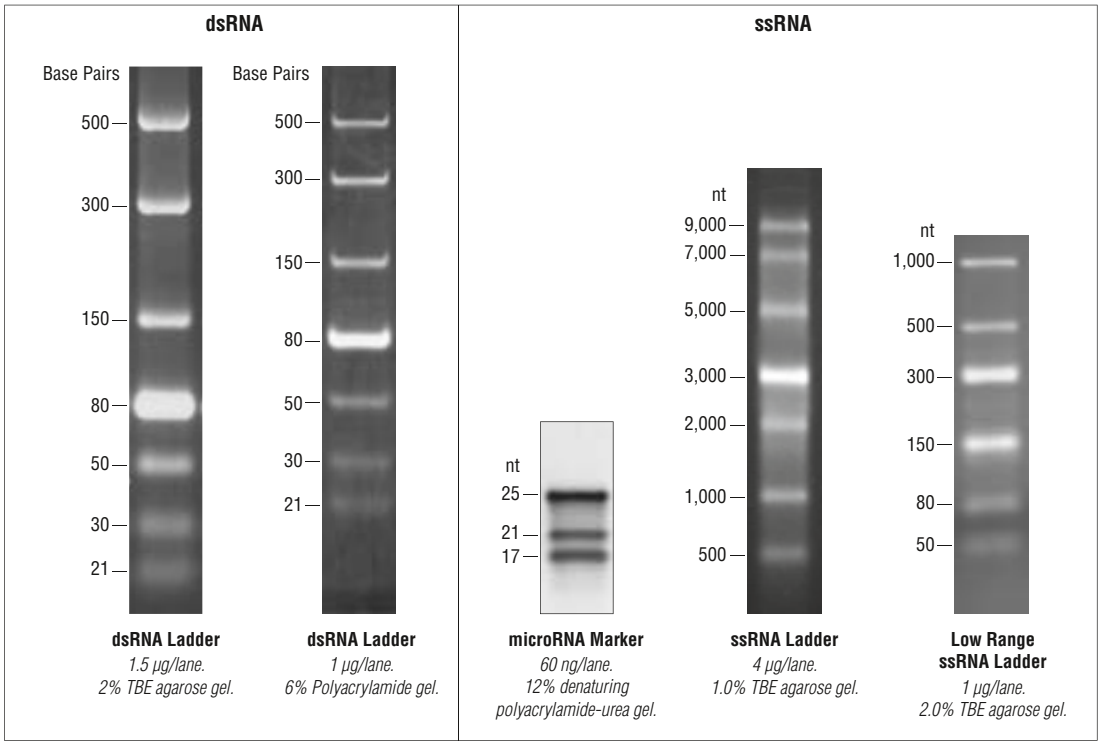
microRNA Marker
#N2102S 100 gel lanes

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 ssRNA ladders are suitable for use as RNA size standards on denaturing or native gels. Both are supplied with 2X Loading Buffer and feature a higher intensity fragment to serve as a reference band. The microRNA Marker, supplied in a ready-to-load denaturing solution, is ideally used as a size marker on polyacrylamide gels or Northern blots and is best visualized stained with ssRNA fluorescent dyes. It is supplied with a 3´-biotinylated 21-mer oligonucleotide probe that can also be labeled with [γ-^{32}P] ATP and T4 PNK (NEB #M0201). The ds RNA Ladder is suitable for use as a size standard in dsRNA analysis on both polyacrylamide and agarose gels.

Concentration: Low Range ssRNA Ladder and dsRNA Ladder are supplied at 500 µg/ml. ssRNA Ladder is supplied at 2,000 µg/ml. MicroRNA Marker is supplied at 12 ng/µl.



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 0.83 nmol

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´.

Reference:

(1) Lau et al. (2001) *Science*, 294, 858–856.

Aim AR camera at this page for a slideshow of artwork on the NEB campus.

The Intersection of Art and Science

Science and art are both driven by curiosity, discovery and a thirst for knowledge. They are both attempts to communicate truth and understanding of ourselves and the world around us. And, even though the preparation and process leading to scientific discovery and artistic expression is actually surprisingly similar, they are often viewed as separate disciplines.

When we are young, we are both scientists and artists, experimenting and creating. We are less likely to think of ourselves as just one or the other. As we get older, our formal education directs us down a narrower path — one can become either an artist or a scientist, but rarely both. The separation in communication between the artistic and scientific cultures has become a great chasm since the Industrial Revolution due to intellectual specialization. However, there is great potential for both cultures to inform and inspire each other.

Don Comb always felt a strong connection between science and art; he once said “Science and art are both trying to say something — science is trying to tell you the truth, and artists are trying to present what they feel is the truth.” Don celebrated his love of art by filling the NEB campus with sculptures, paintings, ceramics and artifacts from all over the world — an eclectic collection that was curated over many years. When approaching the main building at NEB headquarters, one immediately notices the enormous mosaic incorporated into the patina copper exterior, which was selected by an international art design competition. The main lobby includes access to an art gallery, which in addition to some of the work mentioned above, also features revolving art exhibitions from local and regional artists.

NEB also supports many local art-based organizations and schools. One example is Raw Art Works (RAW), an art therapy-based organization for youth — helping kids share their stories. Teen muralists from Raw Art spent time with Don and were treated to his indomitable enthusiasm, warmth and joy. After learning about NEB, they brought their artistic visions to a painted mural that translated their understanding of the company’s science and culture. Additionally, NEB has long supported the youth of RAW with scholarships to attend Monserrat College of Art, another organization supported by NEB.

Art brings joy and can make you happy — when we are happy, we are more creative and productive. Neurobiologist Semir Zeki from the University College in London found that looking at a work of art increased dopamine levels and heightened activity in the brain’s frontal cortex. He concluded that viewing art can give as much pleasure as being in love. Did Don realize that by sharing his love of art, he created an environment that brought happiness and inspiration to the workplace and instigated lively conversations, especially around his more provocatively chosen pieces?

When we allow science to inspire art and art to inform science, we can approach a challenge with a diverse set of intellectual and creative problem-solving skills. It is in this space, where the lines between art and science are blurred, that innovation can lead to unique, groundbreaking discoveries.

Mural created by students at Raw Art Works (RAW).
Credit: Mileidy Rodriguez, NEB

View a slideshow of artwork on the NEB campus.

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-expess proteins.

Featured Products

228 PURExpress® *In Vitro* Protein Synthesis Kit

227 NEBExpress® Cell-free *E. coli* Protein Synthesis System

234 NEBExpress Ni Spin Columns

229 TEV Protease

Featured Tools & Resources

233 Purification Beads, Columns & Resins

350 Enhancing Efficiency

351 Protein Expression with T7 Express Strains

To learn more about NEB's portfolio of products for protein expression and purification, Visit www.neb.com/Protein Expression to learn more.

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

- *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*
- *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*

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 allows for rapid purification of CBD-tagged proteins*
- *Remove affinity tags following your purification with TEV Protease*

NEW

NEBExpress® Cell-free *E. coli* Protein Synthesis System

#E5360S 10 reactions
#E5360L 100 reactions

Companion Product:

NEW
NEBExpress GamS Nuclease Inhibitor
#P0774S 75 µg

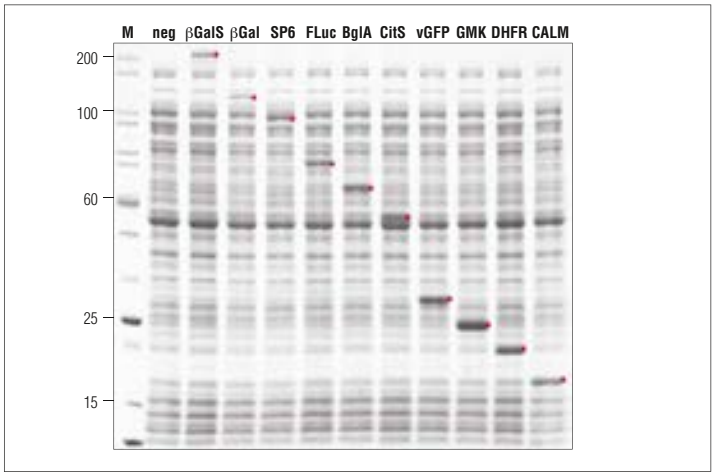
- *Synthesize high yields of protein (typically 0.5 mg/ml)*
- *Protein can be synthesized and visualized in approximately 2–4 hours*
- *Synthesize target proteins ranging from 17 to 230 kDa*
- *Templates can be plasmid DNA, linear DNA, or mRNA*
- *RNase contamination can be inhibited by the supplied 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.

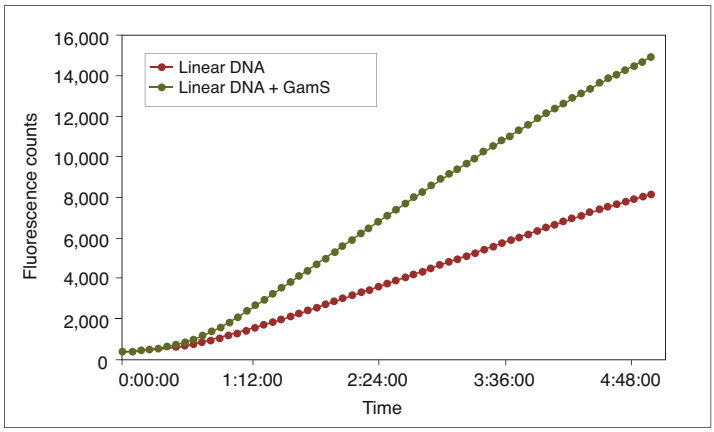
The NEBExpress Cell-free *E. coli* Protein Synthesis System contains all the components required for protein synthesis, except for the target template DNA. It is a combination of a highly active cell extract from a genetically engineered strain, a reaction buffer, and an optimized T7 RNA Polymerase, which together yield robust expression of a wide variety of protein targets ranging from 17 to 230 kDa.

Applications

- Quickly generate analytical amounts of protein for further characterization
- High throughput screening and liquid handling
- 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 Product:

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 nuclease-free 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

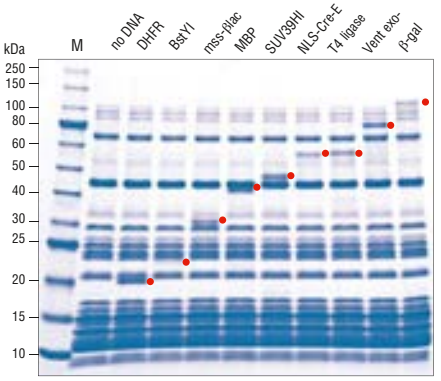
PURExpress Disulfide Bond Enhancer: This proprietary blend of proteins and buffer components is designed to correctly fold target proteins with multiple disulfide bonds produced in PURExpress reactions or *E. coli* S30 extracts. Added at the beginning of a reaction, the components assist with the oxidation 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.

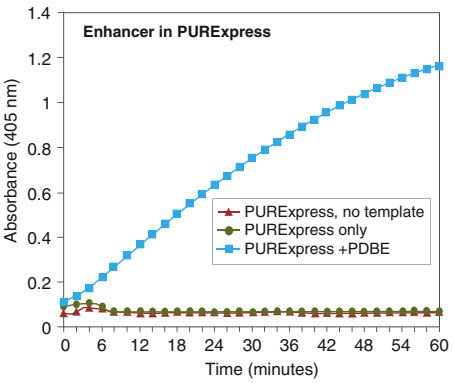
PURExpress Δ RF123 Kit: Release factors are involved in termination of protein translation by recognizing the stop codons in an mRNA sequence. In a ribosome display experiment using PURExpress, lack of release factors could stabilize the ternary complex of mRNA-ribosome-nascent protein. As a result, 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). *E. coli* Ribosome is supplied as a 33.3 mg/ml solution.



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 Ladder.*



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

IN VITRO PROTEIN SYNTHESIS (E6800S)	Δ RIBOSOME (E3313S)	Δ RF 123 (E6850S)	Δ (aa, tRNA) (E6840S)
Solution A	Solution A	Solution A	Solution A (minus aa and tRNA)
Solution B	Factor Mix	Solution B (minus RF1, RF2 and RF3)	Solution B
Control (DHFR) template	Control (DHFR) template	Control (DHFR) template	Control (DHFR) template
	Control Ribosomes	RF1, RF2 and RF3	Amino Acid Mixture
			E. coli tRNA

NEW

NEBExpress MBP Fusion and Purification System

#E8201S

Components Sold Separately:

Amylose Resin
#E8021S 15 ml
#E8021L 100 ml

Anti-MBP Monoclonal Antibody
#E8032S 0.05 ml
#E8032L 0.25 ml

NEW
pMAL-c6T Vector
#N0378S 10 µg

TEV Protease
#P8112S 1,000 units

- *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.

The NEBExpress MBP Fusion and Purification System includes:

- pMAL-c6T Vector
- Amylose Resin
- TEV Protease, TEV Protease Reaction Buffer
- Anti-MBP Monoclonal Antibody
- MBP6 Protein
- MBP6-TEV-paramyosin-ΔSal
- *E. coli* ER2523 (NEB Express) (Glycerol Stock)

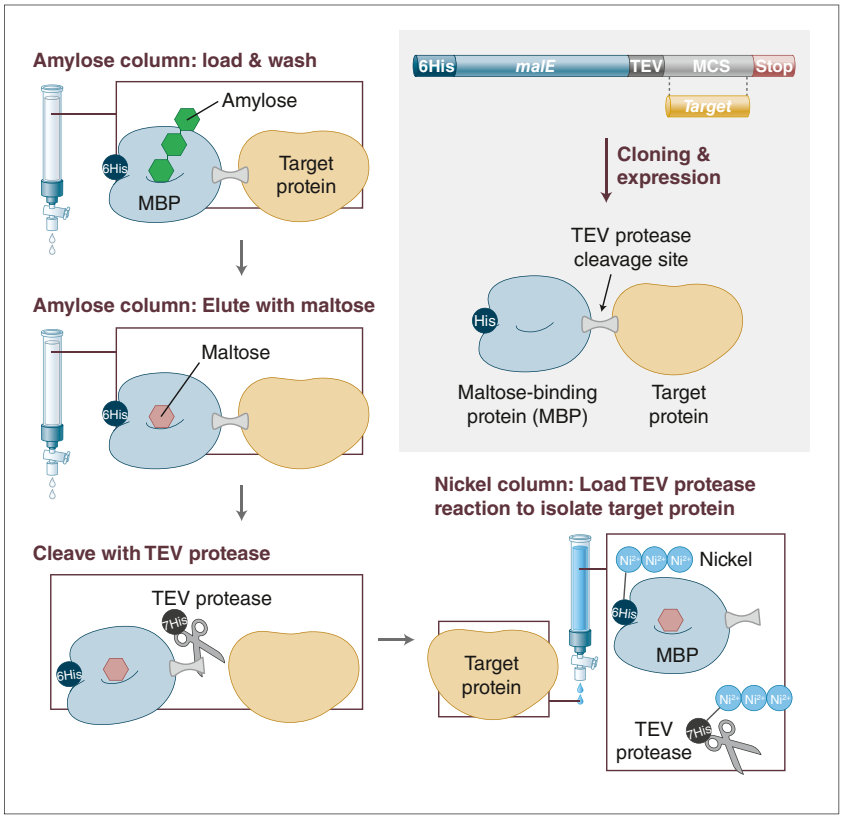


Figure 1: *Schematic illustration of the NEBExpress MBP Fusion and Purification System*

IMPACT™ Kit

#E6901S

Components Sold Separately:

pTXB1 Vector
#N6707S 10 µg

pTYB21 Vector
#N6709S 10 µg

Chitin Resin
#S6651S 20 ml
#S6651L 100 ml

Anti-CBD Monoclonal Antibody
#E8034S 0.05 ml

pTWIN1 Vector
#N6951S 10 µg
(not included with the kit)

- *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*

For a more detailed description and a restriction map of the pTXB1 and pTYB21 vectors, including the MCS, see page 363–364; visit www.neb.com for sequence files.

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.

The IMPACT Kit Includes:

- *E. coli* expression vectors: pTXB1, pTYB21 and pMXB10 Control Vector
- Chitin Beads: Affinity matrix (binding capacity=2 mg/ml)
- Anti-CBD Monoclonal Antibody
- 1, 4-Dithiothreitol (DTT) & SDS Loading Buffer

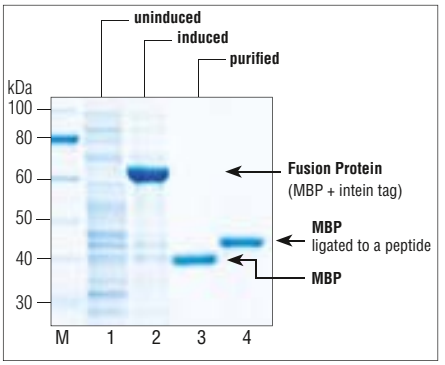


Figure 1: *Purification of Maltose Binding Protein (MBP) in a single affinity purification step: Lane 1: uninduced cell extract. Lane 2: induced cell extract showing expressed fusion protein. Lane 3: MBP fraction eluted after inducing cleavage overnight at 4°C. Lane 4: MBP ligated to a peptide containing an N-terminal cysteine. Marker M is the protein ladder.*

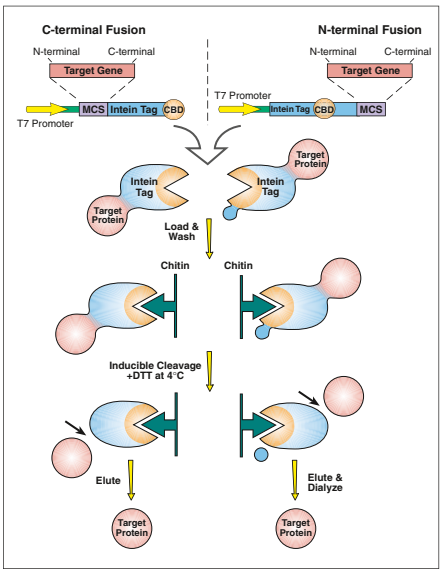


Figure 2: *Schematic of the IMPACT System.*

Table 1: *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[d] 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

Components Sold Separately:

SacII
#R0157S 2,000 units
#R0157L 10,000 units

Yeast Carbon Base Medium Powder
#B9017S 12 g

K. lactis GG799 Competent Cells
#C1001S 5 transformation reactions

- *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*

Restriction map for pKLAC2 can be found on page 358; sequence files are available 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.

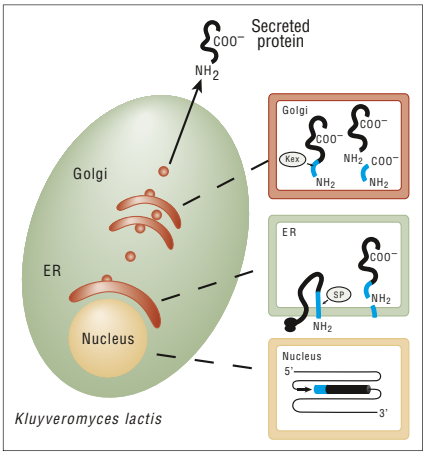
GG799 competent cells are provided in the *K. lactis* Protein Expression Kit. GG799 cells are characterized by very high cell density growth and efficient expression of foreign proteins. GG799 cells have no genetic modifications.

The *K. lactis* Protein Expression Kit Includes:

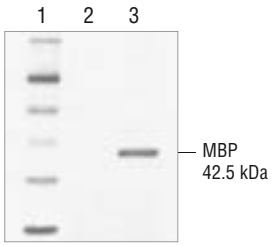
- pKLAC2 Vector and pKLAC1-*malE* Control Plasmid
- SacII
- Integration Primer Set
- rCutSmart™ Buffer
- *K. lactis* GG799 Competent Cells and Transformation Reagent
- Yeast Medium Powder & Acetamide Solution

Note: This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

References: References for properties and applications of these products can be found at www.neb.com.



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.*



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.*

Companion Products:

Enterokinase, light chain
#P8070S 480 units
#P8070L 2,560 units

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

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

Deoxynucleotide (dNTP) Solution Mix
#N0447S 8 µmol of each*
#N0447L 40 µmol of each

*Available in 4 x 0.2 ml aliquots

Competent Cells for Protein Expression

- *Free of animal products*
- *T1 phage resistance (fhuA2)*
- *B strains are deficient in proteases Lon and OmpT*
- *A variety of convenient formats*
- *Bulk sales capabilities with custom packaging*

NEB offers a wide selection of competent cell strains designed for the 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) can be used for expression and purification of His-tagged proteins. NEB Express and T7 Express are offered with varying levels of expression control. Several strains are available with added control by *lacI*^{q}. Only NEB offers exceptional control of T7 expression from 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.

For more information see pages 245–249.

Expression Strains

CHARACTERISTICS	STRAIN	NEB #	SIZE
• Versatile non-T7 expression strain • Protease deficient	NEBExpress Competent *E. coli**	C2523H/I	20 x 0.05 ml/6 x 0.2 ml
• Control of IPTG induced expression from P_{lac}, P_{tac} and P_{trc} • Protease deficient	NEBExpress I^q Competent *E. coli*	C3037I	6 x 0.2 ml
• Most popular T7 expression strain • Protease deficient	T7 Express Competent *E. coli*	C2566H/I	20 x 0.05 ml/6 x 0.2 ml
• T7 expression • Protease deficient • Better reduction of basal expression	T7 Express *lysY* Competent *E. coli*	C3010I	6 x 0.2 ml
• T7 expression • Protease deficient • Highest level of expression control	T7 Express *lysY/I*^{q} Competent *E. coli*	C3013I	6 x 0.2 ml
• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • Protease deficient/B strain	SHuffle® Express Competent *E. coli*	C3028J	12 x 0.05 ml
• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression • Protease deficient/B strain	SHuffle T7 Express Competent *E. coli*	C3029J	12 x 0.05 ml
• 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	SHuffle T7 Express *lysY* Competent *E. coli*	C3030J	12 x 0.05 ml
• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression/K12 strain	SHuffle T7 Competent *E. coli*	C3026J	12 x 0.05 ml
• Routine expression for non-T7 Vectors • Protease deficient	BL21 Competent *E. coli*	C2530H	20 x 0.05 ml
• Routine T7 Expression • Protease deficient	BL21(DE3) Competent *E. coli*	C2527H/I	20 x 0.05 ml/6 x 0.2 ml
• Tunable T7 Expression for difficult targets • Protease deficient	Lemo21(DE3) Competent *E. coli*	C2528J	12 x 0.05 ml
• Expression and purification of His-tagged proteins • Protease deficient	NiCo21(DE3) Competent *E. coli*	C2529H	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.

* NEB Express is the recommended strain for the NEBExpress MBP Fusion and Purification System.

Purification Beads, Columns and Resins

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, and result in a high yield of highly pure substrate. For the full list of products available for protein expression and purification, visit **www.neb.com/ProteinExpression**.

Product Selection Chart

	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	IMMUNOPRECIPITATION	CELL SEPARATION/ CELL SORTING
NEW **NEBExpress Ni-NTA Magnetic Beads** (NEB #S1423)	● (His-tag)			●		●				
NEW **NEBExpress Ni Spin Columns** (NEB #S1427)	● (His-tag)			●		●				
NEW **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)			●		●				
Anti-MBP Magnetic Beads (NEB #E8037)	● (MBP)			●		●				
Chitin Resin (NEB #S6651)	● (intein-CBD tag)	●				●				
Chitin Magnetic Beads (NEB #E8036)	● (intein-CBD tag)			●		●				
Oligo d(T)$_{25}$ Magnetic Beads (NEB #S1419)				●			●	●		
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)				●					●	
Goat Anti-Mouse IgG Magnetic Beads (NEB #S1431)				●					● (Mouse IgGs)	●
Goat Anti-Rabbit IgG Magnetic Beads (NEB #S1432)				●					● (Rabbit IgGs)	●
Goat Anti-Rat IgG Magnetic Beads (NEB #S1433)				●					● (Rat IgGs)	●
Magnetic mRNA Isolation Kit (NEB #S1550)				●				●		

Polyhistidine-tagged Protein Purification

NEW
NEBExpress Ni-NTA Magnetic Beads
#S1423S 1 ml
#S1423L 5 ml

NEW
NEBExpress Ni Resin
#S1428S 25 ml

NEW
NEBExpress Ni Spin Columns
#S1427S 10 columns
#S1427L 25 columns

NEW
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 super-paramagnetic 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 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.

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 Magnetic Beads
#E8037S 1 ml

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 and Anti-MBP Magnetic Beads: Affinity matrices for the small-scale isolation and purification of MBP-fusion proteins. Amylose or monoclonal Anti-MBP are covalently coupled to a paramagnetic particle through a linkage that is stable and leak resistant over a wide pH range.

Support Matrix: Amylose Magnetic Beads – 10 µM superparamagnetic particles; Anti-MBP Magnetic Beads – 1 µM nonporous super paramagnetic particle.

Binding Capacity: 1 mg of Amylose Magnetic Beads will bind ≥ 10 µg of MBP-fusion protein. 1 mg of Anti-MBP Magnetic Beads will bind 5 µg of MBP-paramyosin ΔSal 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

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 on step. Removal of CBD-tag during elutin 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 prepared with 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.

Magnetic 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

Goat Anti-Mouse IgG Magnetic Beads
#S1431S 1 ml

Goat Anti-Rabbit IgG Magnetic Beads
#S1432S 1 ml

Goat Anti-Rat IgG Magnetic Beads
#S1433S 1 ml

- *Small-scale purification or immunoprecipitation of IgG species*
- *No centrifugation required*
- *Regenerate matrix without binding capacity loss*

Oligo $d(T)_{25}$ Magetic 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 nonporous superparamagnetic microparticles

Binding Capacity: ≥ 5 µg rA_{30} 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 nonporous 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 allowing 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 nonporous superparamagnetic microparticles

Binding Capacity: > 280 µg of Human IgG per ml of beads

Goat Anti-Mouse IgG Magnetic Beads: An affinity matrix for the small-scale immunomagnetic separation and purification of mouse IgG. Specifically, the beads consist of Anti-Mouse IgG that is covalently coupled to a 1 µm nonporous superparamagnetic particle.

Support Matrix: 1 µm nonporous superparamagnetic microparticle

Binding Capacity: 5 µg mouse IgG/mg Goat Anti-Mouse IgG Magnetic Beads

Goat Anti-Rabbit IgG Magnetic Beads: An affinity matrix for the small-scale immunomagnetic separation and purification of rabbit IgG. Specifically, the beads consist of Goat Anti-Rabbit IgG that is covalently coupled to a 1 µm nonporous superparamagnetic particle.

Support Matrix: 1 µm nonporous superparamagnetic microparticle

Binding Capacity: 5 µg rabbit IgG/mg Goat Anti-Rabbit IgG Magnetic Beads

Goat Anti-Rat IgG Magnetic Beads: An affinity matrix for the small-scale immunomagnetic separation and purification of rat IgG. Specifically, the beads consist of anti-Rat IgG that is covalently coupled to a 1 µm nonporous superparamagnetic particle.

Support Matrix: 1 µm nonporous superparamagnetic microparticle

Binding Capacity: 5 µg rat IgG/mg Goat Anti-Rat IgG Magnetic Beads

Magnetic Separation Racks

	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.

Sustainable Growth

What does “sustainable growth” mean? Originally conceptualized by Robert C. Higgins, sustainable growth rate refers to the growth rate that can be supported by the financial means of the entity itself, without additional equity or debt. Don Comb was a firm believer in the principles of sustainable growth. Throughout its history, New England Biolabs has grown steadily and sustainably, at times purposefully tempering growth to maintain the company for future generations. And over 45 years later, employees remain confident that the company will be there to support them for years to come — as well as its customers and the scientific community.

Sustainable growth applies to more than just companies and institutions though, it also applies to the populations. In 1972, scientists and economists at MIT published *Limits to Growth*, which outlined that the fate of humanity depends on the preservation of biodiversity and the slowing of economic and population growth. Economic growth means greater industrial output: more consumer goods, factories, oil wells, mines and agricultural clearing — and thus less protection of natural resources and ecosystems. While striving for greater economic growth may lead to wealth, it does not necessarily lead to a better world.

So how do we bring the world’s populations onto a more sustainable path? The first step is to curb wasteful resource consumption and reduce the disparity in living standards between nations. NEB has supported these efforts through contributions to non-profit organizations dedicated to improving lives by helping communities protect, conserve and manage the natural resources upon which their long-term well-being depends. One such organization is Trees, Water & People, which works with communities across Central America to practice watershed protection, sustainable agriculture and reforestation, as well as to distribute clean cookstoves — all in an effort to protect natural resources.

In addition to supporting efforts like Trees, Water & People, NEB houses the Creative Action Institute (CAI), whose mission is to “catalyze community-driven solutions that advance gender equality and build a sustainable planet.” CAI is working around the world to build the capacity of leaders and organizations for innovation, collaboration and resilience to advance conservation, health and human rights.

NEB has always embraced a “small is beautiful” approach, continuing to make scientific and technological advances — while growing sustainably. Additionally, NEB operates its manufacturing facilities and headquarters efficiently, minimizes waste, subsidizes energy usage with solar panels, and recycles aggressively to protect natural resources. But no company can save the world alone, it will take many companies and individuals adopting similar practices. With the combined efforts of many to protect and preserve natural resources, and to slow the growth rate, we can all enjoy this planet for centuries to come.

Sprout spruce seedling emerging from the ground.
Credit: Олег Копьёв, Adobe Stock

Learn more about sustainability at NEB.

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.

Featured Products

242 NEB Cloning Competent *E. coli* Sampler

244 NEB Stable Competent *E. coli*

245 BL21 Competent *E. coli*

248 SHuffle Express Competent *E. coli*

Featured Tools & Resources

350 Enhancing Transformation Efficiency

351 Protein Expression with T7 Express Strains

336 Troubleshooting Guide for Cloning

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.

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	CHEMICAL TRANSFORMATION EFFICIENCY (cfu/µg)	ELECTRO-COMPETENT TRANSFORMATION EFFICIENCY (cfu/µg)	AVAILABLE FORMATS [7]	OUTGROWTH MEDIUM & CONTROL PLASMID INCLUDED?	STRAIN BACKGROUND	LIBRARY CONSTRUCTION
***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]	> 1 x 10^{10}	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	●

Competitor Cross Reference

Using another competent cell strain? Try our competitor cross reference tool to find out which NEB strain is compatible.

Cloning Strain Properties

BLUE/WHITE SCREENING	*lacI*^{q}	*endA*$^-$ (2)	F′	*recA*$^-$	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$^+$

Protein Expression Strain Properties

lacI^q	*lysY*	*endA*$^-$	PROTEASE DEFICIENT (3)	F′	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$^-$

(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) Legend 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.

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 vials x 0.05 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 4 x 25 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.

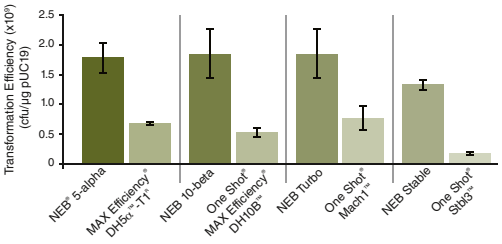
Please refer to the individual datacards for each reagent's recommended use and storage conditions.

Transformation Efficiency:
1–3 x 10^9 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*)

The Sampler Includes:

– NEB Turbo Competent *E. coli* (High Efficiency)
– NEB 5-alpha Competent *E. coli* (High Efficiency)
– NEB 10-beta Competent *E. coli* (High Efficiency)
– NEB Stable Competent *E. coli* (High Efficiency)
– SOC Outgrowth Medium
– NEB 10-beta/Stable Outgrowth Medium
– pUC19 Vector



Benefit from high transformation efficiencies: *Transformation efficiencies were compared using manufacturers' recommended protocols. Values shown are the average of triplicate experiments.*

NEB Turbo Competent *E. coli*

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 4 x 25 ml

- *Tight expression control ($lacI^q$)*
- *Colonies visible after 6.5 hours*
- *Isolate DNA after 4 hrs 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´ *$proA^+B^+$ $lacI^q$ ΔlacZM15 / fhuA2 Δ(lac-proAB) glnV galK16 galE15 R(zgb-210::Tn10)*Tet^S *endA1 thi-1 Δ(hsdS-mcrB)5*

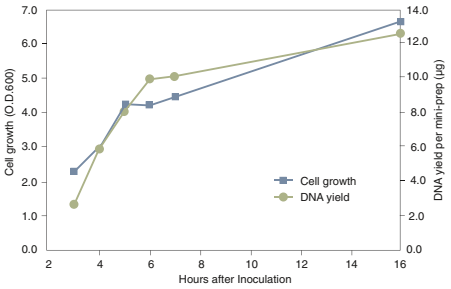
Features:

- Suitable for blue/white screening
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations

Transformation Efficiency:
High Efficiency: 1–3 x 10^9 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Nit

Sensitivity: Amp, Cam, Kan, Spec, Str, Tet



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.

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

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 x 96 well plate

NEB 10-beta Electrocompetent *E. coli*

#C3020K	6 x 0.1 ml

Companion Product:

NEB 10-beta/Stable Outgrowth Medium
#B9035S 4 x 25 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*)

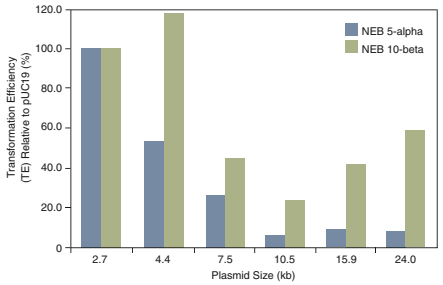
Transformation Efficiency:
High Efficiency: 1–3 x 10^9 cfu/µg pUC19 DNA (NEB #C3019H, #C3019I); 1–3 x 10^8 cfu/µg pUC19 DNA (NEB #C3019P)

Electrocompetent: > 2 x 10^{10} cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA*), Str

Sensitivity: Amp, Cam, Kan, Nit, Spec, Tet

Reagents Supplied:
NEB 10-beta/Stable Outgrowth Medium
pUC19 Control DNA



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 x 96 well plate
#C2987R	1 x 384 well plate
#C2987U	96 x 50 µl/tube

NEB 5-alpha Competent *E. coli*
(Subcloning Efficiency)*

#C2988J	6 x 0.4 ml

NEB 5-alpha Electrocompetent *E. coli*

#C2989K	6 x 0.1 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 4 x 25 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

Electrocompetent: > 1 x 10^{10} cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

* 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*

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 4 x 25 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 ϕ80 Δ(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:
High Efficiency: 1–3 x 10^9 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Tet

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

NEB Stable Competent *E. coli*

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	4 x 25 ml

- *T1 phage resistance (fhuA)*
- *Free of animal products*
- *Carries endA mutation (isolated plasmids are free of EndoI)*

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*)

Applications:

- Cloning unstable inserts
- Isolating and propagating retroviral/lentiviral clones
- Compatible with DNA Assembly reactions, as well as ligation reactions

Transformation Efficiency:
High Efficiency: 1–3 x 10^{9} cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA*), Str, Tet

Sensitivity: Amp, Cam, Kan, Nit, Spec

Reagents Supplied:
NEB 10-beta/Stable Outgrowth Medium
pUC19 Control DNA

dam^{-} / *dcm*$^{-}$ Competent *E. coli*

#C2925H	20 x 0.05 ml
#C2925I	6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium

#B9020S	4 x 25 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: T1 phage (*fhuA31*), Cam, Nit, Str

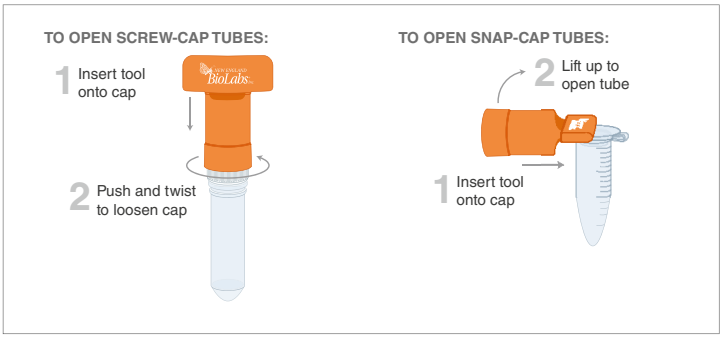
Sensitivity: Amp, Kan, Spec, Tet

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

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 4 x 25 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: T1 phage (*fhuA2*)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

BL21(DE3) Competent *E. coli*

#C2527H 20 x 0.05 ml
#C2527I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 4 x 25 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
- Resistant to phage T1 (*fhuA2*)

Transformation Efficiency:
1–5 x 10^7 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

Lemo21(DE3) Competent *E. coli*

#C2528J 12 x 0.05 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:
High Efficiency: 1–3 x 10^7 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Cam

Sensitivity: Amp, Kan, Nit, Spec, Str, Tet

Reagents Supplied:
L-rhamnose solution
pUC19 control DNA

NiCo21(DE3) Competent *E. coli*

#C2529H 20 x 0.05 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 4 x 25 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: T1 phage (*fhuA2*)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

NEBExpress Competent *E. coli*

NEBExpress Competent *E. coli*
(High Efficiency)
#C2523H 20 x 0.05 ml
#C2523I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 4 x 25 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:
High Efficiency: 0.6–1 x 10^9 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Nit

Sensitivity: Amp, Cam, Kan, Tet, Spec, Str

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

NEBExpress I^q Competent *E. coli*

NEBExpress I^q Competent *E. coli*
(High Efficiency)
#C3037I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 4 x 25 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:
High Efficiency: 0.6–1 x 10^9 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Cam, Nit

Sensitivity: Amp, Kan, Spec, Str, Tet

T7 Express Competent *E. coli*

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 4 x 25 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:
High Efficiency: 0.6–1 x 10^9 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Nit

Sensitivity: Amp, Cam, Kan, Spec, Str, Tet

Reagents Supplied:
SOC Outgrowth Medium
pUC19 Control DNA

T7 Express *lysY* Competent *E. coli*

T7 Express *lysY* Competent *E. coli*
(High Efficiency)
#C3010I 6 x 0.2 ml

Companion Product:
SOC Outgrowth Medium
#B9020S 4 x 25 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 not susceptible to lysis during induction
- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA
- No Cam requirement

Transformation Efficiency:
High Efficiency: 0.6–1 x 10^9 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Cam, Nit

Sensitivity: Amp, Kan, Spec, Str, Tet

T7 Express *lysY/I^q* Competent *E. coli*

T7 Express *lysY/I^q* Competent *E. coli*
(High Efficiency)
#C3013I 6 x 0.2 ml

Companion Product:
SOC Outgrowth Medium
#B9020S 4 x 25 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:
High Efficiency: 0.6–1 x 10^9 cfu/µg pUC19 DNA

Resistance: T1 phage (*fhuA2*), Cam, Nit

Sensitivity: Amp, Kan, Spec, Str, Tet

Disulfide Bonds

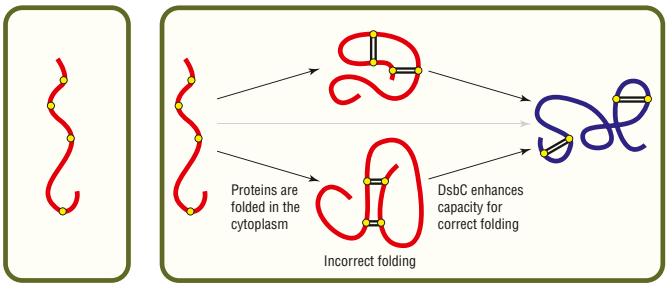
Features of Shuffle® Strains:

- Engineered *E. coli* K12 or B strains promote disulfide bond formation in the cytoplasm
- Constitutively expresses a chromosomal copy of the disufide 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*
- *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: T1 phage (*fhuA2*), Nit, Str*, Spec

Sensitivity: Amp, Cam, Kan, Tet

*Resistance to low levels of streptomycin may be observed.

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: T1 phage (*fhuA2*), Nit, Str*, Spec

Sensitivity: Amp, Cam, Kan, Tet

*Resistance to low levels of streptomycin may be observed.

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: T1 phage (*fhuA2*), Nit, Spec, Str

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: T1 phage (*fhuA2*), Cam, Nit, Str*, Spec

Sensitivity: Amp, Kan, Tet

*Resistance to low levels of streptomycin may be observed.

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 (Table 1). 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).

View our online tutorial for tips on setting up reactions with SHuffle.

Table 1. Percentage of relative solubility of various proteins using SHuffle (K12 and B strains):

PROTEIN	RELATIVE % SOLUBLE K12	B	# CYSTEINES
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)

Reference:

(1) Ke, N. and Berkmen, M. (2014) *Current Protocols Molecular Biology* 16.1B.21.

Aim AR camera at this page
for a musical 3D experience.

Mostly Mozart

It is often said that music is good for the soul. It has a powerful effect on the mind and can influence our moods and emotions. Music can be social and shared collectively with our peers, or it can be deeply personal. In a diverse world, music provides us with a universal language; while musical interpretations can be culture-specific, around the globe people of all ages embrace and are unified with music. And it is well known that musical education has a long-lasting positive impact on children's social and cognitive development. Music activates the region in the frontal cortex that is also activated when performing cooperative altruistic tasks.

"Listening to Mozart makes you smarter," or so the saying goes. As it turns out, any music genre can positively impact our brain, emotional well-being and creativity. But research indicates that listening to Mozart can improve spatial reasoning skills. Studies have shown that exposure to melodic music, notably Mozart's Sonata for Two Pianos, increases dopamine levels and serotonin release — hormones associated with happiness and positive feelings.

Don Comb was a big fan of Mozart, and he was never happier than when he was working at the lab bench with Mozart playing in the background. This became a familiar soundtrack at NEB for those passing by Don's lab or office.

Don's love of Mozart was the inspiration behind an annual "Mostly Mozart" concert — arranged in his honor as a birthday gift from the NEB community. It was so named for two reasons: the program was composed solely of Mozart arrangements, and the concert was held in Mostly Hall — the historic Jacobean summer mansion located on NEB's campus in Ipswich, MA, which was lovingly restored to its original glory and houses NEB's IT, Finance and Marketing Departments. Legend has it, the manor was named Mostly Hall in 1895: When Mattina Riker, the bride of the original estate owner, James Howe Proctor, entered the large hall through the main entry she remarked, "why, it is mostly hall!" — the name has affectionately endured ever since.

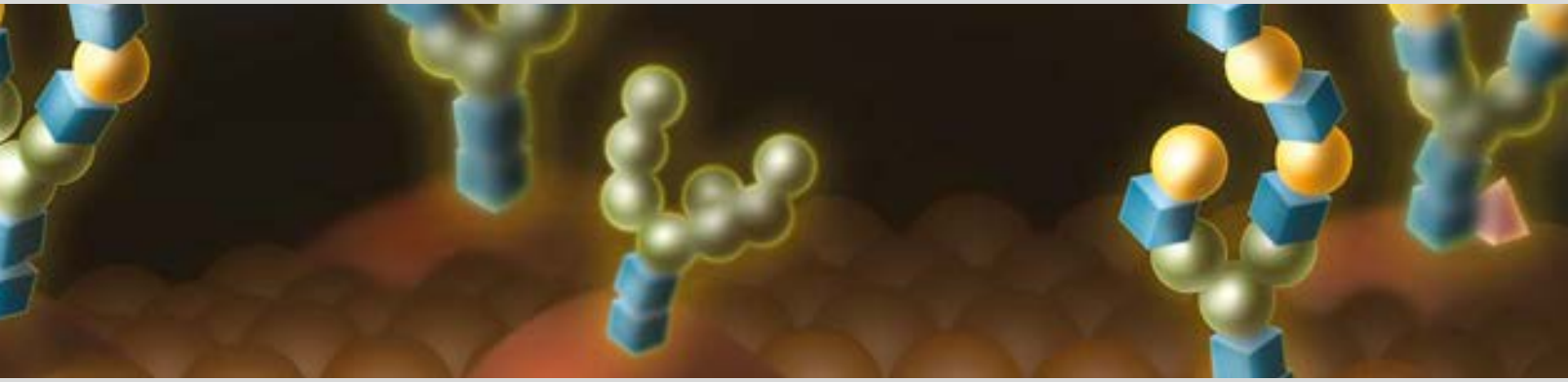
Organized through Rockport Music, a Gloucester, MA-based music organization, these concerts have been held annually for more than ten years. As Mozart's music filled the hall, friends and employees in attendance could witness the joy that music brought to Don's life — he would sit in the front row, with his eyes closed, and enjoy the music with a big smile on his face.

We store musical memories in our implicit memory; they do not require conscious retrieval. This is why we sometimes conjure memories from decades gone by upon hearing just one refrain from a particular song that we attribute to a certain period of our life. Not only do memories come flooding back, but the emotions we associate with those memories can also resurface. For many at NEB, Mozart's music will forever bring a memory of Don Comb and a smile as we go about our day, honoring his legacy and working to continue the prodigious work that he began.

Piano keyboard in the golden light.
Credit: furtseff, Adobe Stock

Experience Mozart's Sonata for Two Pianos.

Glycobiology & Protein Analysis Tools



Trust NEB's expertise in enzymology when you need reagents for glycobiology and protein analysis.

Glycobiology

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.

Glycomics, the study of glycan expression in biological systems, relies on effective enzymatic and analytical techniques for the correlation of glycan structure with function. Glycobiology is a small but rapidly growing field in biology, with relevance to biomedicine, biotechnology, biofuels and basic research. 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

257 Rapid™ PNGase F

268 Thermolabile Proteinase K

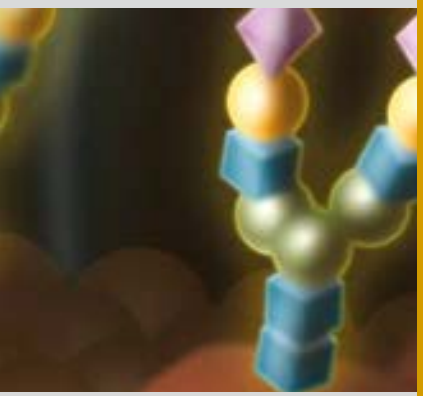
Featured Tools & Resources

Protein Analysis Tools & Glycomics Overview

PNGase F Overview & Selection Chart

Visit www.NEBglycosidase.com to view our online tutorial on *N*- and *O*-linked glycosylation.

(1) Khoury G.A. et al. (2011) *Scientific Reports* 1: 90. PMID: 22034591.
(2) Varki A. (1993) *Glycobiology*, 3, 97–130. PMID: 8490246.
(3) Zhao Y.Y. et al. (2008) *Cancer Sci.* 99, 1304–1310. PMID: 18492092.
(4) Zhao Y. et al. (2008) *FEBS J.* 275, 1939–1948. PMID: 18384383.
(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.
(8) Aarnoudse C.A. et al. (2006) *Curr. Opin. Immunol.* 18, 105–111. PMID: 16303292.
(9) Arnold J.N. (2006) *Immunol. Lett.* 106, 103–110. PMID: 16814399.



RI 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.

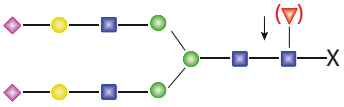
All of our glycosidases are tested for contaminants. Since *p*-nitrophenyl-glycosides are not hydrolyzed by some exoglycosidases, we use only fluorescently-labeled oligosaccharides to screen for contaminating glycosidases.

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.NEBGlycosidase.com** for details.

Endo F2

#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*.

Reagents Supplied:
10X GlycoBuffer 4

Molecular Weight: 39,800 daltons

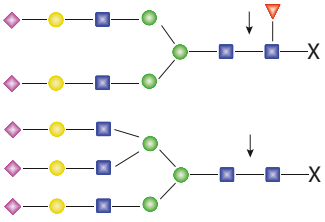
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the carbohydrate from 10 µg Porcine Fibrinogen in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 8,000 units/ml

Heat Inactivation: 65°C for 10 minutes

Endo F3

#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*.

Reagents Supplied:
10X GlycoBuffer 4

Molecular Weight: 38,800 daltons

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.

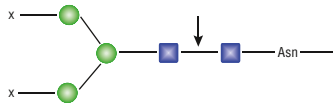
Concentration: 8,000 units/ml

Heat Inactivation: 65°C for 10 minutes

Find an overview of glycobiology.

Endo D

#P0742S 1,500 units
#P0742L 7,500 units



X= (H or oligosaccharide)

- *Removal of paucimannose 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.

Reagents Supplied:
10X DTT
10X GlycoBuffer 2

Molecular Weight: 140,000 daltons

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.

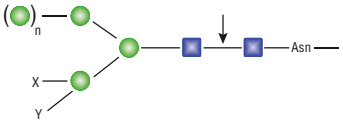
Concentration: 50,000 units/ml

Heat Inactivation: 65°C for 10 minutes

Endoglycosidase 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:
10X Glycoprotein Denaturing Buffer
10X GlycoBuffer 3

Molecular Weight:
Endo H: 29,000 daltons
Endo H_f: 70,000 daltons

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.

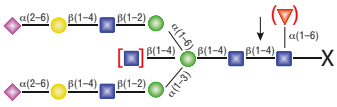
Concentration:
Endo H concentration: 500,000 units/ml
Endo H_f concentration: 1,000,000 units/ml

Usage Notes: Enzymatic activity is not affected by SDS.

To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

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*.

Reagents Supplied:
10X GlycoBuffer 1

Molecular Weight: 136,000 daltons

Unit Definition: 5 µg of IgG in 1X GlycoBuffer 1 are incubated with two-fold dilutions of Endo S for 1 hour at 37°C. Separation of reaction products is visualized by SDS-PAGE.

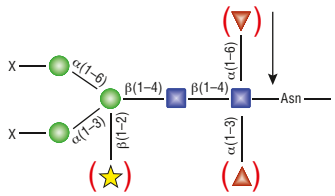
Concentration: 200,000 units/ml

Heat Inactivation: 55°C for 10 minutes

PNGase A

37° 65°

#P0707S 150 units
#P0707L 750 units



PNGase A hydrolyzes N-glycan chains from glycoproteins/peptides regardless of the presence of xylose or fucose. [x = H or Man or GlcNAc].

- *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:
Denature 1 µg of recombinant Avidin in 1X Glycoprotein Denaturing Buffer at 100°C for 10 minutes. After the addition of NP-40 and GlycoBuffer 3, two-fold dilutions of PNGase A are added and the reaction mix is incubated for 1 hour at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X Glycoprotein Denaturing Buffer
10X GlycoBuffer 3
10% NP-40

Molecular Weight: 63,800 daltons

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.

Concentration: 5,000 units/ml

Usage Notes: 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.

PNGase F & PNGase F, Recombinant

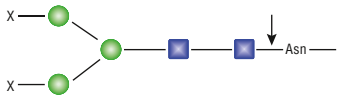
RR 37° 75°

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



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 NEB #P0705 are purified from *Flavobacterium meningosepticum*.

NEB #P0708 and NEB #P0709 are cloned 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:
10X Glycoprotein Denaturing Buffer
10X GlycoBuffer 2
10% NP-40

Molecular Weight: 36,000 daltons

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

Usage Notes: 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.

After addition of NP-40 and Glycobuffer 2, two-fold dilutions of PNGase F are added and the reaction mix is incubated for 1 hour at 37°C.

Companion Products:

RNase B (control substrate)
#P7817S 250 µg

Endoglycosidase Reaction Buffer Pack
10X GlycoBuffer 2, 10X GlycoBuffer 3,
10X Glycoprotein Denaturing Buffer,
10% NP-40 (1 ml of each)
#B0701S

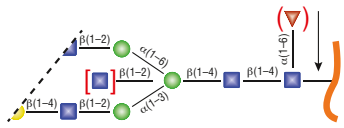
Rapid™ PNGase F & Rapid PNGase F (non-reducing format)

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*

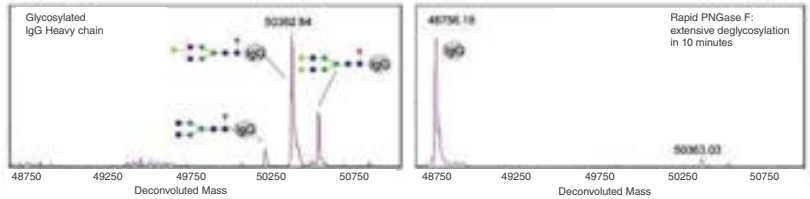
Description: Rapid PNGase F is an improved reagent that allows the complete and rapid deglycosylation of antibodies and fusion proteins in minutes. All *N*-glycans are released rapidly and without bias, and are ready to be prepared for downstream chromatography or mass spectrometry analysis. Rapid PNGase 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:
Rapid PNGase F
Rapid PNGase F Reaction Buffer (5X)
Rapid PNGase F (non-reducing format)
Rapid PNGase F (non-reducing format) Buffer (5X)

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 Rapid PNGase F.

ESI-TOF analysis of an antibody before and after treatment with Rapid PNGase F (non-reducing format).

Remove-iT® PNGase F

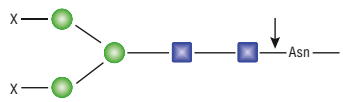
#P0706S 6,750 units
#P0706L 33,750 units

Companion Products:

Chitin Magnetic Beads
#E8036S 5 ml

6-Tube Magnetic Separation Rack
#S1506S 6 tubes

12-Tube Magnetic Separation Rack
#S1509S 12 tubes



Remove-iT 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: 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: Purified from *Flavobacterium meningosepticum.*

Reaction Conditions:
Denature glycoprotein in 1X DTT (40 mM) at 55°C for 10 minutes. Incubate in 1X GlycoBuffer 2 for 1 hour at 37°C. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:
10X DTT
10X GlycoBuffer 2

Molecular Weight: 41,000 daltons

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

Concentration: 225,000 units/ml

Usage Notes: Using typical RNase B denaturing conditions with NEB Glycoprotein Denaturing Buffer containing SDS and DTT, Remove-iT PNGase F yields a higher concentration of 500,000 U/ml.

If using Remove-iT PNGase F under typical PNGase F denaturing conditions, it is essential to have NP-40 in the reaction mixture, as Remove-iT PNGase F is inhibited by SDS. 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.

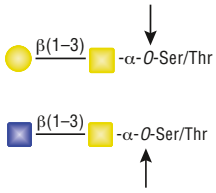
O-Glycosidase

#P0733S 2,000,000 units
#P0733L 10,000,000 units

Companion Products:

O-Glycosidase & Neuraminidase Bundle
#E0540S 1 bundle

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-α-*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*.

Reagents Supplied:
10X Glycoprotein Denaturing Buffer
10X GlycoBuffer 2
10% NP-40

Molecular Weight: 147,000 daltons

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 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).

Concentration: 40,000,000 units/ml

Heat Inactivation: 65°C for 10 minutes

Usage Note: Remove sialic acids if present

Protein Deglycosylation Mix II

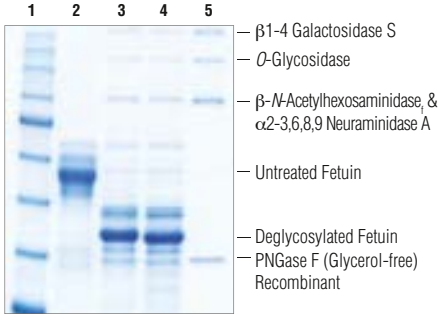
#P6044S 20 reactions

- *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.

Reagents Supplied:
Deglycosylation Mix Buffer 1 (10X)
Deglycosylation Mix Buffer 2 (10X)

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



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: 64 kDa

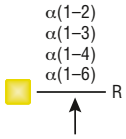
Concentration: 10 mg/ml

Note: 500 µg is enough for approximately 20 reactions. Due to heterogeneous glycosylation, Fetuin runs as a doublet on an SDS-PAGE gel.

α-*N*-Acetylgalactosaminidase

RR BSA 37° 65°

#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: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

Molecular Weight: 47,000 daltons

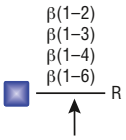
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-D-*N*-Acetylgalactosamine from 1 nmol of (GalNAcα1-3)(Fucα1-2)Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 20,000 units/ml

β-*N*-Acetylglucosaminidase S

RR 37° W6

#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: 1X GlycoBuffer 1. Incubate at 37°C.

Reagents Supplied:
10X GlycoBuffer 1

Molecular Weight: 125,000 daltons

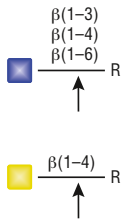
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, non-reducing β-*N*-Acetylglucosamine from 1 nmol GlcNAcβ1-4GlcNAcβ1-4GlcNAc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 4,000 units/ml

β-*N*-Acetylhexosaminidase$_f$

RR 37° 75°

#P0721S 500 units



- *Active only on linear substrates*

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: 1X GlycoBuffer 1. Incubate at 37°C. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1

Molecular Weight: 100,000 daltons

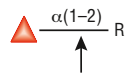
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal β-*N*-Acetylgalactosamine from 1 nmol of GalNAcβ1-4Galβ1-4Glc-AMC, in 1 hour at 37°C in 10 µl volume.

Concentration: 5,000 units/ml

α1-2 Fucosidase

RR BSA 37° 65°

#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: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

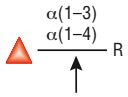
Molecular Weight: 70,000 daltons

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-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

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: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

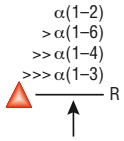
Molecular Weight: 56,000 daltons

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-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 4,000 units/ml

α1-2,3,4,6 Fucosidase

#P0748S 400 units
#P0748L 2,000 units



Description: α1-2,3,4,6 Fucosidase is a broad specificity exoglycosidase that catalyzes the hydrolysis of α1-2, α1-3,α1-4 and α1-6 linked fucose residues from oligosaccharides.

Source: Cloned from bovine kidney and expressed in *E. coli*.

Reaction Conditions: 1X Glycobuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 100°C for 10 minutes.

Reagents Supplied:
10X Glycobuffer 1
100X BSA

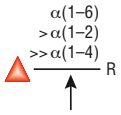
Molecular Weight: 51,800 daltons

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-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 8,000 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: 1X Glycobuffer 1. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X Glycobuffer 1

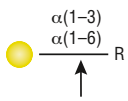
Molecular Weight: 49,000 daltons

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.

Concentration: 2,000 units/ml

α1-3,6 Galactosidase

#P0731S 100 units



Description: α1-3, 6 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-3, 6 linked D-galactopyranosyl residues from oligosaccharides.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli*.

Reaction Conditions: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

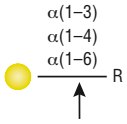
Molecular Weight: 70,000 daltons

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-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 4,000 units/ml

α1-3,4,6 Galactosidase

#P0747S 200 units
#P0747L 1,000 units



Description: α1-3,4,6 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: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

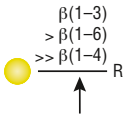
Molecular Weight: 39,700 daltons

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-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

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: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

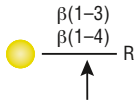
Molecular Weight: 66,000 daltons

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-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 10,000 units/ml

β1-3,4 Galactosidase

#P0746S 400 units



Description: β1-3,4 Galactosidase, cloned from bovine testis 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 testis and expressed in *Pichia pastoris.*

Reaction Conditions: 1X GlycoBuffer 4. Incubate at 37°C.Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 4

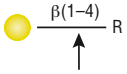
Molecular Weight: 71,000 daltons

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-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

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: 1X GlycoBuffer 1. Incubate at 37°C. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1

Molecular Weight: 231,000 daltons

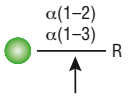
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, β-D-galactose from 1 nmol Galβ1-4GlcNAcβ1-3Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 8,000 units/ml

α1-2,3 Mannosidase

RR BSA 37° 55°

#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: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 55°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

Molecular Weight: 90,000 daltons

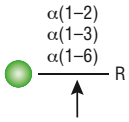
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-D-Mannose from 1 nmol of Manα1-3Manβ1-4GlcNAc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 32,000 units/ml

α1-2,3,6 Mannosidase

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: 1X GlycoBuffer 4 and 1X Zinc. Incubate at 37°C. Heat inactivation: 95°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 4
10X Zinc

Molecular Weight: 110,000 daltons

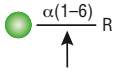
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal mannose from 1 nmol of Man(α1,3)-Man(β1,4)-GlcNAc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 2,000 units/ml

α1-6 Mannosidase

RR BSA 37° 65°

#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: 1X GlycoBuffer 1. Supplement with 100 µg/ml BSA. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1
100X BSA

Molecular Weight: 58,000 daltons

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-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

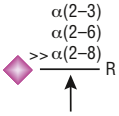
Concentration: 40,000 units/ml

Note: *p*-nitrophenyl-α-D-mannopyranoside is NOT a substrate for this enzyme.

α2-3,6,8 Neuraminidase

RR 37° 65°

#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: 1X GlycoBuffer 1. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1

Molecular Weight: 43,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol of Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC, in 5 minutes at 37°C in a total reaction volume of 10 µl.

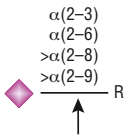
Concentration: 50,000 units/ml

Note: This enzyme shows a preference for α2,3 and α2,6 linkages over α2,8 linkages.

α2-3,6,8,9 Neuraminidase A

RR 37° 65°

#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: 1X GlycoBuffer 1. Incubate at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1

Molecular Weight: 100,000 daltons

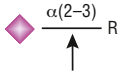
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 20,000 units/ml

α2-3 Neuraminidase S

RR 37° 75°

#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: 1X GlycoBuffer 1. Incubate at 37°C. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:
10X GlycoBuffer 1

Molecular Weight: 74,000 daltons

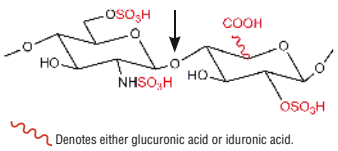
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the α-Neu5Ac from 1 nmol of Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 8,000 units/ml

Ann-Marie has been with NEB for 5 years as a Technical Account Manager for our subsidiary office in the UK. She enjoys working with customers across the south of London in both industry and academia. In her spare time, Ann-Marie likes to travel and spend time exploring the outdoors with her family.

Bacteroides Heparinase I

#P0735S 240 units
#P0735L 600 units



Denotes either 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:
10 µg heparin substrate, 10 µl *Bacteroides* Heparinase Reaction Buffer and H_2O in a total reaction volume of 100 µl. Incubate reaction at 30°C.
Heat inactivation: 100°C for 1 minute.

Reagents Supplied:
10X *Bacteroides* Heparinase Reaction Buffer

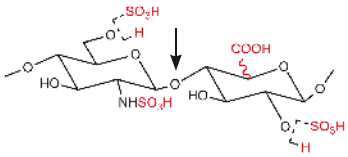
Molecular Weight: 42,000 daltons

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. For unit assay conditions, visit www.neb.com.

Concentration: 12,000 units/ml

Bacteroides Heparinase II

#P0736S 80 units
#P0736L 200 units



Denotes either 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:
10 µg heparin substrate, 10 µl *Bacteroides* Heparinase Reaction Buffer and H_2O in a total reaction volume of 100 µl. Incubate reaction at 30°C.
Heat inactivation: 100°C for 1 minute.

Reagents Supplied:
10X *Bacteroides* Heparinase Reaction Buffer

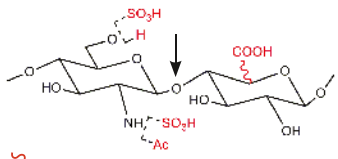
Molecular Weight: 86,000 daltons

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. For unit assay conditions, visit www.neb.com.

Concentration: 4,000 units/ml

Bacteroides Heparinase III

#P0737S 14 units
#P0737L 35 units



Denotes either 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:
10 µg heparan sulfate substrate, 10 µl *Bacteroides* Heparinase Reaction Buffer and H_2O in a total reaction volume of 100 µl. Incubate reaction at 30°C.
Heat inactivation: 100°C for 1 minute.

Reagents Supplied:
10X *Bacteroides* Heparinase Reaction Buffer

Molecular Weight: 75,000 daltons

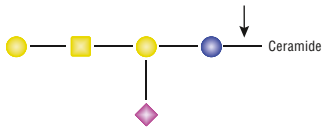
Unit Definition: One unit is defined as the amount of enzyme that will liberate 1.0 µmol unsaturated oligosaccharides from heparan sulfate per minute at 30°C and pH 7.0 in a total reaction volume of 100 µl. For unit assay conditions, visit www.neb.com.

Concentration: 700 units/ml

Cloned at NEB | RR Recombinant Enzyme | BSA Requires BSA | Enzymes for Innovation | 65 Heat Inactivation

Endoglycoceramidase I (EGCase I)

#P0773S 150 mU



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.

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: EGCase I is isolated from a strain of *E. coli*, which contains the cloned EGCase I gene from *Rhodococcus triatomea*.

Reaction Conditions: 1X EGCase I Reaction Buffer. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied:
10X EGCase I Reaction Buffer

Molecular Weight: 50,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to hydrolyze 1 µmol of ganglioside GM1a per minute at 37°C.

Concentration: 6 units/ml

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 cloned from *Streptococcus equi* subspecies *zooepidemicus* 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*.

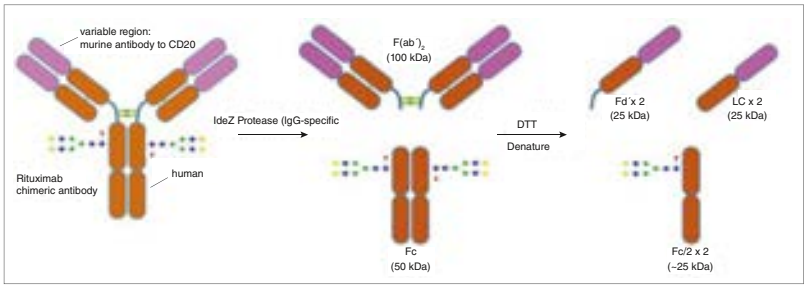
Reaction Conditions:
1X GlycoBuffer 2. Incubate at 37°C.
Heat inactivation: 65°C for 10 minutes.

Reagents Supplied with Enzyme:
10X GlycoBuffer 2

Molecular Weight: 35,578 daltons

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.

Concentration: 80,000 units/ml

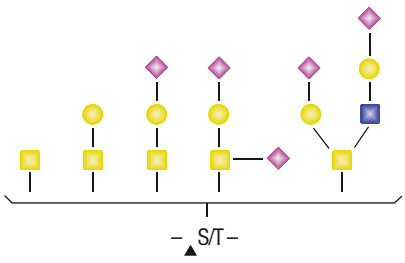


Digestion of IgG with IdeZ Protease (IgG-specific), followed by denaturation.

NEW

O-Glycoprotease

#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 TrisHCl, pH 8.0. Incubate at 37°C.
Heat inactivation: 95°C for 10 minutes.

Molecular Weight: 96,727 daltons

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 90% of 2 µM FAM-labeled O-glycopeptide in 2 hours at 37°C in a total reaction volume of 20 µl.

Concentration: 1,000 units/ml

Gal Glc Man GalNAc GlcNAc Fuc NeuAc **R** = any sugar

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 analyses. 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.

Trypsin-ultra™, Mass Spectrometry Grade

#P8101S	100 µg

Lys/Arg▼XXX

- *Digestion of proteins for proteomic analysis by mass spectrometry*
- *Protein and peptide identification*
- *TPCK treatment eliminates chymotryptic activity*
- *Free of contaminating proteases*

Description: Trypsin-ultra, Mass Spectrometry Grade is a serine endopeptidase. It selectively cleaves peptide bonds C-terminal to lysine and arginine residues. Trypsin-ultra, Mass Spectrometry Grade is treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inactivate any remaining chymotryptic activity. It is modified by acetylation of the ε-amino groups of lysine residues to prevent autolysis. Trypsin-ultra, Mass Spectrometry Grade cleaves at Lys-Pro and Arg-Pro bonds at a much slower rate than other amino acid residues.

Source: Isolated from bovine (*Bos taurus*) pancreas.

Reaction Conditions:
1X Trypsin Ultra Reaction Buffer. Incubate at 37°C.

Reagents Supplied with Enzyme:
2X Trypsin-ultra Reaction Buffer

Molecular Weight: 23,675 daltons

Reconstitution: Trypsin-ultra, Mass Spectrometry Grade should be reconstituted by the addition of 20–200 µl of high purity water. Rapid autolysis is a function of enzyme concentration.

Notes: Can be stored frozen in solution at –20°C for up to 1 week. A decrease in activity will occur if stored in solution. Use only freshly reconstituted protease for best results.

NEW

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: 19,800 daltons

Concentration: 0.4 mg/ml

Notes: 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.

John has been with NEB for 10 years, and serves as Packaging Supervisor for NEB's OEM and Customized Solutions Team. His strict attention to detail ensures that your custom product will be packaged quickly and according to your requirements. When not at work, John enjoys boating and coaching youth sports.

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 double-distilled water to make a 100 ng/µl solution in 10 mM Tris-HCl, pH 8.0. Rapid autolysis is a function of enzyme concentration. The solution can be stored at 4°C for several days or in single-use aliquots at –20°C for several months.

Note: LysC is compatible for simultaneous co-digestion with Trypsin-ultra, Mass Spectrometry Grade (NEB #P8101).

Endoproteinase GluC

RR 37°

#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 in *Bacillus subtilis.*

Reaction Conditions:
1X GluC Reaction Buffer. Incubate at 37°C.

Reagents Supplied with Enzyme:
2X 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. Rapid autolysis is a function of enzyme concentration.

Note: 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.

Endoproteinase AspN

RR 37°

#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:
1X AspN Reaction Buffer. Incubate at 37°C.

Reagents Supplied with Enzyme:
2X AspN Reaction Buffer

Molecular Weight: 40,089.9 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.

Notes: 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.

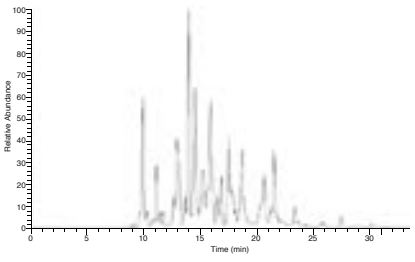
Trypsin-digested BSA MS Standard (CAM Modified)

#P8108S 500 pmol

- *Standardization range: 500 to 2400 Da*

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-ultra, Mass Spectrometry Grade, (NEB #P8101).



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 1,600 units

- *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)*

Reaction Conditions: Proteinase K is active in a wide range of buffers including all NEB specific restriction endonuclease buffers. It is highly active between pH 7.5 and 12.0 and temperatures between 20 and 60°C. Proteinase K is also active in chelating agents such as EDTA and activity is stimulated in up to 2% SDS or 4 M urea. Calcium is important for thermostability of Proteinase K but it is not required for catalysis, therefore Proteinase K is also active in buffers containing chelating agents such as EDTA.

Molecular Weight: 28,900 daltons

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.

Concentration: 800 units/ml

Thermolabile Proteinase K

RR 25° 55°

#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 K. *lactis*.

Molecular Weight: 29,000 daltons

Reaction Conditions: Thermolabile Proteinase K is active in a wide range of buffers. It is highly active between pH 7.0-9.5 and temperatures 20-40°C. It is active in chelating agents such as EDTA up to 10 mM.

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.

Concentration: 120 units/ml

TEV Protease

RR 30° 65°

#P8112S 1,000 units

ENLYFQ▼(G/S/M)

- *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-(Gly/Ser/Met) and cleaves between the Gln and Gly/Ser/Met 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*.

Molecular Weight: 28,000 daltons

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.

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: Purified from bovine plasma and activated by treatment with the activating enzyme from Russell's viper venom.

Molecular Weight: The predominant form of Factor Xa has a molecular weight of approximately 43,000 daltons, consisting of two disulfide-linked chains of approximately 27,000 daltons and 16,000 daltons. On SDS-PAGE, the reduced chains have apparent molecular weights of 30,000 daltons and 20,000 daltons.

Unit Definition: 1 µg of Factor Xa will cleave 50 µg of test substrate to 95% completion in 6 hours or less.

Concentration: 1 mg/ml

Removal: Factor Xa will bind specifically to benzamidine-agarose.

Enterokinase, light chain

RR

#P8070S 480 units
#P8070L 2,560 units

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: Purified from *Pichia pastoris* containing a clone of the light chain of the bovine enterokinase gene.

Molecular Weight: The molecular weight of the light chain of enterokinase is 26,300 daltons. Its apparent molecular weight on SDS-PAGE is 31 kDa.

Unit Definition: One 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.

Concentration: 16,000 U/ml

Removal: Enterokinase will bind specifically to trypsin inhibitor agarose (e.g., Sigma T-0637).

Furin

RR

#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 (kindly provided by R. Fuller).

Molecular Weight: The calculated molecular weight of truncated human furin is 52,700 daltons. Its apparent molecular weight in SDS-PAGE gels is 57,000 daltons.

Unit Definition: One unit is defined as the amount of furin 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.

Concentration: 2,000 units/ml

Lambda Protein Phosphatase (Lambda PP)

RR 30° 65°

#P0753S	20,000 units
#P0753L	100,000 units

Companion Products:

p-Nitrophenylphosphate (PNPP)
Non-specific substrate for protein, alkaline and acid phosphatases

#P0757S	1 ml
#P0757L	5 ml

Sodium Orthovanadate (Vanadate)
General inhibitor for protein phosphotyrosyl specific phosphatases

#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-PPase is active on phosphorylated histidine residues.

Source: Isolated from a strain of *E. coli* that carries the bacteriophage lambda ORF221 open reading frame (kindly provided by Dr. D. Barford).

Reaction Conditions:
1X NEBuffer for Protein MetalloPhosphatases (PMP). Supplement with 1 mM $MnCl_2$. Incubate at 30°C. Heat inactivation: 65°C for 1 hour in the presence of 50 mM EDTA.

Reagents Supplied with Enzyme:
10X NEBuffer for Protein MetalloPhosphatases (PMP)
10X $MnCl_2$ (10 mM)

Molecular Weight: 25,000 daltons

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.

Concentration: 400,000 units/ml

Lea has been with NEB for 6 years as a Production Scientist in our DNA Polymerase Group. She is involved in all facets of the manufacturing process for some of our most popular products. From planning to production and functional testing, Lea helps to make sure these products are always available for our customers.

Protein Kinases

The reversible addition of phosphate groups to proteins is important for the transmission of signals within eukaryotic cells and, as a result, protein phosphorylation and dephosphorylation regulate many diverse cellular processes. As the number of known protein kinases has increased at an ever-accelerating pace, it has become more challenging to determine which protein kinases interact with which substrates in the cell. The determination of consensus phosphorylation site motifs by amino acid sequence alignment of known substrates has proven useful in this pursuit. These motifs can be helpful for predicting phosphorylation sites for specific protein kinases within a potential protein substrate.

Since the determinants of protein kinase specificity involve complex 3-dimensional interactions, these motifs, short amino-acid sequences describing the primary structure around the phosphoacceptor residue, are a significant over-simplification of the issue. They do not take into account possible secondary and tertiary structural elements, or determinants from other polypeptide chains or from distant locations within the same chain. Furthermore, not all of the residues described in a particular specificity motif may carry the same weight in determining recognition and phosphorylation by the kinase. As a consequence, they should be used with some caution.

On the other hand, many of the residues within these consensus sequences have in fact proven to be crucial recognition elements, and the very simplicity of these motifs has made them useful in the study of protein kinases and their substrates. In addition to the prediction of phosphorylation sites, short synthetic oligopeptides based on consensus motifs are often excellent substrates for protein kinase activity assays.

The table below summarizes the specificity motifs for protein kinases that are available from NEB. Phosphoacceptor residue is indicated in red, amino acids which can function interchangeably at a particular residue are separated by a slash (/), and residues which do not appear to contribute strongly to recognition are indicated by an "X".

PROTEIN KINASE	NEB #	RECOGNITION DETERMINANT	SIZE
cAMP-dependent Protein Kinase (PKA), Catalytic Subunit	P6000S/L	R - R - X - S / T Y Y= hydrophobic residue	100,000/500,000 units
Casein Kinase II (CK2)	P6010S/L	S - X - X - E / D	10,000/50,000 units

D = aspartic acid, E = glutamic acid, R = arginine, S = serine, T = threonine, Y = tyrosine, 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.

Companion Product:

Adenosine-5´ Triphosphate (ATP)

#P0756S	1.0 ml
#P0756L	5.0 ml

Ph.D.™ Phage Display Peptide Library Kits

Ph.D.-7 Kit
10 Panning Experiments
#E8100S

Ph.D.-12 Kit
10 Panning Experiments
#E8110S

Ph.D.-C7C Kit
10 Panning Experiments
#E8120S

Components Sold Separately:

Ph.D.-12 Phage Display Peptide Library
50 Panning Experiments
#E8111L

The Ph.D. Kits Include:

- *Sufficient Phage Display Library for 10 separate panning experiments, complexity of 10^9 clones*
- *28 gIII Sequencing Primer (100 pmol)*
- *96 gIII Sequencing Primer (100 pmol)*
- *Host E. coli strain ER2738*
- *Control Target (Streptavidin) and Elutant (Biotin)*
- *Detailed Protocols*

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.

New England Biolabs 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.

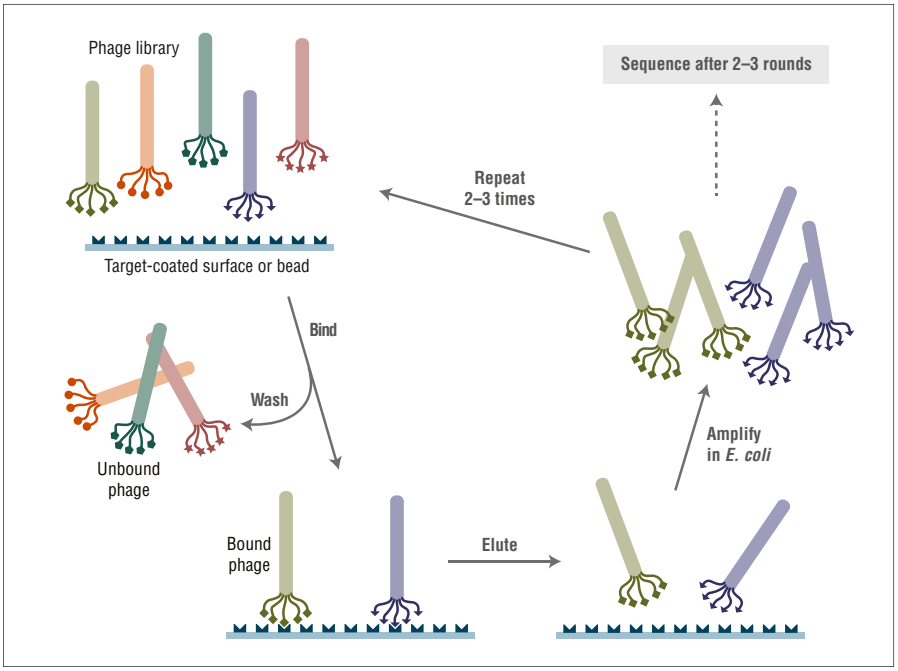


Figure 1: Routine Phage Display Workflow. *Round 1 : Incubate 10^{11} pfu Ph.D. library + target incubation, wash away non-binders, elute bound phage, enrich selected phage with amplification in E. coli. Carry out 3-4 rounds of selection and then proceed with sequencing and/or phage-ELISA.*

β-endorphin:

Y	G	G	F	M	T	S	E	K	Q	T	P…

1st round sequences:

Y	G	W	I	S	P	P	L	H	L	P	T
Y	Q	P	D	N	P	S	R	Q	I	A	N
Y	W	P	A	H	I	R	A	V	P	M	I
R	L	D	D	I	K	N	T	L	A	F	S
S	S	D	V	Y	S	L	Y	P	F	I	M
E	F	F	P	H	P	M	L	H	N	S	R
D	N	W	P	Y	R	P	S	F	S	L	S
S	H	N	T	Y	S	A	P	R	P	S	A
S	L	L	H	Y	A	S	S	L	S	L	M
F	N	Q	N	A	E	P	F	S	S	R	P
H	P	R	Q	L	L	H	H	P	L	S	P

2nd round sequences:

Y	G	G	F	L	I	G	L	Q	D	A	S
Y	G	G	F	H	Y	K	E	T	G	A	L
Y	Q	P	D	N	P	S	R	Q	I	A	N
V	Y	C	Y	I	N	Q	S	M	I	G	N
H	H	D	T	E	Y	R	T	T	Q	L	S
N	L	K	F	P	T	N	P	K	A	M	W
L	P	N	L	T	W	A	L	M	P	R	A
D	N	W	P	Y	R	P	S	F	S	L	S
S	H	N	T	Y	S	A	P	R	P	S	A
S	L	L	H	Y	A	S	S	L	S	L	M
V	T	M	N	T	K	T	P	G	P	M	P

3rd round sequences:

Y	G	G	F	M	T	T	P	S	H	V	P
Y	G	G	F	M	T	T	P	S	H	V	P
Y	G	G	F	I	S	Q	T	Q	H	Y	S
Y	G	G	F	I	S	Q	T	Q	H	Y	S
Y	G	G	F	G	N	S	L	V	M	P	V
Y	G	G	F	S	M	P	F	L	P	A	L
Y	G	A	F	D	V	T	T	G	V	T	S
Y	G	V	F	N	P	H	Y	L	P	S	L
A	P	S	T	D	K	Q	A	T	M	P	L
A	S	V	A	V	S	S	R	Q	D	A	A

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 α-amino group of the N-terminal tyrosine is part of the epitope.

Companion Product:

Anti-M13 pIII Monoclonal Antibody

Anti-M13 pIII Monoclonal Antibody (mouse isotype IgG2a) is derived from the A23 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with the C-terminal half of M13 coat protein III (residues 259–406).

Note: ELISA against whole phage using this antibody is not recommended since the epitope lies in a region that is not accessible on intact M13 phage virions.

#E8033S 0.1 ml

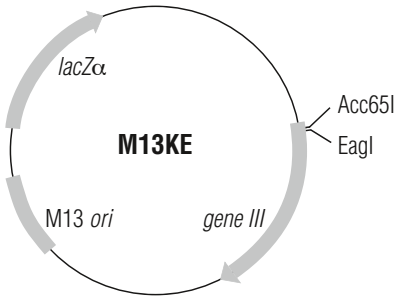
Ph.D. Peptide Display Cloning System

Ph.D. Peptide Display Cloning System

20 µg M13KE gIII Cloning Vector
16 µg Extension Primer

#E8101S

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.



View of the Copper Beech Tree adjacent to NEB's laboratory facility. Credit: Mileidy Rodriguez, NEB

Promoting Environmental Sustainability

Don Comb was certainly a lover of nature. Growing up, one of his hobbies was entomology, and a formative insect-related observation contributed to his life-long commitment to protecting nature. As a child, Don noticed his parent's car windshield splattered with insects at the end of a journey. However, as an adult, he saw a marked reduction in the insect population, and knew this was a reflection of the health of entire ecosystems. Don was not one to passively make note of a less-than-ideal situation — instead he was a person who would ask big questions, and then search for solutions that he could implement. He did this in both his personal and professional life.

When Don selected a new location for NEB's headquarters in Ipswich, MA, his priority was to minimize and, where possible, mitigate the impact NEB had on the environment. As such, the company implemented sustainable solutions in every aspect of its operations. The campus lies on a historic property comprised of an idyllic 160-acre expanse of mixed forest, wetlands and agricultural land. The beautiful grounds include public trails for hiking, skiing, bird-watching and horseback riding, and is home to a vast array of wildlife species.

In 2003, NEB hosted a competition, inviting architects to submit innovative designs for a 140,000 sq. ft state-of-the-art laboratory facility. The only rule was that they had to design the building around three Copper Beech trees. In fact, NEB installed underground retaining walls to protect the Copper Beech tree's root system to prevent compaction of surrounding soil by heavy construction machinery as the facility was being built.

The laboratory facility is LEED® (Leadership in Energy and Environmental Design) and ISO 14001 certified, which dictates a framework of strict, environmentally sound regulations, including a specific percentage of recycled materials used in construction and limits as to how far those materials should be transferred. No exotic woods were used — only common, local wood such as Maple, White Oak and domestic Ash. The main hallway is mostly constructed of glass, optimizing use of natural light and enabling a view of the majestic surroundings from almost anywhere.

The campus is situated within the Ipswich River Watershed, a complex and sensitive ecosystem that provides drinking water to 350,000 people and thousands of businesses in 14 communities. All of NEB's water needs are pulled from the Ipswich River, and we felt it was important to protect this resource. For this reason, another pioneering sustainable feature was installed on campus — a Solar Aquatics Wastewater System® that processes wastewater through a series of physical and biological filters, essentially utilizing and accelerating processes found naturally in streams and wetlands. Water is discharged from the facility back into the groundwater cleaner than when it came in.

Don believed that all companies should be responsible for protecting the environment. This commitment helped to make NEB a pioneer in eco-friendly building and operating practices, and set a precedent for other biotechnology companies to follow. As NEB continues to expand, we choose to make decisions with the mindset of protecting the planet for future generations.

Take a tour of the NEB campus.

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 40 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

278 EpiMark 5-hmC and 5-mC Analysis Kit

279 EpiMark Methylated DNA Enrichment Kit

283 EpiMark Nucleosome Assembly Kit

289 NEBNext Enzymatic Methyl-seq

Featured Tools & Resources

Videos of NEB Scientists Discussing Epigenetics

Epigenetics-related FAQs

Feature Articles

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

EpiMark® 5-hmC and 5-mC Analysis Kit

#E3317S 20 reactions

- *Reproducible quantitation of 5-hmC and 5-mC within a specific loci*
- *Easy-to-follow protocols*
- *Compatible with existing techniques (PCR)*
- *Amenable to high throughput*

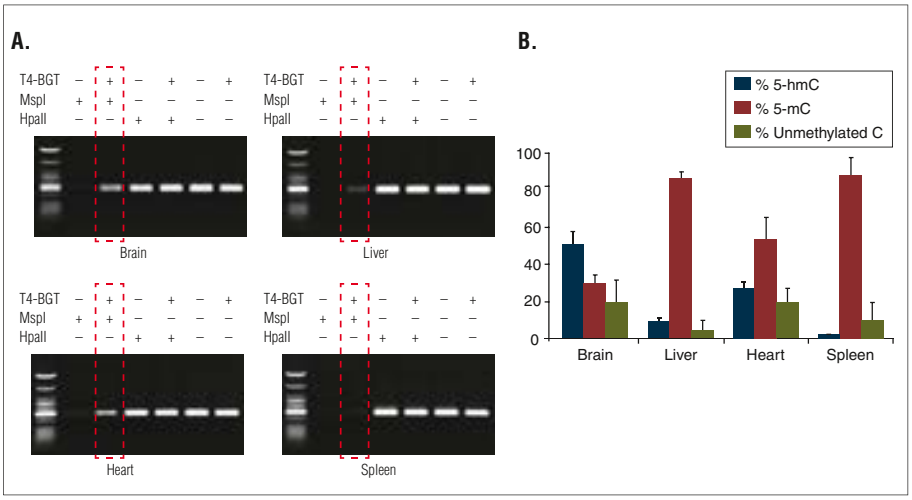
Visit **EpiMark.com** to view a video tutorial for this kit.

Description: The EpiMark 5-hmC and 5-mC Analysis Kit is a simple and robust method for the identification and quantitation of 5-hydroxymethylcytosine (5-hmC) and 5-methylcytosine (5-mC) within a specific DNA locus. This enzymatic approach utilizes the differential methylation sensitivity of the isoschizomers MspI and HpaII in a simple 3-step protocol.

Briefly, genomic DNA is treated with T4 phage β-glucosyltransferase and UDP-glucose, which glucosylates all 5-hmC present. DNA is digested with MspI and HpaII, two isoschizomers with different methylation sensitivity. Endpoint or real time PCR can then be used to identify and quantitate the different methylation states. Designed to simplify methylation analysis, the EpiMark Kit expands the potential for new biomarker discovery.

The EpiMark 5-hmC and 5-mC Analysis Kit Includes:

- T4 Phage β-glucosyltransferase
- UDP-Glucose
- MspI
- HpaII
- Proteinase K
- Control DNA (unmodified, 5-mC and 5-hmC)
- Forward and reverse control primer mix
- NEBuffer 4



Analysis of the different methylation states in Balb/C mouse tissue samples (locus 12) using the EpiMark 5-hmC and 5-mC Analysis Kit. *A) Endpoint PCR of the 6 different reactions needed for methylation analysis. The boxed lanes indicate the presence of 5-hmC. B) Real time PCR data was used to determine amounts of 5-hmC and 5-mC present. The results demonstrate a variation in 5-hmC levels in the tissue sources indicated.*

T4 Phage β-glucosyltransferase

#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*

Learn about 5-hmC detection in Balb/C brain tissue.

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: 1X NEBuffer 4 and 40 µM UDP-Glucose. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied with Enzyme:
10X NEBuffer 4
50X UDP-Glucose (2 mM)

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

EPIGENETICS

Cloned at NEB | RR Recombinant Enzyme | Epi EpiMark Validated | NEB4 Optimal Buffer | BSA Requires BSA | 37° Incubation Temperature

EpiMark Methylated DNA Enrichment Kit

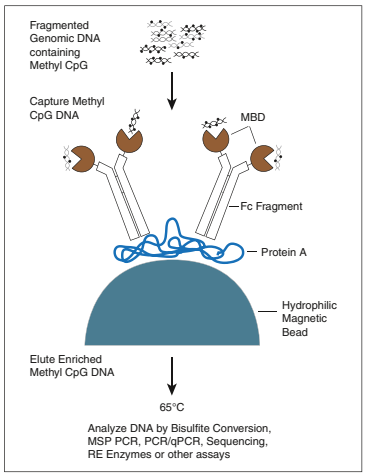
Epi

#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.

Enrichment Workflow



The EpiMark Methylated DNA Enrichment Kit Includes:

- MBD2-Fc protein
- Protein A Magnetic Beads
- Bind/wash buffer
- High-salt elution buffer
- Fragmented HeLa DNA
- Line element primers for methylated-locus controls
- RPL30 primers for unmethylated locus contols
- MirA locus control primers

EpiMark N6-Methyladenosine Enrichment Kit

Epi

See page 220 for more information.

#E1610S 20 reactions

Description: The EpiMark N6-Methyladenosine Enrichment 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.

EpiMark Bisulfite Conversion Kit

Epi

#E3318S 48 reactions

- *Complete conversion of unmodified cytosine to uracil*
- *Easy-to-follow protocol*
- *Reliable and consistent results*
- *Purification columns included*

Description: This technique can reveal the methylation status of every cytosine residue, and it is amenable to massively parallel sequencing methods. Bisulfite conversion involves the conversion of unmodified cytosines to uracil, leaving the modified bases 5-mC and 5-hmC. The EpiMark Bisulfite Conversion Kit is designed for the detection of methylated cytosine, using a series of alternating cycles of thermal denaturation, followed by incubation with sodium bisulfite. This kit includes all the reagents necessary for complete bisulfite conversion, including spin columns. Amplification of bisulfite-treated samples can then be performed using EpiMark Hot Start *Taq* DNA Polymerase.

The EpiMark Bisulfite Conversion Kit Includes:

- Sodium metabisulfite
- Solubilization buffer
- Desulphonation reaction buffer
- EpiMark spin columns with 2 ml collection tubes
- Binding buffer
- Wash buffer
- Elution buffer

EpiMark Hot Start *Taq* DNA Polymerase

RR Epi

See page 71 for more information.

#M0490S 100 reactions
#M0490L 500 reactions

Description: EpiMark Hot Start *Taq* DNA Polymerase 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 assembly of reactions at room temperature. An advantage of the aptamer-based hot start mechanism is that it does not require a separate high temperature incubation step to activate the enzyme. The advanced aptamer-based hot-start activity coupled with the supplied optimized reaction buffer makes the EpiMark Hot Start *Taq* DNA Polymerase an excellent choice for use on bisulfite-converted DNA.

dilA Diluent Buffer 65 Heat Inactivation

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.

Reference:

(1) Cohen-Karni, D. et al. (2011) *PNAS*, 108, 11040–11045.

AbaSI

rCutSmart RR Epi dilC 25° 65°

#R0665S 1,000 units

5′...^{ghm}C N_{11-13}▼N_{9-10} G...3′
3′..... G N_{9-10} ▲N_{11-13} ^{x}C...5′

^{x}C = ^{ghm}C, ^{hm}C, ^{m}C or C

Description: AbaSI is a DNA modification-dependent endonuclease that recognizes 5-glucosylhydroxymethylcytosine (^{ghm}C) in double-stranded DNA and cleaves 11–13 bases 3′ from the modified C leaving a 2–3 base 3′ overhang. The enzyme only cleaves if there is a G residue 20–23 nucleotides 3′ from the modified C. AbaSI also recognizes 5-hydroxymethylcytosine (^{hm}C) at a much lower efficiency. It does not recognize DNA with 5-methylcytosine (^{m}C) or unmodified cytosine.

Reaction Conditions: 1X rCutSmart Buffer + 1 mM DTT (supplied), 25°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

Note: This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

FspEI

rCutSmart RR Epi dilB 37° 80°

#R0662S 200 units

5′...C^{m}C $(N)_{12}$▼...3′
3′...G G $(N)_{16}$▲...5′

Description: FspEI is a modification-dependent endonuclease which recognizes C^{m}C sites and generates a double-stranded DNA break on the 3′ side of the modified cytosine at N_{12}/N_{16}. Recognized cytosine modifications include C5-methylation (5-mC) and C5-hydroxymethylation (5-hmC).

Reaction Conditions: 1X rCutSmart Buffer + 1X Enzyme Activator Solution (supplied), 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

Note: Star activity may result from extended digestion.

This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

LpnPI

rCutSmart RR Epi dilB 37° 65°

#R0663S 200 units

5′...C^{m}C D G $(N)_{10}$▼...3′
3′...G G H C $(N)_{14}$▲...5′

Description: LpnPI is a modification-dependent endonuclease which recognizes C^{m}CDG sites and generates a double-stranded DNA break on the 3′ side of the modified cytosine at N_{10}/N_{14}. Recognized cytosine modifications include C5-methylation (5-mC) and C5-hydroxymethylation (5-hmC).

Reaction Conditions: 1X rCutSmart Buffer + 1X Enzyme Activator Solution (supplied), 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Note: Star activity may result from extended digestion.

This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

MspJI

rCutSmart RR Epi dilB 37° 65°

#R0661S 200 units
#R0661L 1,000 units

5′...^{m}C N N R $(N)_{9}$▼...3′
3′... G N N Y $(N)_{13}$▲...5′

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: 1X rCutSmart Buffer + 1X Enzyme Activator Solution (supplied), 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Note: Star activity may result from extended digestion.

This product will be supplied with Recombinant Albumin-containing buffer. For details, visit page 20 or www.neb.com/BSA-free.

Cloned at NEB | RR Recombinant Enzyme | Epi EpiMark Validated | NEB4 Optimal Buffer | BSA Requires BSA | 37° Incubation Temperature | dilA Diluent Buffer | 65° Heat Inactivation

McrBC

#M0272S 500 units
#M0272L 2,500 units

5´...Pu^{m}C ($N_{40–3000}$) Pu^{m}C... 3´

- *Determination of the methylation state of CpG dinucleotides*
- *Detection of cytosine methylated DNA*

Description: McrBC is an endonuclease that cleaves DNA containing methylcytosine* on one or both strands. McrBC will not act upon unmethylated DNA. Sites on the DNA recognized by McrBC consist of two half-sites of the form (G/A)^{m}C. These half-sites can be separated by up to 3 kb, but the optimal separation is 55–103 base pairs. McrBC requires GTP for cleavage, but in the presence of a non-hydrolyzable analog of GTP, the enzyme will bind to methylated DNA specifically, without cleavage. McrBC will act upon a pair of Pu^{m}CG sequence elements, thereby detecting a high proportion of methylated CpGs, but will not recognize HpaII/MspI sites (CCGG) in which the internal cytosine is methylated.

* 5-methylcytosine, 5-hydroxymethylcytosine or N4-methylcytosine

Reaction Conditions: 1X NEBuffer 2 + 200 µg/ml BSA + 1 mM GTP, 37°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied with Enzyme:
10X NEBuffer 2
100X BSA
100X GTP (100 mM)
Control Plasmid DNA

Unit Definition: One unit is defined as the amount of enzyme required to cleave 0.5 µg of a plasmid containing multiple McrBC sites in 1 hour at 37°C in a total reaction volume of 50 µl.

Concentration: 10,000 units/ml

Note: McrBC makes one cut between each pair of half-sites, cutting close to one half-site or the other, but cleavage positions are distributed over several base pairs approximately 30 base pairs from the methylated base. Therefore, when multiple McrBC half-sites are present in DNA (as is the case with cytosine-methylated genomic DNA), the flexible nature of the recognition sequence results in an overlap of sites, producing a smeared, rather than a sharp, banding pattern.

Additional Restriction Enzymes for Epigenetic Analysis

See pages 36, 40, 43 for more information.

Visit page 321 for more information about Dam, Dcm and CpG methylation.

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.

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

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

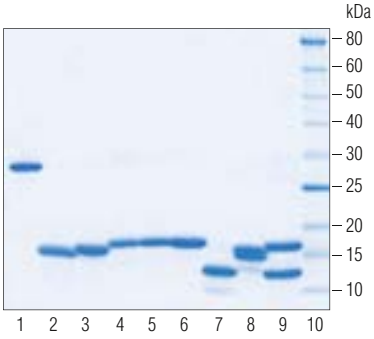
Histones

- *Purification and characterization of enzymes that modify histone proteins*
- *Octamer and nucleosome modification studies*
- *Carrier chromatin immunoprecipitation (CChIP)*
- *High throughput studies*

In eukaryotes, nuclear DNA is assembled into chromatin by nucleoprotein complexes. The primary unit of chromatin, a nucleosome core particle (NCP), is an octamer complex made up of two molecules each of Histone H2A, H2B, H3 and H4 and approximately 147 base pairs of nuclear DNA. Histone H1 further condenses the DNA by binding the linker segments between NCP complexes (1,2). Histones undergo diverse post-translational modification including acetylation, phosphorylation, mono-, di- or tri-methylation, ubiquitination, isomerization and ADP-ribosylation. Through their potential combinatorial sequences on a given histone and their reversibility, these modifications dynamically restrict or recruit numerous other proteins or protein complexes onto chromatin (3). The study of their roles in gene regulation (4), cellular stress events (4), aging and DNA repair (5) is revealing the multiple functions of histone modifications in the fate of a cell. Additional variability is incorporated into the system by histone variants. Acting individually or combinatorially in conjunction with DNA modification, histone modifications and histone variants are thought to establish an epigenetic code or epigenetic mechanism of gene regulation (3).

In total, seven human histones, including three histone H3 variants (see alignment below), have been individually cloned and expressed in *E. coli* and then highly purified from cell extracts at NEB. Mass spectrometry analysis demonstrates that these recombinant histones are free of post-translational modifications. These histones are ideal substrates for the purification and characterization of histone modifying enzymes.

To aid in studying intact nucleosomes, we now offer the EpiMark Nucleosome Assembly Kit. The precise mixing of a 2:1 ratio of Histone H2A/H2B Dimer to Histone H3.1/H4 Tetramer generates a recombinant human histone octamer, and in the presence of DNA forms nucleosomes (7,8). Enzymes that are unable to modify individual histones or DNA may be active on these nucleosome core particles, the histone dimer, or the histone tetramer (9,10). The NCPs also may be used as carrier chromatin in CChIP (carrier chromatin immunoprecipitation) assays (11). The recombinant human histone dimer and recombinant human histone tetramer are also available as separate products.



Experience the purity of Histones from NEB, *SDS-PAGE analysis of the histones available from NEB.*

1: Histone H1° (NEB #M2501) 1 µg
2: Histone H2A (NEB #M2502) 1 µg
3: Histone H2B (NEB #M2505) 1 µg
4: Histone H3.1 (NEB #M2503) 1 µg
5: Histone H3.2 (NEB #M2506) 1 µg
6: Histone H3.3 (NEB #M2507) 1 µg
7: Histone H4 (NEB #M2504) 1 µg
8: Histone H2A/H2B Dimer (NEB #M2508) 2 µg
9: Histone H3.1/H4 Tetramer (NEB #M2509) 2 µg
10: NEB Protein Ladder

References:

(1) Kornberg, R.D. (1977) *Annu. Rev. Biochem.*, 46, 931–954.
(2) van Holde, K.E. (1989) *Chromatin*, 1–497.
(3) Kim, J.K., Samaranayake, M. and Pradhan, S. (2009) *Cell. Mol. Life Sci.*, 66, 596–612.
(4) Huang, J. et al. (2006) *Nature*, 444, 629–632.
(5) Pahlich, S., Zakaryan, R.P. and Gehring, H. (2006) *Biochim. Biophys. Acta.*, 1764, 1890–1903.
(6) Hake, S.B. et al. (2006) *J. Biol. Chem.*, 281, 559–568.
(7) Luger et al. (1999) *Methods in Mol. Biol.*, 119, 1–16.
(8) Comb, D. and Mersha, F. unpublished observations.
(9) Li, Yan et al. (2009) *J. Biol. Chem.*, 284, 34283–34295.
(10) Fung, J. et al. (2002) *Current Biology*, 12, 1086–1099.
(11) O'Neill et al. (2006) *Nature Genetics,* 38, 835–841.


```
       10         20         30         40         50         60         70
ARTKQTARKS TGGKAPRKQL ATKAARKSAP ATGGVKKPHR YRPGTVALRE IRRYQKSTEL LIRKLPFQRL   histone H3.1
ARTKQTARKS TGGKAPRKQL ATKAARKSAP ATGGVKKPHR YRPGTVALRE IRRYQKSTEL LIRKLPFQRL   histone H3.2
ARTKQTARKS TGGKAPRKQL ATKAARKSAP STGGVKKPHR YRPGTVALRE IRRYQKSTEL LIRKLPFQRL   histone H3.3
       80          90        100        110        120        130
VREIAQDFKT DLRFQSSAVM ALQEACEAYL VGLFEDTNLC AIHAKRVTIM PKDIQLARRI RGERA         histone H3.1
VREIAQDFKT DLRFQSSAVM ALQEASEAYL VGLFEDTNLC AIHAKRVTIM PKDIQLARRI RGERA         histone H3.2
VREIAQDFKT DLRFQSAAIG ALQEASEAYL VGLFEDTNLC AIHAKRVTIM PKDIQLARRI RGERA         histone H3.3
```

Sequence alignment of Human Histone variants H3.1, H3.2 and H3.3. *Human Histone H3.1, 3.2 and 3.3 vary by only a few amino acids (changes are highlighted in* ***red****), but are associated with different biological functions (6).*

EpiMark Nucleosome Assembly Kit

RR Epi

#E5350S 20 reactions

Components Sold Separately:

Histone H3.1/H4 Tetramer Human, Recombinant
#M2509S 1 nmol

Histone H2A/H2B Dimer Human, Recombinant
#M2508S 2 nmol

Nucleosome Control DNA
#N1202S 0.2 nmol

- *Highly pure, recombinant system*
- *Pre-formed histone dimer and tetramer complexes simplify octamer formation*
- *Components stable for one year*
- *Ideal for ChIP Assay, HAT Assay and enzyme modification assays (e.g., methylation studies)*

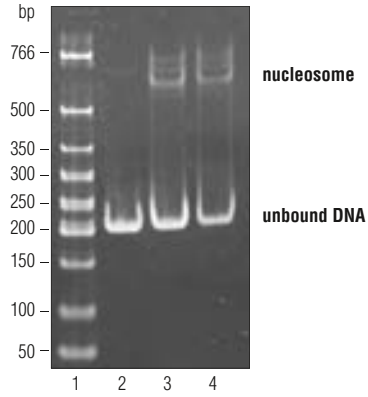
Description: This kit contains the components necessary to form an unmodified recombinant human nucleosome using your own target DNA or the supplied control DNA. The protocol requires the mixing of already formed and purified recombinant human Histone H2A/H2B Dimer and Histone H3.1/H4 Tetramer in the presence of DNA in high salt, followed by dialysis down to low salt, to make nucleosomes. One tetramer associates with two dimers to form the histone octamer on the DNA, generating a nucleosome. A method for assaying nucleosome formation by gel shift assay is also provided. These nucleosomes may serve as a better substrate for enzymes that are inactive on the DNA or one of the core histones alone. Each described reaction creates nucleosomes from ~50 pmol of a 208 bp DNA and may be scaled depending on the experiment.

Histone H2A/H2B Dimer Human, Recombinant is generated by refolding the denatured, purified subunits H2A and H2B, followed by gel filtration. Histone H3.1/H4 Tetramer Human, Recombinant is generated by refolding the denatured, purified subunits H3.1 and H4, followed by gel filtration. Both the dimer and tetramer are highly pure and are available separately for histone modification studies.

The Nucleosome Control DNA is a 208 base pair fragment from *Lytechinus variegates* 5SrDNA, and can be used for mononucleosome formation.

The EpiMark Nucleosome Assembly Kit Includes:

– Histone H2A/H2B Dimer
– Histone H3.1/H4 Tetramer
– Control DNA



Gel shift assay to visualize nucleosome formation.
Samples from nucleosome assembly reactions were run on 6% polyacrylamide gel in 0.5X TBE. Lane 1: Low Molecular Weight DNA Ladder (NEB #N3233). Lane 2: Nucleosome Control DNA. Lane 3: 0.5:1 ratio of Octamer to DNA. Lane 4: 1:1 ratio of Octamer* to DNA.*

**Octamer = 2:1 mix of Histone H2A/H2B Dimer and Histone H3.1/H4 Tetramer.*

Histone H1^0 Human, Recombinant

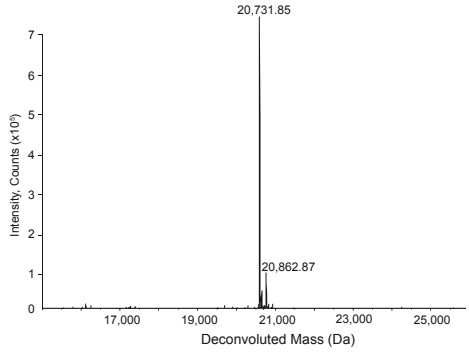
RR Epi

#M2501S 100 µg

Description: Histone H1 acts on the linker region of polynucleosome DNA to condense the chromatin into structures of ~30 nm. It is not necessary for octamer or nucleosome core particle formation. Eight different Histone H1 proteins have been identified in the human genome. Histone H1^0 is a non replication-dependent histone that is highly expressed in terminally differentiated cells.

Synonyms: Histone H1.0, Histone H1(0), Histone H1'

Concentration: 1 mg/ml



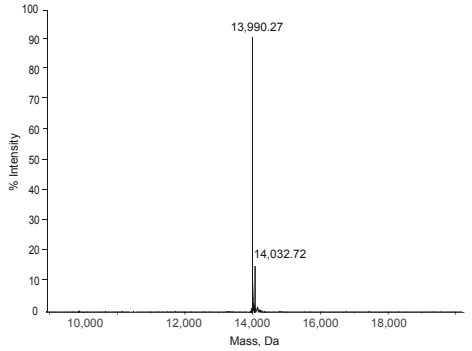
Mass Spectrometry Analysis of Histone H1^0 Human, Recombinant. The average mass calculated from primary sequence is 20,732 Da.

Histone H2A Human, Recombinant

#M2502S 100 µg

Description: Histone H2A combines with Histone H2B to form the H2A-H2B heterodimer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer. Histone H2A is also modified by various enzymes and can act as a substrate for them. These modifications have been shown to be important in gene regulation.

Concentration: 1 mg/ml



Mass Spectrometry Analysis of Histone H2A Human, Recombinant. The average mass calculated from primary sequence is 13,990 Da.

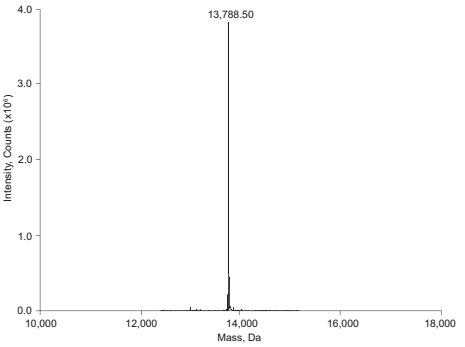
Histone H2B Human, Recombinant

#M2505S 100 µg

Description: Histone H2B combines with Histone H2A to form the H2A-H2B heterodimer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer. Histone H2B is also modified by various enzymes and can act as a substrate for them. These modifications have been shown to be important in gene regulation.

Synonyms: Histone H2B/8, Histone H2B.1, Histone H2B-GL105

Concentration: 1 mg/ml



Mass Spectrometry Analysis of Histone H2B Human, Recombinant. The average mass calculated from primary sequence is 13,789 Da.

Histone H3.1 Human, Recombinant

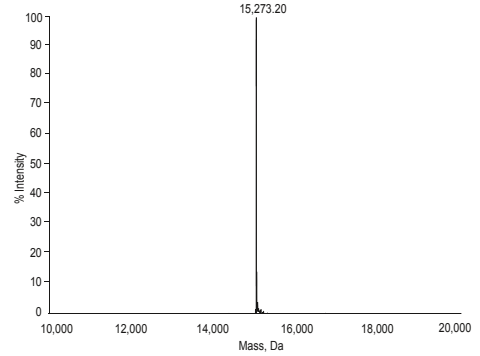
#M2503S 100 µg

Description: Histone H3 combines with Histone H4 to form the H3/H4 tetramer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer. It is also modified by various enzymes and can act as a substrate for them. These modifications have been shown to be important in gene regulation.

Histone H3.1, an H3 variant that has thus far only been found in mammals, is replication dependent and is associated with gene activation and gene silencing.

Synonyms: Histone H3/a

Concentration: 1 mg/ml



Mass Spectrometry Analysis of Histone H3.1 Human, Recombinant. The average mass calculated from primary sequence is 15,273 Da.

Cloned at NEB | Recombinant Enzyme | EpiMark Validated | Optimal Buffer | Requires BSA | Incubation Temperature | Diluent Buffer | Heat Inactivation

Histone H3.2 Human, Recombinant

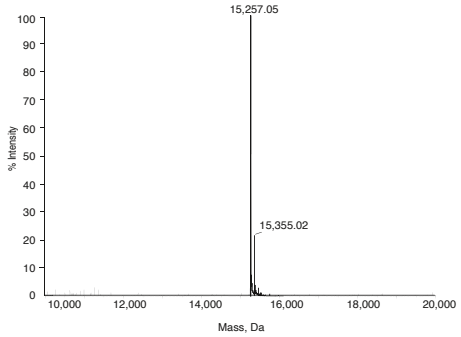
#M2506S 100 µg

Description: Histone H3 combines with Histone H4 to form the H3/H4 tetramer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer. It is also modified by various enzymes and can act as a substrate for them. These modifications have been shown to be important in gene regulation.

Histone H3.2, an H3 variant that is found in all eukaryotes except budding yeast, is replication dependent and is associated with gene silencing.

Synonyms: Histone H3/m, H3/o

Concentration: 1 mg/ml



Mass Spectrometry Analysis of Histone H3.2 Human, Recombinant. The average mass calculated from primary sequence is 15,257 Da.

Histone H3.3 Human, Recombinant

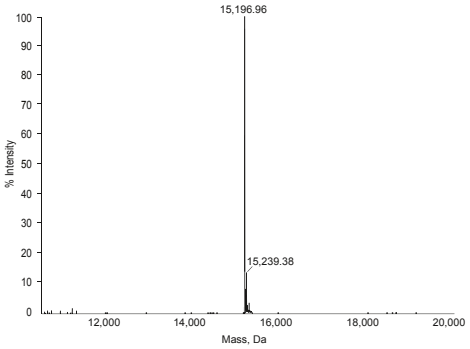
#M2507S 100 µg

Description: Histone H3 combines with Histone H4 to form the H3/H4 tetramer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer. It is also modified by various enzymes and can act as a substrate for them. These modifications have been shown to be important in gene regulation.

Histone H3.3, an H3 variant that is found in all eukaryotes from yeast to human, is replication and cell cycle phase independent and is the most common H3 in non-dividing cells. It has been shown to be enriched in covalent modifications associated with gene activation.

Synonyms: Histone H3.3A, H3F3, H3.3B

Concentration: 1 mg/ml



Mass Spectrometry Analysis of Histone H3.3 Human, Recombinant. The average mass calculated from primary sequence is 15,197 Da.

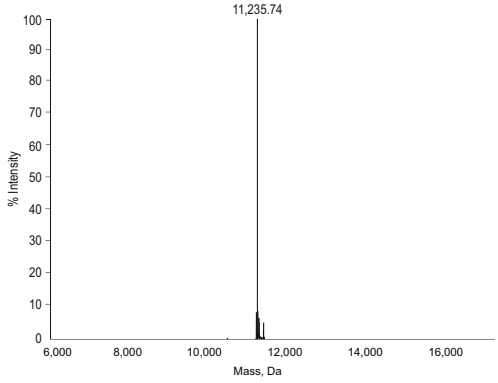
Histone H4 Human, Recombinant

#M2504S 100 µg

Description: Histone H3 combines with Histone H4 to form the H3/H4 tetramer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer. Histone H4 is also modified by various enzymes and can act as a substrate for them. These modifications have been shown to be important in gene regulation.

Synonyms: For HIST2H4 gene: H4/N, H4F2, H4FN

Concentration: 1 mg/ml



Mass Spectrometry Analysis of Histone H4 Human, Recombinant. The average mass calculated from primary sequence is 11,236 Da.

DNA Methyltransferases

RR

See pages 124-126 for more information.

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

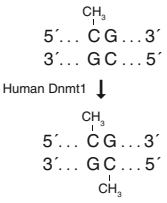
MspI Methyltransferase
#M0215S 100 units

*Taq*I Methyltransferase
#M0219S 1,000 units

Human DNA (cytosine-5) Methyltransferase (Dnmt1)

RR Epi SAM 37° 65°

#M0230S 50 units
#M0230L 250 units



Description: Dnmt1 methylates cytosine residues in hemimethylated DNA at 5´...CG...3´. Mammalian Dnmt1 is believed to be involved in carcinogenesis, embryonic development and several other biological functions. The bulk of the methylation takes place during DNA replication in the S-phase of the cell cycle.

Reaction Conditions: 1X Dnmt1 Reaction Buffer. Supplement with 100 µg/ml BSA and 160 µM S-adenosylmethionine. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied with Enzyme:
10X Dnmt1 Reaction Buffer
100X BSA
32 mM S-adenosylmethionine (SAM)

Unit Definition: One unit is the amount of enzyme required to catalyze the transfer of 1 pmol of methyl group to poly dI.dC substrate in a total reaction volume of 25 µl in 30 minutes at 37°C.

Concentration: 2,000 units/ml

Note: For DNA modification and protection applications, M.SssI (NEB #M0226) is preferred because it efficiently methylates both unmethylated and hemimethylated DNA substrates.

HeLa Genomic DNA

#N4006S 15 µg

- *PCR, SNP analysis and southern blotting*
- *Genomic DNA library construction*
- *Control DNA for Methylation-specific PCR (MSP), Bisulfite sequencing, Methylation-sensitive Single-Nucleotide Primer Extension (MS-SNuPE,) Combined Bisulfite Restriction Analysis (COBRA), Bisulfite treatment and PCR-single strand Confirmation Polymorphism Analysis (Bisulfite-PCR-SSCP/BiPS)*

Description: HeLa (cervix adenocarcinoma) cells were grown to confluency in DMEM plus 10% fetal bovine serum. Genomic DNA was isolated by a standard genomic purification protocol, phenol extracted and equilibrated to 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA.

Note: NEB is one of the only suppliers of this product.

$A_{260}/_{280}$ Ratio: 1.87

5-methyl-dCTP

#N0356S 1 µmol in 0.1 ml

- *Generation of fully methylated cytosine-substituted DNA*

Description: Cytosine modification at carbon 5 (C5) represents an important epigenetic modification. It is also believed to be the starting substrate for the Ten-Eleven 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)

Concentration: 10 mM solution

Molecular weight: 481.1 (acid form)

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)]

MILLI-Q® is a registered trademark of Millipore, Inc.

As Supply Chain Manager, Dan is responsible for making sure that NEB has uninterrupted access to the critical components needed to manufacture all its products. His attention to detail ensures that our products are ready to ship when your order is placed. In his spare time, Dan spends as much time as possible mountain biking.

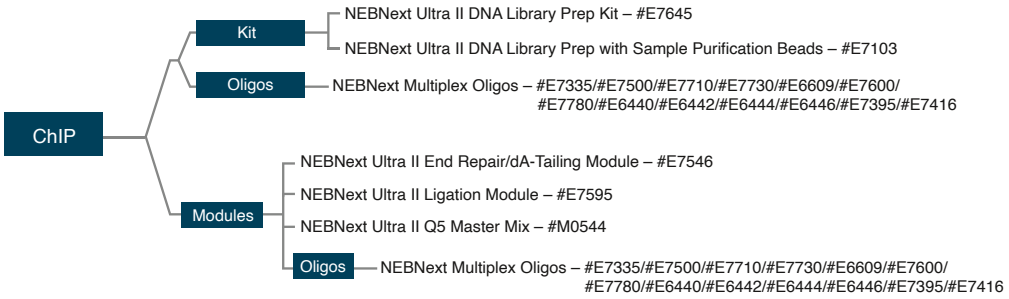
Single Letter Code:								
R = A or G	**Y** = C or T	**M** = A or C	**K** = G or T	**S** = C or G	**W** = A or T	**H** = A or C or T (not G)	**B** = C or G or T (not A)	
V = A or C or G (not T)	**D** = A or G or T (not C)	**N** = A or C or G or T						

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 on NEBNext reagents for library preparation, see pages 146–175.



Katherine is a Development Associate for our Organic Synthesis Team. In this role, she works to develop, process and purify oligos for our products that help support ongoing research in genetic sequencing and screening. When not at work, Katherine spends time enjoying local trails and beaches in the summer and skiing in the winter.

Cloned at NEB | RR Recombinant Enzyme | Epi EpiMark Validated | NEB4 Optimal Buffer | BSA Requires BSA | 37° Incubation Temperature | dilA Diluent Buffer | 65° Heat Inactivation

NEBNext Enzymatic Methyl-seq

NEBNext Enzymatic Methyl-seq Kit
#E7120S 24 reactions
#E7120L 96 reactions

NEBNext Enzymatic Methyl-seq Conversion Module
#E7125S 24 reactions
#E7125L 96 reactions

NEBNext Q5U Master Mix
#M0597S 50 reactions
#M0597L 250 reactions

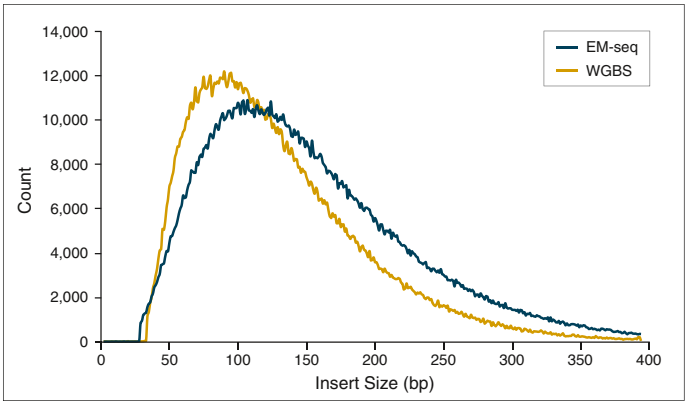
NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)
#E7140S 24 reactions
#E7140L 96 reactions

- *Superior sensitivity of detection of 5-mC and 5-hmC*
- *Larger library insert sizes*
- *More uniform GC coverage*
- *Greater mapping efficiency*
- *High-efficiency library preparation*

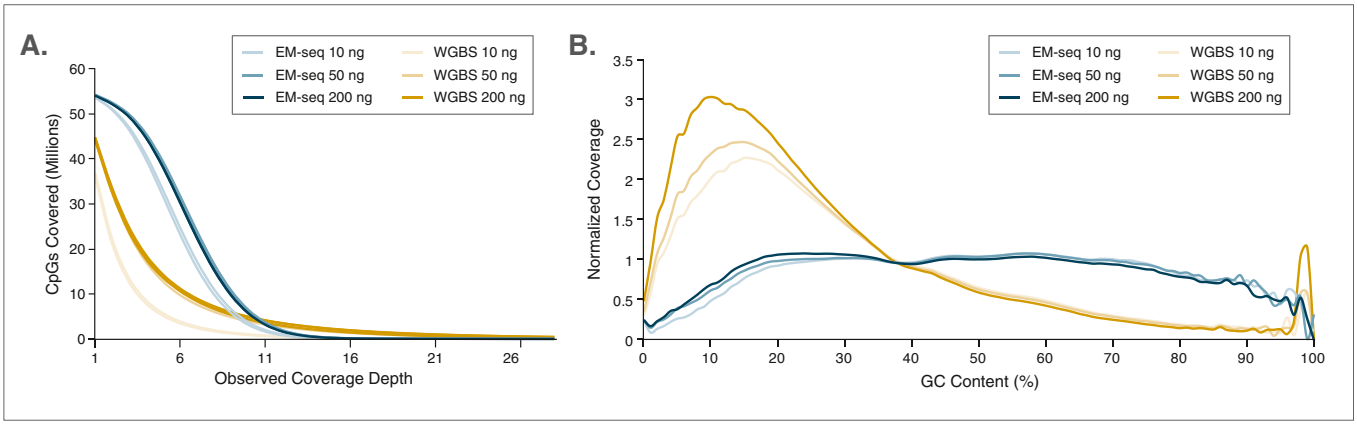
NEBNext Enzymatic Methyl-seq is an enzymatic alternative to bisulfite conversion with superior performance. For more information, including extensive performance data, visit **NEBNext.com**.

Description: While bisulfite sequencing has been the gold standard for the study of DNA methylation, this conversion treatment is damaging to DNA, resulting in DNA fragmentation, loss and GC bias. The NEBNext Enzymatic Methyl-seq Kit (EM-seq™) provides an enzymatic alternative to whole genome bisulfite sequencing (WGBS), combined with high-efficiency streamlined library preparation suitable for Illumina sequencing.

The highly effective EM-seq enzymatic conversion minimizes damage to DNA and, in combination with the supplied NEBNext Ultra II library preparation workflow reagents, results in high quality libraries that enable superior detection of 5-mC and 5-hmC from fewer sequencing reads.



NEBNext Enzymatic Methyl-seq libraries have larger insert sizes *50 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris® S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold™ kit for bisulfite conversion. Libraries were sequenced on an Illumina MiSeq (2 x 76 bases) and insert sizes were determined using Picard 2.18.14. The normalized frequency of each insert size was plotted, illustrating that library insert sizes are larger for EM-seq than for WGBS, and indicating that EM-seq does not damage DNA as bisulfite treatment does in WGBS.*



EM-seq identifies more CpGs than WGBS, at lower sequencing coverage depth with superior uniformity of GC coverage. *10, 50 and 200 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold Kit for bisulfite conversion. Libraries were sequenced on an Illumina NovaSeq® 6000 (2 x 100 bases). Reads were aligned to hg38 using bwa-meth 0.2.2.*

A: *Coverage of CpGs with EM-seq and WGBS libraries was analyzed using 324 million paired end reads, and each top and bottom strand CpGs were counted independently, yielding a maximum of 56 million possible CpG sites. EM-seq identifies more CpGs at lower depth of sequencing.*

B: *GC coverage was analyzed using Picard 2.17.2 and the distribution of normalized coverage across different GC contents of the genome (0-100%) was plotted. EM-seq libraries have significantly more uniform GC coverage, and lack the AT over-representation and GC under-representation typical of WGBS libraries.*

Giving Back

In these times of uncertainty, it is vital to think of others and to help when we can. Philanthropy, through foundation and corporate giving, offers a way to marshal resources to help address the broad array of challenges facing our planet. Don Comb's love of the natural world and his philanthropic impulse compelled him to establish or support many organizations over the years. NEB continues these efforts, supporting organizations throughout the world that help advance science, protect the environment, promote education, and help the less fortunate. NEB creates many opportunities for employees to give back, which, in turn, has created a generous culture throughout.

In 1982, inspired by his deep love of nature and fascination with the arts and diverse cultures, Don created the New England Biolabs Foundation (NEBF) as a private, charitable foundation to address problems including loss of biodiversity and public health challenges in many regions of the world. Today, NEBF's mission is to foster community-based conservation of landscapes and seascapes and the biocultural diversity found in these places, working in countries of Central America, Andean South America and West Africa, and also locally on the North Shore of Massachusetts. NEBF provides small grants to grassroots organizations in these regions for projects such as coral reef conservation, watershed restoration, supporting food sovereignty through agroecology, and helping communities sustain their traditional ecological knowledge and linguistic diversity.

Don believed in a simple but powerful premise that was well ahead of its time: "if you put small amounts of money in the hands of the people closest to the biodiversity, you can make a big impact." His commitment to invest in small and grassroots organizations working to protect their landscapes, livelihoods and biodiversity shaped the foundation's approach early on and remains its touchstone to this day.

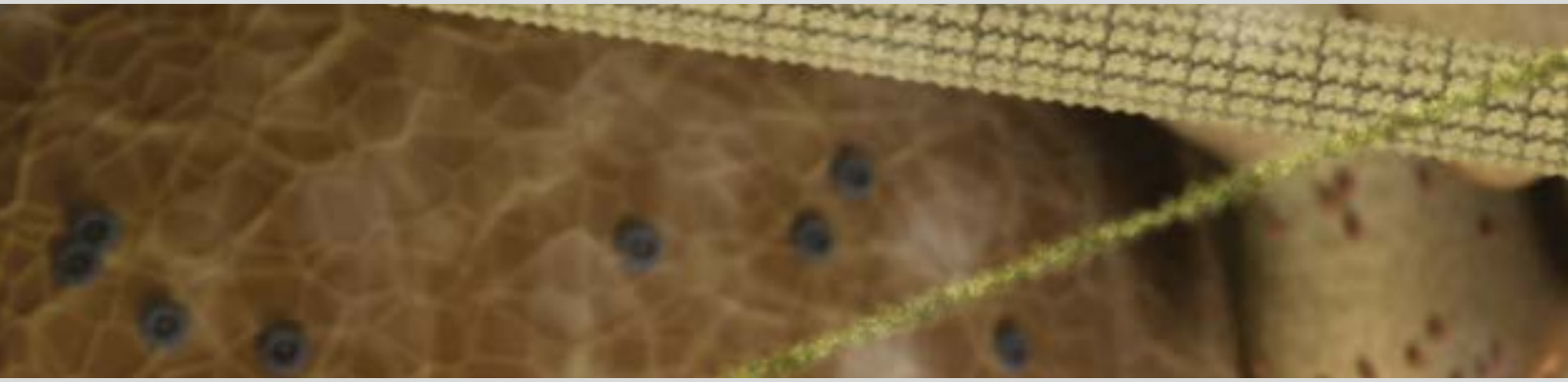
In the early days of the foundation, Don felt it was essential to get out in the field and visit the communities and projects that NEBF supported in many different parts of the world. He was intrigued by Papua New Guinea (PNG) and listened to the local people when they expressed concern for conserving their environment and maintaining their traditions. In response, NEBF funded various innovative projects in PNG, including a forest-friendly means for communities to build housing and canoes, and the establishment of the Wau Biological Station, which aimed to protect local plants and trees and establish a butterfly range. Don's commitment to visiting projects in the field laid the groundwork for NEBF's approach to the present day in which the foundation's staff and board make an effort to visit projects and spend time in the field with NEBF's partners.

Building on these early roots, today NEBF's grant-making extends to places as diverse as Peru, Ghana and Belize. NEBF continues to fund in areas that are central to the crucible of threats facing our planet and humanity today: the intersection of climate change, biodiversity loss, extinction of cultures and languages, social injustice and food insecurity. The foundation complements its grant-making with capacity-building opportunities, such as workshops, enabling grantees to develop new skills and strategies and build relationships with each other – as a result, a growing network of NEBF grantees and partners work closely together to tackle these problems.

Don Comb crosses a shaky vine bridge in the Southern Highlands of Papua New Guinea, 1985.
Credit: Dave Comb

Learn more about the New England Biolabs Foundation.

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

296 SNAP-Cell 647-SiR

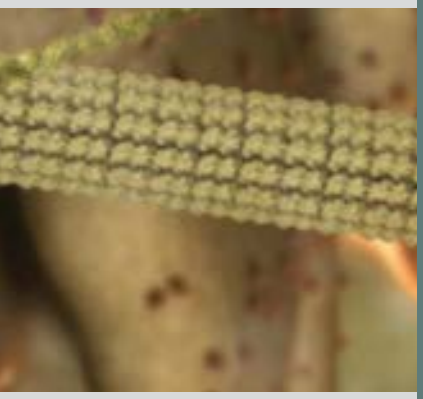
296 SNAP-Surface® 649

Featured Tools & Resources

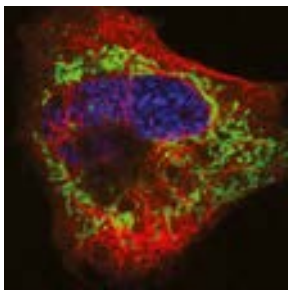
354 Troubleshooting Guide for SNAP-tag Technology

Application Notes

Videos and Tutorials: SNAP-tag Technology



Cellular Imaging & Analysis



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.

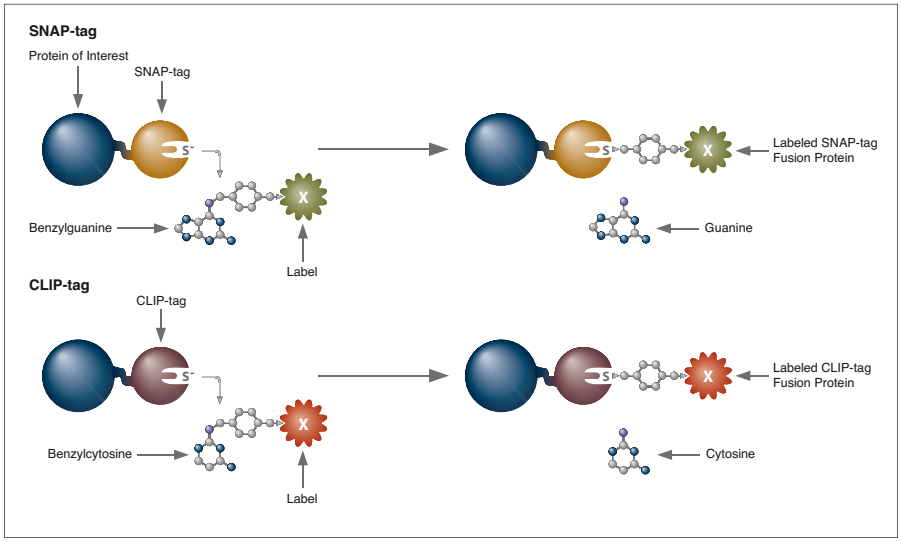
Features of SNAP-tag and CLIP-tag:

- *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*

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 proteins. 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 (gold) and CLIP-tag (purple). *The SNAP- or CLIP-tag is fused to the protein of interest (blue). Labeling occurs through covalent attachment to the tag, releasing either a guanine or a cytosine moeity.*

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 O^6-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. Non-fluorescent, non-cell-permeable blocking agent is also available.

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. Non-fluorescent cell-permeable blocking agent is also available.

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

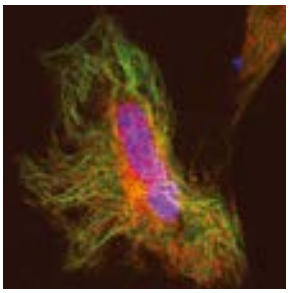
While SNAP/CLIP-tag technologies are complementary to GFP, there are several applications for which SNAP- and CLIP-tag self-labeling technologies are advantageous.

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

Sam Brown works as a Production Technician at our Rowley Production Facility. In this role, he helps with fermentation of several of the GMP-grade* enzymes. In his spare time, Sam enjoys skiing, sailing and reading about history.

*Learn more about GMP-grade enzymes on page 7.

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. SNAP-tag substrates consist of a fluorophore conjugated to guanine or chloropyrimidine leaving groups via a benzyl linker, while CLIP-tag substrates consist of a fluorophore conjugated to a cytosine leaving group via a benzyl linker. These substrates will label their respective tags without the need for additional enzymes. Cell-permeant substrates (SNAP- and CLIP-Cell) are suitable for both intracellular and cell-surface labeling, whereas non-cell-permeant substrates (SNAP- and CLIP-Surface) are specific for fusion proteins expressed on the cell surface only.

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	APPLICATIONS	NEB #	EXCITATION*	EMISSION*(1)	SIZE
	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.

This table lists all currently available fluorescent substrates for SNAP-tag and CLIP-tag, along with excitation and emission wavelengths (determined from a labeled fusion tag, rather than the unreacted substrate).

Blocking Agents

- *Irreversible blocking*
- *Ideal for pulse-chase applications*

Blocking agents are non-fluorescent substrates that block the reactivity of the SNAP- or CLIP-tag intracellularly (SNAP-Cell Block and CLIP-Cell Block) or on the surface of live cells (SNAP-Surface Block and CLIP-Cell Block). They can be used to generate inactive controls in live cell and *in vitro* labeling experiments performed with SNAP- or CLIP-tag fusion proteins.

SNAP- and CLIP-Cell Block are highly membrane permeant and once inside the cell react with the SNAP- or CLIP-tag, irreversibly inactivating them 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 and *in vitro*	100 nmol
CLIP-Cell Block	S9220S	Block CLIP-tag inside or on the surface of live cells and *in vitro*	100 nmol
SNAP-Surface Block	S9143S	Block SNAP-tag on the surface of live 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

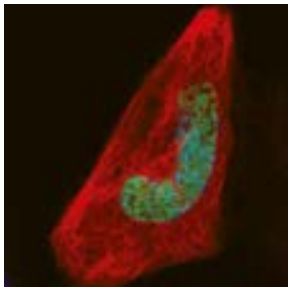
#P9312S 50 µg

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

Lovely works at our subsidiary office in Australia. As a member of Customer Service, she oversees shipments to New Zealand. Lovey also enjoys baking and is a budding organist.

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 SNAP$_f$ and CLIP$_f$ 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. SNAP$_f$-tag and CLIP$_f$-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 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. Plasmids have been purified free of endotoxins for efficient transfection

Concentration: 500 µg/ml

Restriction Map: For a more detailed description and restriction map of pSNAP$_f$ Vector, see page 362. See www.neb.com for sequence and map files for all expression and control plasmids.

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

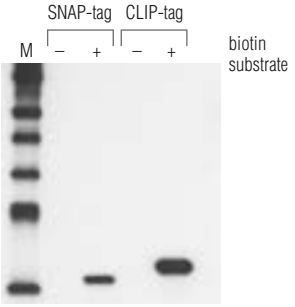
Biotin Labels

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 based on biotin with an amidocaproyl linker. Biotin labels 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.

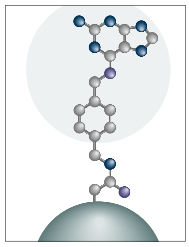


Western blot analysis of biotin labeling reactions using anti-Biotin Antibody (CST #7075). SNAP-tag and CLIP-tag (5 µM) labeled with a biotin-containing substrate (10 µM). Marker M is Biotinylated protein ladder (CST #7727).

SNAP-Capture

- *Selectively capture SNAP-tag fusion proteins from solution*
- *Ideal for protein pull-down experiments or proteomic analysis*

SNAP-Capture products are agarose or 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.



Substrate structure on SNAP-Capture Pull-Down Resin

PRODUCT	NEB #	SIZE
SNAP-Capture Pull-Down Resin	S9144S	2 ml
SNAP-Capture Magnetic Beads	S9145S	2 ml

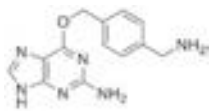
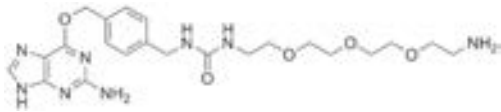
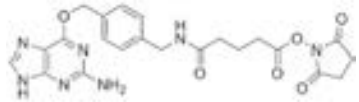
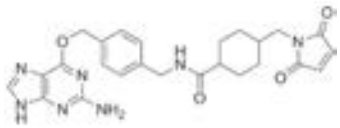
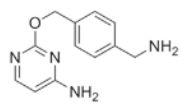
Building Blocks

- *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 and CLIP-tag labeling technologies, building blocks are available for linkage of the core benzylguanine (BG) and benzylcytosine (BC) moieties 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.

References:
References for enzyme properties and applications for this product can be found at www.neb.com.

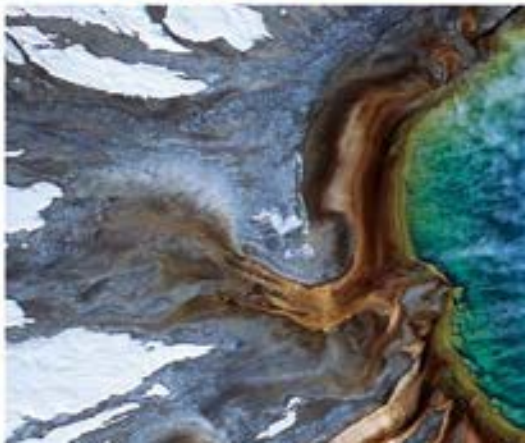
PRODUCT	NEB #	STRUCTURE	APPLICATION	SIZE
BG-NH2	S9148S		SNAP-tag substrate. Suitable for linkage to NHS esters and other activated carboxylic esters.	2 mg
BG-PEG-NH2	S9150S		SNAP-tag substrate. PEG-linker gives superior flexibility. Particularly suited for immobilization on solid surfaces.	2 mg
BG-GLA-NHS	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
BC-NH2	S9236S		CLIP-tag substrate. Suitable for linkage to NHS esters and other activated carboxylic esters.	2 mg

NEW ENGLAND

BioLabs

RESTRICTION ENDONUCLEASES
NUCLEIC ACIDS
tRNA's
DNA LIGASE
BACTERIAL CELLS

1975 - 1976
CATALOG



And the NEB Catalog

When one thinks of a catalog, they might think of a list of products that are available for sale. As a user, one might browse a catalog to find a particular product, or just to see what is new from their favorite brand. But to NEB, the catalog is so much more — it is a reflection of who we are, the products we offer, and the resources we can provide to help advance science.

In 1975, the first NEB catalog was released, featuring 7 restriction enzymes, 7 DNA or RNA modifying enzymes and 6 transfer RNAs — meager in content but with promise for more. Over the next few years, NEB contemplated how to add value to this list of products, and the answer was simple — share the science. In the early 1980s, NEB began curating technical information related to restriction enzymes and released the first Technical Reference section. This included information on how enzymes behaved and how to set up reactions properly — information that is now easily accessible through the internet, but back then was found mostly in books. Over time, this content expanded to include other cloning-related applications. This idea of a "Benchtop Reference Tool" was unique at the time, and as a result, the NEB Catalog and Technical Reference became a mainstay on the lab benches of undergraduate and graduate researchers around the world. It was the beginning of a collaborative relationship between scientists and NEB.

The catalog also provides an opportunity to share ideas that Don Comb and NEB felt passionate about. Each edition focuses on a theme and contains a collection of minireviews that addresses various scientific, environmental and/or humanitarian challenges facing the world. This represents a unique opportunity to reflect on ideas that are important to NEB, and to think about how we can do better as scientists, and in life.

Over the years, the catalog has expanded to thousands of products, supporting a wide variety of applications. There are even products that may not have an application yet — Don always loved to make unique enzymes available to scientists, with hopes that they would find new applications for them. Sir Richard Roberts, Nobel Laureate and NEB's Chief Scientific Officer, took this a step further and spearheaded the "Enzymes for Innovation" initiative — sharing enzymes with unique activities that have the potential to lead to new discoveries.

Is it possible to build a relationship through a catalog? And are catalogs even necessary in this digital age? We think so! A catalog can be much more than a transactional exercise — it can be part of a sustainable relationship based on the sharing of knowledge. Sure, your device can give you instant answers, but there is something comforting about flipping through a book, complete with earmarks and notes in the sidebar about how you modified an experiment, or flagged products to look at later. We have often heard scientists recall their first NEB Catalog, or which cover was their favorite — in fact, some have even saved them!

In our minds, the NEB Catalog is more than a book — it embodies the ethos of NEB. The passion that our scientists put into developing our products, the humility in the way the products and data are presented, and the genuine way the stories reflect who we are as a company and what we feel is important — empowering science, preserving the environment, and collaborating with our peers.

Can you guess what year these catalog cover images came from?

A collection of past NEB Catalog covers.

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 still stands, and 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 product 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 the scientists at NEB. Several of our product lines have designated technical support scientists assigned to servicing customers in those application areas. Any 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 often 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.

Learn more about NEB's tech support program.

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.

Online Interactive Tools

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.

Ligase Fidelity Tools

This set of beta 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 Spit DNA sequences for scarless high-fidelity assembly with SplitSet™.

NEB 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

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® V2.1

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 Tools (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.

Read Coverage Calculator

This tool allows for easy calculation of values associated with read coverage in NGS protocols.

PCR Fidelity Estimator

Estimate the percentage of correct DNA copies (those without base substitution errors) per cycle of PCR for selected DNA polymerases.

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.

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.

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.

Mobile Apps

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.

NEB Tool for Augmented Reality

Download the NEB Augmented Reality (AR) app for iPhone or iPad at the Apple® App Store or for Android on Google Play™.

NEBnow Locator

NEBnow Freezer Programs are ideally suited for researchers in academics and industry looking for on-site access to the world's finest restriction enzymes and related products. NEB freezers offer you convenience, flexibility and value.

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 NEBuffer	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, Double Digest Finder** and **NEBcloner**


TIPS FOR SETTING UP RE DIGESTS

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
- 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
- Recombinant Albumin is included in NEBuffer r1.1, r2.1, r3.1 and rCutSmart™ Buffer. BSA is included in NEBuffer 1.1, 2.1, 3.1 and CutSmart Buffer. In either case, no additional BSA is needed.
- Restriction enzymes that do not require BSA for optimal activity are not adversely affected if BSA is present in the reaction

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

	RESTRICTION ENZYME*	DNA	10X NEBUFFER
10 µl rxn**	1 unit	0.1 µg	1 µl
25 µl rxn	5 units	0.5 µg	2.5 µl
50 µl rxn	10 units	1 µg	5 µl

* Restriction enzymes can be diluted using the recommended diluent buffer when smaller amounts are needed

** 10 µl rxns should not be incubated for longer than 1 hour to avoid evaporation.

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**

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

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 or CutSmart Buffer, making double digestion simple. If you are using an enzyme that is not supplied with rCutSmart or CutSmart Buffer, the Performance Chart for Restriction Enzymes (pages 309–314) 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 or CutSmart 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 (see page 306). The final concentration of glycerol in any reaction should be less than 5% to minimize the possibility of star activity (see page 316). 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 three enzymes with unique buffers: EcoRI, SspI and DpnII. In most cases, DpnII requires a sequential digest. Note that EcoRI and SspI have HF versions (NEB #R3101 and NEB #R3132, respectively) which is supplied with rCutSmart or CutSmart Buffer.

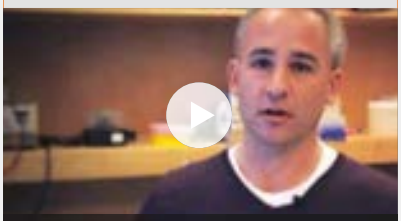
Setting up a Sequential Digestion

- 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 pages 132–141.

TOOLS & RESOURCES

Visit www.neb.com/nebtools for:

- Help choosing double digest conditions using NEB's, **Double Digest Finder** and **NEBcloner®**



TIPS FOR SETTING UP DOUBLE DIGESTS

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 assymetric 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

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
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 or 3.1) may be salt sensitive, so 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.
	Inhibition by PCR components	• Clean up the PCR fragment prior to restriction digest
	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 supercolied 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 PCR & DNA Cleanup Kit (NEB #T1030),
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 or 3.1) 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 > 215 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. Note that Recombinant Albumin or BSA is included in all NEBuffers, and is not provided as a separate tube. 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

SAM Supplied with a separate vial of S-adenosylmethionine (SAM). To obtain 100% activity, SAM should be added to the 1X reaction mix as specified on the product data card.

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 or CutSmart 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.

Note: Starting April 2021, NEB will begin the transition to supply all restriction enzymes with Recombinant Albumin (rAlbumin) -containing buffers (r1.1, r2.1, r3.1 & rCutSmart). There is no difference in enzyme performance when using rAlbumin- or BSA-containing buffers. We have reported the supplied rAlbumin-containing buffer, but show % activity for both buffer sets.

			ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS r1.1 1.1	r2.1 2.1	r3.1 3.1	rCUTSMART CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	UNIT SUBSTRATE	METHYLATION SENSITIVITY	NOTE(S)
RR	e		AatII	rCutSmart	< 10	50*	50	100	37°	80°	B	Lambda	CpG	
RR			AbaSI	rCutSmart + DTT	25	50	50	100	25°	65°	C	T4 wt Phage		e
RR	e		AccI	rCutSmart	50	50	10	100	37°	80°	A	Lambda	CpG	
RR	e		Acc65I	r3.1	10	75*	100	25	37°	65°	A	pBC4	dcm CpG	
RR	e		AciI	rCutSmart	< 10	25	100	100	37°	65°	A	Lambda	CpG	d
RR	e		AclI	rCutSmart	< 10	< 10	< 10	100	37°	No	B	Lambda	CpG	
RR	e		AcuI	rCutSmart	50	100	50	100	37°	65°	B	Lambda		1, b, d
RR			AfeI	rCutSmart	25	100	25	100	37°	65°	B	pXba	CpG	
RR	e		AflII	rCutSmart	50	100	10	100	37°	65°	A	phiX174		
RR			AflIII	r3.1	10	50	100	50	37°	80°	B	Lambda		
RR			AgeI	r1.1	100	75	25	75	37°	65°	C	Lambda	CpG	
RR	e	e	AgeI-HF	rCutSmart	100	50	10	100	37°	65°	A	Lambda	CpG	
RR	e		AhdI	rCutSmart	25	25	10	100	37°	65°	A	Lambda	CpG	a
RR			AleI-v2	rCutSmart	< 10	< 10	< 10	100	37°	65°	B	Lambda	CpG	
RR	e		AluI	rCutSmart	25	100	50	100	37°	80°	B	Lambda		b
RR			AlwI	rCutSmart	50	50	10	100	37°	No	A	Lambda dam-	dam	1, b, d
RR	e		AlwNI	rCutSmart	10	100	50	100	37°	80°	A	Lambda	dcm	
RR	e		ApaI	rCutSmart	25	25	< 10	100	25°	65°	A	pXba	dcm CpG	
RR	e		ApaLI	rCutSmart	100	100	10	100	37°	No	A	Lambda HindIII	CpG	
RR	e		ApeKI	r3.1	25	50	100	10	75°	No	B	Lambda	CpG	
RR	e		ApoI	r3.1	10	75	100	75	50°	80°	A	Lambda		
RR	e	e	ApoI-HF	rCutSmart	10	100	10	100	37°	80°	B	Lambda		
RR	e		AscI	rCutSmart	< 10	10	10	100	37°	80°	A	Lambda	CpG	
RR	e		AseI	r3.1	< 10	50*	100	10	37°	65°	B	Lambda		3
RR			AsiSI	rCutSmart	100	100	25	100	37°	80°	B	pXba (Xho digested)	CpG	2, b
RR	e		AvaI	rCutSmart	< 10	100	25	100	37°	80°	A	Lambda	CpG	
RR	e		AvaII	rCutSmart	50	75	10	100	37°	80°	A	Lambda	dcm CpG	
RR	e		AvrII	rCutSmart	100	50	50	100	37°	No	B	Lambda HindIII		
RR	e		BaeI	rCutSmart + SAM	50	100	50	100	25°	65°	A	Lambda	CpG	e
RR	e		BaeGI	r3.1	75	75	100	25	37°	80°	A	Lambda		
RR	e		BamHI	r3.1	75*	100*	100	100*	37°	No	A	Lambda		3
RR	e	e	BamHI-HF	rCutSmart	100	50	10	100	37°	No	A	Lambda		
RR			BanI	rCutSmart	10	25	< 10	100	37°	65°	A	Lambda	dcm CpG	1

Performance Chart for Restriction Enzymes (continued)

ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS r1.1 1.1	r2.1 2.1	r3.1 3.1	rCUTSMART CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	UNIT SUBSTRATE	METHYLATION SENSITIVITY	NOTE(S)
BanII	rCutSmart	100	100	50	100	37°	80°	A	Lambda		2
BbsI	r2.1	100	100	25	75	37°	65°	B	Lambda		
BbsI-HF	rCutSmart	10	10	< 10	100	37°	65°	B	Lambda		
BbvI	rCutSmart	100	100	25	100	37°	65°	B	pBR322		3
BbvCI	rCutSmart	10	100	50	100	37°	No	B	Lambda	CpG	1, a
BccI	rCutSmart	100	50	10	100	37°	65°	A	pXba		3, b
BceAI	r3.1	100*	100*	100	100*	37°	65°	A	pBR322	CpG	1
BcgI	r3.1	10	75*	100	50*	37°	65°	A	Lambda	dam CpG	e
BciVI	rCutSmart	100	25	< 10	100	37°	80°	C	Lambda		b
BclI	r3.1	50	100	100	75	50°	No	A	Lambda dam-	dam	
BclI-HF	rCutSmart	100	100	10	100	37°	65°	B	Lambda dam-	dam	
BcoDI	rCutSmart	50	75	75	100	37°	No	B	Lambda	CpG	
BfaI	rCutSmart	< 10	10	< 10	100	37°	80°	B	Lambda		2, b
BfuAI	r3.1	< 10	25	100	10	50°	65°	B	Lambda	CpG	3
BglI	r3.1	10	25	100	10	37°	65°	B	Lambda	CpG	
BglII	r3.1	10	10	100	< 10	37°	No	A	Lambda		
BlpI	rCutSmart	50	100	10	100	37°	No	A	Lambda		d
BmgBI	r3.1	< 10	10	100	10	37°	65°	B	Lambda	CpG	3, b, d
BmrI	r2.1	75	100	75	100*	37°	65°	B	Lambda HindIII		b
BmtI	r3.1	100	100	100	100+	37°	65°	B	pXba		2
BmtI-HF	rCutSmart	50	100	10	100	37°	65°	B	pXba		
BpmI	r3.1	75	100	100	100*	37°	65°	B	Lambda		2, d
Bpu10I	r3.1	10	25	100	25	37°	80°	B	Lambda		3, b, d
BpuEI	rCutSmart	50*	100	50*	100	37°	65°	B	Lambda		d
BsaI-HFv2	rCutSmart	100	100	100	100	37°	80°	B	pXba	dcm CpG	
BsaAI	rCutSmart	100	100	100	100	37°	No	C	Lambda	CpG	
BsaBI	rCutSmart	50	100	75	100	60°	80°	B	Lambda dam-	dam CpG	2
BsaHI	rCutSmart	50	100	100	100	37°	80°	C	Lambda	dcm CpG	
BsaJI	rCutSmart	50	100	100	100	60°	80°	A	Lambda		
BsaWI	rCutSmart	10	100	50	100	60°	80°	A	Lambda		
BsaXI	rCutSmart	50*	100*	10	100	37°	No	C	Lambda		e
BseRI	rCutSmart	100	100	75	100	37°	80°	A	Lambda		d
BseYI	r3.1	10	50	100	50	37°	80°	B	Lambda	CpG	d
BsgI	rCutSmart	25	50	25	100	37°	65°	B	Lambda		d
BsiEI	rCutSmart	25	50	< 10	100	60°	No	A	Lambda	CpG	
BsiHKAI	rCutSmart	25	100	100	100	65°	No	A	Lambda		
BsiWI	r3.1	25	50*	100	25	55°	65°	B	phiX174	CpG	
BsiWI-HF	rCutSmart	50	100	10	100	37°	No	B	phiX174	CpG	
BsII	rCutSmart	50	75	100	100	55°	No	A	Lambda	dcm CpG	b
BsmI	rCutSmart	25	100	< 10	100	65°	80°	A	Lambda		
BsmAI	rCutSmart	50	100	100	100	55°	No	B	Lambda	CpG	
BsmBI-v2	r3.1	< 10	50	100	25	55°	80°	B	Lambda	CpG	
BsmFI	rCutSmart	25	50	50	100	65°	80°	A	pBR322	dcm CpG	1
BsoBI	rCutSmart	25	100	100	100	37°	80°	A	Lambda		
Bsp1286I	rCutSmart	25	25	25	100	37°	65°	A	Lambda		3
BspCNI	rCutSmart	100	75	10	100	37°	80°	A	Lambda		b
BspDI	rCutSmart	25	75	50	100	37°	80°	A	Lambda	dam CpG	
BspEI	r3.1	< 10	10	100	< 10	37°	80°	B	Lambda dam-	dam CpG	
BspHI	rCutSmart	10	50	25	100	37°	80°	A	Lambda	dam	
BspMI	r3.1	10	50*	100	10	37°	65°	B	Lambda		
BspQI	r3.1	100*	100*	100	100*	50°	80°	B	Lambda		3

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.

ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS r1.1 1.1	r2.1 2.1	r3.1 3.1	rCUTSMART CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	UNIT SUBSTRATE	METHYLATION SENSITIVITY	NOTE(S)
BsrI	r3.1	< 10	50	100	10	65°	80°	B	phiX174		b
BsrBI	rCutSmart	50	100	100	100	37°	80°	A	Lambda	CpG	d
BsrDI	r2.1	10	100	75	25	65°	80°	A	Lambda		3, d
BsrFI-v2	rCutSmart	25	25	0	100	37°	No	C	pBR322	CpG	
BsrGI	r2.1	25	100	100	25	37°	80°	A	Lambda		
BsrGI-HF	rCutSmart	10	100	100	100	37°	80°	A	Lambda		
BssHII	rCutSmart	100	100	100	100	50°	65°	B	Lambda	CpG	
BssSI-v2	rCutSmart	10	25	< 10	100	37°	No	B	Lambda		
BstAPI	rCutSmart	50	100	25	100	60°	80°	A	Lambda	CpG	b
BstBI	rCutSmart	75	100	10	100	65°	No	A	Lambda	CpG	
BstEII	r3.1	10	75*	100	75*	60°	No	A	Lambda		3
BstEII-HF	rCutSmart	< 10	10	< 10	100	37°	No	A	Lambda		
BstNI	r3.1	10	100	100	75	60°	No	A	Lambda		a
BstUI	rCutSmart	50	100	25	100	60°	No	A	Lambda	CpG	b
BstXI	r3.1	< 10	50	100	25	37°	80°	B	Lambda	dcm	3
BstYI	r2.1	25	100	75	100	60°	No	A	Lambda		
BstZ17I-HF	rCutSmart	100	100	10	100	37°	No	A	Lambda	CpG	b
Bsu36I	rCutSmart	25	100	100	100	37°	80°	C	Lambda HindIII		b
BtgI	rCutSmart	50	100	100	100	37°	80°	B	pBR322		
BtgZI	rCutSmart	10	25	< 10	100	60°	80°	A	Lambda	CpG	3, b, d
BtsI-v2	rCutSmart	100	100	25	100	37°	No	A	Lambda		1
BtsIMutI	rCutSmart	100	50	10	100	55°	80°	A	pUC19		b
BtsCI	rCutSmart	10	100	25	100	50°	80°	B	Lambda		
Cac8I	rCutSmart	50	75	100	100	37°	65°	B	Lambda	CpG	b
ClaI	rCutSmart	10	50	50	100	37°	65°	A	Lambda dam-	dam CpG	
CspCI	rCutSmart	10	100	10	100	37°	65°	A	Lambda		e
CviAII	rCutSmart	50	50	10	100	25°	65°	C	Lambda		
CviKI-1	rCutSmart	25	100	100	100	37°	No	A	pBR322		1, b
CviQI	r3.1	75	100*	100	75*	25°	No	C	Lambda		b
DdeI	rCutSmart	75	100	100	100	37°	65°	B	Lambda		
DpnI	rCutSmart	100	100	75	100	37°	80°	B	pBR322	CpG	b
DpnII	U	25	25	100*	25	37°	65°	B	Lambda dam-	dam	
DraI	rCutSmart	75	75	50	100	37°	65°	A	Lambda		
DraIII-HF	rCutSmart	< 10	50	10	100	37°	No	B	Lambda	CpG	b
DrdI	rCutSmart	25	50	10	100	37°	65°	A	pUC19	CpG	3
EaeI	rCutSmart	10	50	< 10	100	37°	65°	A	Lambda	dcm CpG	b
EagI	r3.1	10	25	100	10	37°	65°	B	pXba	CpG	
EagI-HF	rCutSmart	25	100	100	100	37°	65°	B	pXba	CpG	
EarI	rCutSmart	50	10	< 10	100	37°	65°	B	Lambda	CpG	b, d
EciI	rCutSmart	100	50	50	100	37°	65°	A	Lambda	CpG	2
Eco53kI	rCutSmart	100	100	< 10	100	37°	65°	A	pXba	CpG	3, b
EcoNI	rCutSmart	50	100	75	100	37°	65°	A	Lambda		b
Eco0109I	rCutSmart	50	100	50	100	37°	65°	A	Lambda HindIII	dcm	3
EcoP15I	r3.1 + ATP	75	100	100	100	37°	65°	A	pUC19		e
EcoRI	U	25	100*	50	50*	37°	65°	C	Lambda	CpG	
EcoRI-HF	rCutSmart	10	100	< 10	100	37°	65°	C	Lambda	CpG	
EcoRV	r3.1	10	50	100	10	37°	80°	A	Lambda	CpG	
EcoRV-HF	rCutSmart	25	100	100	100	37°	65°	B	Lambda	CpG	
Esp3I	rCutSmart	100	100	< 10	100	37°	65°	B	Lambda	CpG	
FatI	r2.1	10	100	50	50	55°	80°	A	pUC19		
FauI	rCutSmart	100	50	10	100	55°	65°	A	Lambda	CpG	3, b, d

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 buffer.

Performance Chart for Restriction Enzymes (continued)

ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS r1.1 1.1	r2.1 2.1	r3.1 3.1	rCUTSMART CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	UNIT SUBSTRATE	METHYLATION SENSITIVITY	NOTE(S)
Fnu4HI	rCutSmart	< 10	< 10	< 10	100	37°	No	A	Lambda	CpG	a
FokI	rCutSmart	100	100	75	100	37°	65°	A	Lambda	dcm CpG	3, b, d
FseI	rCutSmart	100	75	< 10	100	37°	65°	B	pBC4	dcm CpG	
FspI	rCutSmart	10	100	10	100	37°	No	C	Lambda	CpG	b
FspEI	rCutSmart + Enz. Activ.	< 10	< 10	< 10	100	37°	80°	B	pBR322	dcm	1, e
HaeII	rCutSmart	25	100	10	100	37°	80°	A	Lambda	CpG	
HaeIII	rCutSmart	50	100	25	100	37°	80°	A	Lambda		
HgaI	r1.1	100	100	25	100*	37°	65°	A	phiX174	CpG	1
HhaI	rCutSmart	25	100	100	100	37°	65°	A	Lambda	CpG	
HincII	r3.1	25	100	100	100	37°	65°	B	Lambda	CpG	
HindIII	r2.1	25	100	50	50	37°	80°	B	Lambda		2
HindIII-HF	rCutSmart	10	100	10	100	37°	80°	B	Lambda		
HinfI	rCutSmart	50	100	100	100	37°	80°	A	Lambda	CpG	
HinP1I	rCutSmart	100	100	100	100	37°	65°	A	Lambda	CpG	
HpaI	rCutSmart	< 10	75*	25	100	37°	No	A	Lambda	CpG	1
HpaII	rCutSmart	100	50	< 10	100	37°	80°	A	Lambda	CpG	
HphI	rCutSmart	50	50	< 10	100	37°	65°	B	Lambda	dam CpG	1, b, d
Hpy99I	rCutSmart	50	10	< 10	100	37°	65°	A	Lambda	CpG	
Hpy166II	rCutSmart	100	100	50	100	37°	65°	C	pBR322	CpG	
Hpy188I	rCutSmart	25	100	50	100	37°	65°	A	pBR322	dam	1, b
Hpy188III	rCutSmart	100	100	10	100	37°	65°	B	pUC19	dam CpG	3, b
HpyAV	rCutSmart	100	100	25	100	37°	65°		Lambda	CpG	3, b, d
HpyCH4III	rCutSmart	100	25	< 10	100	37°	65°	A	Lambda		b
HpyCH4IV	rCutSmart	100	50	25	100	37°	65°	A	pUC19	CpG	
HpyCH4V	rCutSmart	50	50	25	100	37°	65°	A	Lambda		
I-CeuI	rCutSmart	10	10	10	100	37°	65°	B	pBHS ScaI-linearized		
I-SceI	rCutSmart	10	50	25	100	37°	65°	B	pGPS2 NotI-linearized		
KasI	rCutSmart	50	100	50	100	37°	65°	B	pBR322	CpG	3
KpnI	r1.1	100	75	< 10	50	37°	No	A	pXba		1
KpnI-HF	rCutSmart	100	25	< 10	100	37°	No	A	pXba		
LpnPI	rCutSmart + Enz. Activ.	< 10	< 10	< 10	100	37°	65°	B	pBR322		1, e
MboI	rCutSmart	75	100	100	100	37°	65°	A	Lambda dam-	dam CpG	
MboII	rCutSmart	100*	100	50	100	37°	65°	C	Lambda dam-	dam	b
MfeI	rCutSmart	75	50	10	100	37°	No	A	Lambda		2
MfeI-HF	rCutSmart	75	25	< 10	100	37°	No	A	Lambda		
MluI	r3.1	10	50	100	25	37°	80°	A	Lambda	CpG	
MluI-HF	rCutSmart	25	100	100	100	37°	No	A	Lambda	CpG	
MluCI	rCutSmart	100	10	10	100	37°	No	A	Lambda		
MlyI	rCutSmart	50	50	10	100	37°	65°	A	Lambda		b, d
MmeI	rCutSmart	50	100	50	100	37°	65°	B	phiX174	CpG	b, c
MnlI	rCutSmart	75	100	50	100	37°	65°	B	Lambda		b
MscI	rCutSmart	25	100	100	100	37°	80°	C	Lambda	dcm	
MseI	rCutSmart	75	100	75	100	37°	65°	A	Lambda		
MslI	rCutSmart	50	50	< 10	100	37°	80°	A	Lambda		
MspI	rCutSmart	75	100	50	100	37°	No	A	Lambda		
MspA1I	rCutSmart	10	50	10	100	37°	65°	B	Lambda	CpG	
MspJI	rCutSmart + Enz. Activ.	< 10	< 10	< 10	100	37°	65°	B	pBR322		1, e
MwoI	rCutSmart	< 10	100	100	100	60°	No	B	Lambda	CpG	
NaeI	rCutSmart	25	25	< 10	100	37°	No	A	pXba	CpG	b
NarI	rCutSmart	100	100	10	100	37°	65°	A	pXba	CpG	
Nb.BbvCI	rCutSmart	25	100	100	100	37°	80°	A	pUB		e

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.

ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS r1.1 1.1	r2.1 2.1	r3.1 3.1	rCUTSMART CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	UNIT SUBSTRATE	METHYLATION SENSITIVITY	NOTE(S)
Nb.BsmI	r3.1	< 10	50	100	10	65°	80°	A	pBR322		e
Nb.BsrDI	rCutSmart	25	100	100	100	65°	80°	A	pUC19		e
Nb.BssSI	r3.1	10	100	100	25	37°	No	B	pUC19		e
Nb.BtsI	rCutSmart	75	100	75	100	37°	80°	A	phiX174		e
NciI	rCutSmart	100	25	10	100	37°	No	A	Lambda	CpG	b
NcoI	r3.1	100	100	100	100+	37°	80°	A	Lambda		
NcoI-HF	rCutSmart	50	100	10	100	37°	80°	B	Lambda		
NdeI	rCutSmart	75	100	100	100	37°	65°	A	Lambda		
NgoMIV	rCutSmart	100	50	10	100	37°	No	A	pXba	CpG	1
NheI	r2.1	100	100	10	100+	37°	65°	C	Lambda HindIII	CpG	
NheI-HF	rCutSmart	100	25	< 10	100	37°	80°	C	Lambda HindIII	CpG	
NlaIII	rCutSmart	< 10	< 10	< 10	100	37°	65°	B	phiX174		
NlaIV	rCutSmart	10	10	10	100	37°	65°	B	pBR322	dcm CpG	
NmeAIII	rCutSmart	10	10	< 10	100	37°	65°	B	phiX174		c
NotI	r3.1	< 10	50	100	25	37°	65°	C	pBC4	CpG	
NotI-HF	rCutSmart	25	100	25	100	37°	65°	A	pBC4	CpG	
NruI	r3.1	< 10	10	100	10	37°	No	A	Lambda	dam CpG	b
NruI-HF	rCutSmart	0	25	50	100	37°	No	A	Lambda	dam CpG	
NsiI	r3.1	10	75	100	25	37°	65°	B	Lambda		
NsiI-HF	rCutSmart	< 10	20	< 10	100	37°	80°	B	Lambda		
NspI	rCutSmart	100	100	< 10	100	37°	65°	A	Lambda		
Nt.AlwI	rCutSmart	10	100	100	100	37°	80°	A	pUC101 dam-dcm-	dam	e
Nt.BbvCI	rCutSmart	50	100	10	100	37°	80°	A	pUB	CpG	e
Nt.BsmAI	rCutSmart	100	50	10	100	37	65°	A	pBR322	CpG	e
Nt.BspQI	r3.1	< 10	25	100	10	50°	80°	B	pUC19		e
Nt.BstNBI	r3.1	0	10	100	10	55°	80°	A	T7		e
Nt.CviPII	rCutSmart	10	100	25	100	37°	65°	A	pUC19	CpG	e
PacI	rCutSmart	100	75	10	100	37°	65°	A	pNEB193		
PaeR7I	rCutSmart	25	100	10	100	37°	No	A	Lambda HindIII	CpG	
PaqCI	rCutSmart	< 10	100	10	100	37°	65°	B	Lambda	CpG	1
PciI	r3.1	50	75	100	50*	37°	80°	B	pXba		
PflFI	rCutSmart	25	100	25	100	37°	65°	A	pBC4		b
PflMI	r3.1	0	100	100	50	37°	65°	A	Lambda	dcm	3, b, d
PI-PspI	U	10	10	10	10	65°	No	B	pAKR XmnI		
PI-SceI	U	10	10	10	10	37°	65°	B	pBSvdeX XmnI		
PleI	rCutSmart	25	50	25	100	37°	65°	A	Lambda	CpG	b, d
PluTI	rCutSmart	100	25	< 10	100	37°	65°	A	pXba	CpG	
PmeI	rCutSmart	< 10	50	10	100	37°	65°	A	Lambda	CpG	
PmlI	rCutSmart	100	50	< 10	100	37°	65°	A	Lambda HindIII	CpG	
PpuMI	rCutSmart	< 10	< 10	< 10	100	37°	No	B	Lambda HindIII	dcm	
PshAI	rCutSmart	25	50	10	100	37°	65°	A	Lambda	CpG	
PsiI-v2	rCutSmart	25	50	10	100	37°	65°	B	Lambda		3
PspGI	rCutSmart	25	100	50	100	75°	No	A	T7	dcm	3
PspOMI	rCutSmart	10	10	< 10	100	37°	65°	B	pXba	dcm CpG	
PspXI	rCutSmart	< 10	100	25	100	37°	No	B	Lambda HindIII	CpG	
PstI	r3.1	75	75	100	50*	37°	80°	C	Lambda		
PstI-HF	rCutSmart	10	75	50	100	37°	No	C	Lambda		
PvuI	r3.1	< 10	25	100	< 10	37°	No	B	pXba	CpG	
PvuI-HF	rCutSmart	25	100	100	100	37°	No	B	pXba	CpG	
PvuII	r3.1	50	100	100	100*	37°	No	B	Lambda		
PvuII-HF	rCutSmart	< 10	< 10	< 10	100	37°	No	B	Lambda		

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 buffer.

Performance Chart for Restriction Enzymes (continued)

ENZYME	SUPPLIED NEBUFFER	% ACTIVITY IN NEBUFFERS r1.1 1.1	r2.1 2.1	r3.1 3.1	rCUTSMART CUTSMART	INCUB. TEMP. (°C)	INACTIV. TEMP. (°C)	DIL.	UNIT SUBSTRATE	METHYLATION SENSITIVITY		NOTE(S)
RsaI	rCutSmart	25	50	< 10	100	37°	No	A	Lambda		CpG	
RsrII	rCutSmart	25	75	10	100	37°	65°	C	Lambda		CpG	
SacI	r1.1	100	50	10	100+	37°	65°	A	Lambda HindIII			
SacI-HF	rCutSmart	10	50	< 10	100	37°	65°	A	Lambda HindIII		CpG	
SacII	rCutSmart	10	100	10	100	37°	65°	A	pXba		CpG	
SalI	r3.1	< 10	< 10	100	< 10	37°	65°	A	Lambda HindIII		CpG	
SalI-HF	rCutSmart	10	100	100	100	37°	65°	A	Lambda HindIII		CpG	
SapI	rCutSmart	75	50	< 10	100	37°	65°	B	Lambda			
Sau3AI	r1.1	100	50	10	100+	37°	65°	A	Lambda		CpG	b
Sau96I	rCutSmart	50	100	100	100	37°	65°	A	Lambda	dcm	CpG	
SbfI	rCutSmart	50	25	< 10	100	37°	80°	A	Lambda			3
SbfI-HF	rCutSmart	50	25	< 10	100	37°	80°	B	Lambda			
ScaI-HF	rCutSmart	100	100	10	100	37°	80°	B	Lambda			
ScrFI	rCutSmart	100	100	100	100	37°	65°	C	Lambda	dcm	CpG	2, a
SexAI	rCutSmart	100	75	50	100	37°	65°	A	pBC4 dcm-	dcm		3, b, d
SfaNI	r3.1	< 10	75	100	25	37°	65°	B	phiX174		CpG	3, b
SfcI	rCutSmart	75	50	25	100	37°	65°	B	Lambda			3
SfiI	rCutSmart	25	100	50	100	50°	No	C	pXba	dcm	CpG	
SfoI	rCutSmart	50	100	100	100	37°	No	B	Lambda HindIII	dcm	CpG	
SgrAI	rCutSmart	100	100	10	100	37°	65°	A	Lambda		CpG	1
SmaI	rCutSmart	< 10	< 10	< 10	100	25°	65°	B	Lambda HindIII		CpG	b
SmlI	rCutSmart	25	75	25	100	55°	No	A	Lambda			b
SnaBI	rCutSmart	50*	50	10	100	37°	80°	A	T7		CpG	1
SpeI	rCutSmart	75	100	25	100	37°	80°	C	Adenovirus-2			
SpeI-HF	rCutSmart	25	50	10	100	37°	80°	C	pXba			
SphI	r2.1	100	100	50	100+	37°	65°	B	Lambda			2
SphI-HF	rCutSmart	50	25	10	100	37°	65°	B	Lambda			
SrfI	rCutSmart	10	50	0	100	37°	65°	B	pNEB193-SrFI		CpG	
SspI	U	50	100	50	50	37°	65°	C	Lambda			
SspI-HF	rCutSmart	25	100	< 10	100	37°	65°	B	Lambda			
StuI	rCutSmart	50	100	50	100	37°	No	A	Lambda	dcm		
StyD4I	rCutSmart	10	100	100	100	37°	65°	B	Lambda	dcm	CpG	
StyI	r3.1	10	25	100	10	37°	65°	A	Lambda			b
StyI-HF	rCutSmart	25	100	25	100	37°	65°	A	Lambda			
SwaI	r3.1	10	10	100	10	25°	65°	B	pXba			b, d
TaqI-v2	rCutSmart	50	100	50	100	65°	No	B	Lambda	dam		
TfiI	rCutSmart	50	100	100	100	65°	No	C	Lambda		CpG	
TseI	rCutSmart	75	100	100	100	65°	No	B	Lambda		CpG	3
Tsp45I	rCutSmart	100	50	< 10	100	65°	No	A	Lambda			
TspMI	rCutSmart	50*	75*	50*	100	75°	No	B	pUCAdeno		CpG	d
TspRI	rCutSmart	25	50	25	100	65°	No	B	Lambda			
Tth111I	rCutSmart	25	100	25	100	65°	No	B	pBC4			b
XbaI	rCutSmart	< 10	100	75	100	37°	65°	A	Lambda HindIII dam-	dam		
XcmI	r2.1	10	100	25	100*	37°	65°	C	Lambda			2
XhoI	rCutSmart	75	100	100	100	37°	65°	A	Lambda HindIII			b
XmaI	rCutSmart	25	50	< 10	100	37°	65°	A	pXba		CpG	3
XmnI	rCutSmart	50	75	< 10	100	37°	65°	A	Lambda			b
ZraI	rCutSmart	100	25	10	100	37°	80°	B	Lambda		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.

Activity of Enzymes at 37°C

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
AbaSI	25°	0
ApaI	25°	100*
ApeKI	75°	10
ApoI	50°	50
BaeI	25°	100
BclI	50°	50
BfuAI	50°	25
BsaBI	60°	25
BsaJI	60°	25
BsaWI	60°	50
BsiEI	60°	10
BsiHKAI	65°	10
BsiWI	55°	25
BslI	55°	50
BsmAI	55°	50
BsmBI-v2	55°	10
BsmFI	65°	100
BsmI	65°	10

ENZYME	OPTIMAL TEMP. (°C)	% ACTIVITY AT 37°C
BspQI	50°	50
BsrI	65°	10
BsrDI	65°	50
BssHII	50°	100
BstAPI	60°	25
BstBI	65°	10
BstEII	60°	10
BstNI	60°	25
BstUI	60°	10
BstYI	60°	10
BtgZI	60°	50
BtsCI	50°	25
BtsIMutI	55°	50
CviAII	25°	10
CviQI	25°	25
FatI	55°	100
FauI	55°	50
MwoI	60°	25

ENZYME	OPTIMAL TEMP. (°C)	% ACTIVITY AT 37°C
Nb.BsmI	65°	100
Nb.BsrDI	65°	50
Nt.BspQI	50°	50
Nt.BstNBI	55°	50
PI-PspI	65°	10
PspGI	75°	25
SfiI	50°	10
SmaI	25°	25
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

*ApaI has 100% activity at 37°C, however the half-life of this enzyme at 37°C is only 30 minutes.

Activity of DNA Modifying Enzymes in rCutSmart Buffer

A selection of DNA modifying enzymes were assayed in rCutSmart or CutSmart 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 or CutSmart Buffer replacing the supplied buffer.

ENZYME	ACTIVITY IN rCUTSMART OR CUTSMART	REQUIRED SUPPLEMENTS
Antarctic Phosphatase	+++	Requires Zn^{2+}
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+}
E. coli DNA Ligase	+++	Requires NAD
Endonuclease III (Nth), recombinant	+++	
Endonuclease VIII	+++	
Exonuclease I	+++	
Exonuclease III	+++	
Exonuclease VII	+++	
Exonuclease V (Rec BCD)	+++	Requires ATP
GpC Methyltransferase (M. CviPI)	+	Requires DTT
Hi-T4 DNA Ligase	+++	Requires ATP
Lambda Exonuclease	++	
McrBC	+++	
Micrococcal Nuclease	+++	Requires Ca^{2+}

ENZYME	ACTIVITY IN rCUTSMART OR CUTSMART	REQUIRED SUPPLEMENTS
Nuclease Bal-31	+++	
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 ExoI	+++	
USER Enzyme, recombinant	+++	

+++ 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 (4), ethylene glycol, dimethylacetamide, dimethylformamide, sulphalane (5)]	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. (1986) *Nucleic Acids Res.* 14, 811.

TOOLS & RESOURCES

Visit NEBRestrictionEnzymes.com to find:

- Online tutorials on how to avoid star activity, and for setting up restriction enzyme digests
- Access to troubleshooting guides & usage guidelines

RESTRICTION ENZYME STAR ACTIVITY

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 degredation 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

WHAT IS A HIGH-FIDELITY ENZYME?

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
++ Enzyme is active 4–8 hours
+ Enzyme is active 2–4 hours
– No benefit from digesting over 1 hour
N/A Not Available

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	+
AgeI-HF	++
AhdI	+++
AleI-v2	N/A
AluI	++
AlwI	+
AlwNI	+++
ApaI @25°C	+++
ApaLI	+++
ApeKI @75°C	+++
ApoI @50°C	+++
ApoI-HF	+++
AscI	+++
AseI	+++
AsiSI	+++
AvaI	++
AvaII	++
AvrII	+++
BaeI @25°C	+
BaeGI	+
BamHI	+
BamHI-HF	+
BanI	+++
BanII	+
BbsI	++
BbsI-HF	–

ENZYME	SURVIVAL
BbvI	–
BbvCI	+++
BccI	+
BceAI	+++
BcgI	+
BciVI	–
BclI @50°C	+
BclI-HF	N/A
BcoDI	+++
BfaI	+
BfuAI @50°C	++
BglI	+++
BglII	++
BlpI	+
BmgBI	–
BmrI	–
BmtI	+
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	+++

ENZYME	SURVIVAL
BsmI @65°C	+
BsmAI @55°C	++
BsmBI-v2 @55°C	+
BsmFI @65°C	+++
BsoBI	+++
Bsp1286I	+
BspCNI @25°C	–
BspDI	++
BspEI	+++
BspHI	+++
BspMI	++
BspQI @50°C	–
BsrI @65°C	++
BsrBI	+
BsrDI @65°C	+
BsrFI-v2	+++
BsrGI	+++
BsrGI-HF	+++
BssHII @50°C	+
BssSI-v2	+++
BstAPI @60°C	++
BstBI @65°C	+++
BstEII @60°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	++

ENZYME	SURVIVAL
ClaI	+
CspCI	+
CviAII @25°C	–
CviKI-1	–
CviQI @25°C	++
DdeI	+++
DpnI	+++
DpnII	+++
DraI	++
DraIII-HF	+++
DrdI	+++
EaeI	+++
EagI	+++
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	+++
FspEI	+++
HaeII	–
HaeIII	++
HgaI	–
HhaI	++

Survival in a Reaction (continued)

ENZYME	SURVIVAL
HincII	+++
HindIII	+++
HindIII-HF	+++
HinfI	++
HinP1I	++
HpaI	++
HpaII	++
HphI	++
Hpy99I	–
Hpy166II	++
Hpy188I	–
Hpy188III	++
HpyAV	–
HpyCH4III	+++
HpyCH4IV	++
HpyCH4V	++
I-CeuI	++
I-SceI	++
KasI	–
KpnI	++
KpnI-HF	+
LpnPI	–
MboI	+
MboII	–
MfeI	++
MfeI-HF	++
MluI	+++
MluI-HF	+++
MluCI	–
MlyI	–
MmeI	–
MnlI	++
MscI	+
MseI	+++
MslI	–

ENZYME	SURVIVAL
MspI	–
MspA1I	++
MspJI	+++
MwoI @60°C	+++
NaeI	+
NarI	–
Nb.BbvCI	+++
Nb.BsmI @65°C	++
Nb.BsrDI @65°C	++
Nb.BssSI	+++
Nb.BtsI	++
NciI	+
NcoI	++
NcoI-HF	++
NdeI	+++
NgoMIV	+++
NheI	++
NheI-HF	+++
NlaIII	+
NlaIV	+
NmeAIII	–
NotI	++
NotI-HF	+++
NruI	+++
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	+++

ENZYME	SURVIVAL
PaeR7I	+++
PaqCI	N/A
PciI	++
PflFI	+++
PflMI	+
PI-PspI @65°C	+++
PI-SceI	+++
PleI	+
PluTI	+
PmeI	–
PmlI	+
PpuMI	+++
PshAI	–
PsiI-v2	+++
PspGI @75°C	++
PspOMI	+++
PspXI	+++
PstI	+
PstI-HF	+
PvuI	+++
PvuI-HF	+++
PvuII	+++
PvuII-HF	–
RsaI	++
RsrII	++
SacI	+++
SacI-HF	+++
SacII	+++
SalI	+++
SalI-HF	++
SapI	–
Sau3AI	+
Sau96I	++
SbfI	+
SbfI-HF	–

ENZYME	SURVIVAL
ScaI-HF	++
ScrFI	++
SexAI	++
SfaNI	+
SfcI	–
SfiI @50°C	++
SfoI	–
SgrAI	–
SmaI @25°C	+++
SmlI @55°C	++
SnaBI	+
SpeI	++
SpeI-HF	+
SphI	+++
SphI-HF	–
SrfI	+++
SspI	++
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	++
XbaI	+++
XcmI	+++
XhoI	+++
XmaI	+
XmnI	++
ZraI	+

Cleavage of Supercoiled DNA

Restriction enzymes cleave different DNA substrates with varying efficiency. Restriction enzymes were tested for their ability to cleave various plasmids (pBR322, pUC19 and pLITMUS) under standard reaction conditions. Single sites were tested on each of these plasmids, depending on availability, and average values were taken when there was more than one data point available. Lambda DNA was used as the standard (1 unit to cleave in all cases).

ENZYME	UNITS TO CLEAVE PLASMID
AatII	3
AccI	4
Acc65I	1
AflII	2
AflIII	1
AgeI	1
AhdI	1
AlwNI	2
ApaI	1
ApoI	1
AseI	0.3
AvaI	10
AvrII	1
BaeI	3
BamHI	3
BanII	1
BglII	8

ENZYME	UNITS TO CLEAVE PLASMID
BpmI	1
BsaAI	20
BsgWI	3
BsaWI	3
BsaXI	2
BsgI	1
BsmI	1
BspDI	1
BspEI	1
BspMI	**
BspQI	3
BsrGI	1
BssHI	4
BstZ17I-HF	1
BtgI	5
ClaI	5
EagI	10

ENZYME	UNITS TO CLEAVE PLASMID
EcoNI	3
Eco0109I	8
EcoRI	3
EcoRV	1
HincII	4
HindIII	5
KasI	4
KpnI	2
MluI	2
NarI	10
NcoI	1
NdeI	3
NheI	5
NmeAIII	**
NruI	1
NsiI	1
PaqCI	1
PciI	3

ENZYME	UNITS TO CLEAVE PLASMID
PsiI-v2	0.25
PstI	1
PvuI	2
PvuII	2
SacI	5
SalI	10
SapI	1
SmaI	1
SnaBI	1
SpeI	1
SphI	3
SspI	4
StuI	3
TspMI	1
Tth111I	2
XbaI	2
XhoI	10
XmnI	5

** BspMI and NmeAIII require 2 copies of its recognition sequence for cleavage to occur. Thus, the single BspMI site in pBR322 and pUC19 as well as the single NmeAIII site in pUC19 are resistant to cleavage. A 100-fold overdigestion with BspMI cuts less than half the DNA present, while a 10-fold overdigestion with NmeAIII cuts less than half the DNA present. Other plasmids may also be resistant to BspMI and NmeAIII cleavage.

Compatible Cohesive Ends

Restriction enzymes that produce compatible cohesive ends often produce recleavable ligation products. The combinations listed were identified by computer analysis, 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**

Enzymes that have degenerate recognition sequences (e.g., recognize more than one sequence) are followed by a specific sequence in parentheses and are only listed if a non-degenerate equivalent does not exist. Be aware that these degenerate enzymes will cleave sequences in addition to the one listed.
A "—" denotes a ligation product that cannot be recleaved.

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	— TaqI
AciI (C/CGC)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI BsaHI (GR/CGCC), HinP1I, NarI HpaII	— AciI HpaII
AclI (AA/CGTT)	AccI (GT/CGAC), AciI, ClaI, BstBI, HinP1I, HpaII, NarI, TaqI	— —
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, MluCI	ApoI, MluCI ApoI, EcoRI, MluCI MluCI
AscI (GG/CGCGCC)	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, XhoI TaqI 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

ENZYME	LIGATED TO	RECLEAVED BY
BfaI (C/TAG)	AseI, Csp6I, MseI, NdeI	—
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 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 AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	TaqI —

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 AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	TaqI —
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, MluCI	ApoI, EcoRI, MluCI ApoI, MluCI MluCI
FatI (/CATG)	BspHI, NcoI, PciI	FatI, NlaIII
HinP1I (G/CGC)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI AciI, BsaHI (GR/CGCC), NarI BsaHI (GR/CGTC) HpaII	— HhaI HgaI AciI
HpaII/MspI (C/CGG)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI 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, MluCI	MluCI
MluI (A/CGCGT)	AscI, BssHII	BstUI, HhaI
MluCI (/AATT)	ApoI (R/AATTY), EcoRI, MfeI	MluCI
MseI (T/TAA)	AseI BfaI, Csp6I, NdeI	MseI —
NarI (GG/CGCC)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI 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
PluTI (GGCGC/C)	HaeII	HaeII
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
PstI (CTGCA/G)	BsiHKAI, Bsp1286I (GTGCA/C) NsiI SbfI	BsgI — PstI
PvuI (CGAT/CG)	AsiSI PacI BsiEI (CGAT/CG)	DpnII, 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
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 (T/CGA)	AccI (GT/CGAC), ClaI, BstBI AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	TaqI —
XbaI (T/CTAGA)	AvrII, NheI, SpeI, StyI (C/CTAGG)	BfaI
XhoI (TliI) (C/TCGAG)	PspXI SalI	XhoI, TliI TaqI
XmaI (C/CCGGG)	AgeI, BsaWI, BspEI, BsrFI, NgoMIV, SgrAI AvaI (C/CCGGG)	HpaII, NciI, ScrFI AvaI, BsaJI, HpaII, NciI, ScrFI, SmaI, XmaI

Dam (G^mATC), Dcm (C^mCWGG) 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^6A)GTGC and GCAC(N^6A)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 un-methylated 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 on page 309–314, 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, r305–307.

Kelly works as a Production Technician at our Rowley Production Facility, helping with the formulation of enzymes for catalog and custom products. In her spare time, Kelly enjoys surfing, playing soccer and hiking.

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 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

* 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.

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
	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 260/280 ratio of DNA template
	Presence of inhibitor in reaction	• Further purify starting template by alcohol precipitation, drop dialysis or commercial clean up kit (NEB #T1030) • 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
	Complex template	• Use Q5 High-Fidelity (NEB #M0491) or One*Taq* DNA Polymerase (NEB #M0482) • For GC-rich templates, use One*Taq* DNA Polymerase (NEB #M0480) with One*Taq* GC Reaction Buffer (plus One*Taq* High GC Enhancer, if necessary) or Q5 High-Fidelity DNA Polymerase (NEB #M0491) with the High GC Enhancer • 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. 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.

Optimization Tips for Luna® qPCR

TIPS FOR OPTIMIZATION

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 page 325.

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

TIPS FOR OPTIMIZATION

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 page 324.

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 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
- 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

Luna qPCR Troubleshooting Guide

PROBLEM	PROBABLE CAUSE(S)	SOLUTION(S)
qPCR traces show low or no amplification	Reagent omitted from qPCR assay Reagent added improperly to qPCR assay	• Verify all steps of the protocol were followed correctly
	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 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/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

Brian is a member of our Digital Marketing Team. He is responsible for uploading content to the many pages found on our website. When not working in his home office, Brian can be found tending to his farm and his pet pig.

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 Reagent added improperly to RT-qPCR assay	• Verify all steps of the protocol were followed correctly
	Incorrect channel selected for the qPCR thermal cycler	• Verify correct optical settings 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 RT-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/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
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/ 1pmol 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.

Chart Legend

–	0%	+	0–20%
++	20–50%	+++	50–100%
NT	not tested		

	BASE PAIRS FROM END				
ENZYME	1 bp	2 bp	3 bp	4 bp	5 bp
AccI*	–	–	–	–	–
AciI	–	+	+	++	+++
AgeI	+++	+++	+++	+++	+++
AgeI-HF	++	+++	+++	+++	+++
AleI-v2	+++	+++	+++	+++	+++
AluI	–	+++	+++	+++	+++
ApaI	+++	+++	+++	+++	+++
AscI	+++	+++	+++	+++	+++
AvrII	++	++	+++	+++	+++
BamHI	+	++	+++	+++	+++
BamHI-HF	+	+	+++	+++	+++
BbsI-HF	+++	+++	+++	+++	+++
BclI-HF	–	–	+++	+++	+++
BglII	++	+++	+++	+++	+++
BmtI	+++	+++	+++	+++	+++
BmtI-HF	+++	+++	+++	+++	+++
BsaI-HFv2	+++	+++	+++	+++	+++
BsiWI	++	+++	+++	+++	+++
BsiWI-HF	+++	+++	+++	+++	+++
BsmBI-v2	+++	+++	+++	+++	+++
BsrGI	+++	+++	+++	+++	+++
BssHII	+	+++	+++	+++	+++
BstZ17I-HF	+	+++	+++	+++	+++
ClaI	–	–	+	+++	+++
DdeI	+++	+++	+++	+++	+++
DpnI	–	++	++	NT	NT
DraIII-HF	+++	+++	+++	+++	+++
EagI	++	+++	+++	+++	+++
EagI-HF	+	+++	+++	+++	+++
EcoRI	+	+	++	++	+++
EcoRI-HF	+	+	++	+++	+++
EcoRV	++	++	++	++	+++
EcoRV-HF	+	++	++	++	+++
Esp3I	+++	+++	+++	+++	+++
FseI	+	++	+++	+++	+++
HindIII	–	+	+++	+++	+++
HindIII-HF	–	+	+++	+++	+++
HpaI	+++	+++	+++	+++	+++
KpnI	+	+++	+++	+++	+++
KpnI-HF	+	+++	+++	+++	+++
MfeI	+	++	+++	+++	+++
MfeI-HF	+	++	+++	+++	+++
MluI	+	++	+++	+++	+++

* AccI requires at least 13 base pairs beyond the end of its recognition sequence to cleave efficiently.

	BASE PAIRS FROM END				
ENZYME	1 bp	2 bp	3 bp	4 bp	5 bp
MseI	+++	+++	+++	+++	+++
NcoI	–	++	+++	+++	+++
NcoI-HF	+	++	+++	+++	+++
NdeI	+	+	+++	+++	+++
NheI	+	++	+++	+++	+++
NheI-HF	++	++	+++	+++	+++
NlaIII	++	+++	+++	+++	+++
NotI	++	++	++	++	++
NotI-HF	++	++	++	++	++
NsiI	+	+	+++	+++	+++
NspI	–	–	+	+	+++
PacI	+++	+++	+++	+++	+++
PaqCI	++	+++	–	–	–
PciI	+++	+++	+++	+++	+++
PmeI	+++	+++	+++	+++	+++
PstI	+	+++	+++	+++	+++
PstI-HF	++	+++	+++	+++	+++
PvuI	+++	+++	+++	+++	+++
PvuI-HF	+++	+++	+++	+++	+++
PvuII	++	++	++	+++	+++
PvuII-HF	–	++	++	+++	+++
RsaI	+	+++	+++	+++	+++
SacI	–	++	+++	+++	+++
SacI-HF	–	+	+++	+++	+++
SacII	+++	+++	+++	+++	+++
SalI	–	++	+++	+++	+++
SalI-HF	–	++	+++	+++	+++
SapI	+++	+++	+++	+++	+++
Sau3AI	+++	+++	+++	+++	+++
SbfI	++	+++	+++	+++	+++
SbfI-HF	++	+++	+++	+++	+++
ScaI-HF	+	+++	+++	+++	+++
SfiI	+++	+++	+++	+++	+++
SmaI	+++	+++	+++	+++	+++
SpeI	+	++	++	++	++
SpeI-HF	+	++	++	++	++
SphI	+++	+++	+++	+++	+++
SphI-HF	++	++	+++	+++	+++
SspI	+	+++	+++	+++	+++
SspI-HF	+	+++	+++	+++	+++
StuI	+++	+++	+++	+++	+++
StyI-HF	+	+++	+++	+++	+++
XbaI	++	++	++	++	++
XhoI	++	++	++	+++	+++
XmaI	+++	+++	+++	+++	+++

Activity of Restriction Enzymes in PCR Buffers

Frequently, a PCR product needs to be digested directly restriction enzymes. For convenience, digestion can be performed directly in the PCR mix without any purification of the DNA. 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, Standard *Taq* 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.

* 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.

Chart Legend

Cleavage in extension mix with 5 units of enzyme:

+++ complete cleavage ++ ~ 50% cleavage

\+ ~ 25% cleavage – no cleavage

** It has been shown that the addition of 1X Restriction Enzyme Buffer may help to improve the ability of some enzymes to cleave.

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	+++	+	+++	+++	<+
AgeI-HF	+++	<+	++	+++	+++
AhdI	<+	–	–	<+	<+
AleI-v2	–	–	–	+	+
AluI	+++	+	+++	+++	+++
AlwI	–	<+	<+	<+	<+
AlwNI	<+++	+	+++	<+	+
ApaI	+++	<+	<+	<+	–
ApaLI	+++	<+	<+	+++	+++
ApeKI	<+++	++	+++	<+	+
ApoI	+++	++	+++	++	+++
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	<+++	–	+	<+	–
BglI	<+	++	+	<+	<+
BglII	<+	+	++	<+	<+
BlpI	<+++	<+	<+	<+	–
BmgBI	–	++	+	<+	<+
BmrI	<+++	<+	+++	+++	+++
BmtI	+++	<+	++	+++	+++
BmtI-HF	++	<+	+	++	+++
BpmI	<+	<+	+++	<+++	<+++
BpuEI	+++	–	++	<+++	<+++
Bpu10I	<+	<+	+++	++	+++
BsaAI	+++	++	+++	+++	+++
BsaBI	+	<+	++	++	+++
BsaHI	+++	+	+++	+++	+++
BsaI-HFv2	+	<+	+	+	++
BsaJI	+++	<+	++	+++	+++
BsaWI	<+++	<+	++	+	+
BsaXI	<+++	<+	<+	<+	<+
BseRI	+++	<+	++	++	+
BseYI	+++	++	++	+++	+++
BsgI	<+	<+	+	<+	<+
BsiEI	+++	<+	++	++	++
BsiHKAI	–	++	+	–	–
BsiWI	+++	<+	+++	+++	+++
BsiWI-HF	–	–	–	–	–
BsmI	+++	++	+++	+++	+++
BsmAI	+++	++	+++	<+	<+
BsmBI	<+++	+	++	<+	<+
BsmFI	<+	+++	++	+	+
BsnI	+++	+	<+	+++	+
BsoBI	+++	+++	+++	++	+++
BspCNI	<+	<+	+	–	–
BspDI	<+++	<+	++	+++	+++
BspEI	–	<+	<+	–	–
BspHI	+++	<+	+++	+++	+++
Bsp1286I	<+	<+	<+	<+	<+
BspMI	+++	<+	++	<+	<+
BspQI	+	++	+++	+++	+++
BsrBI	+++	<+	+	+++	+++
BsrDI	<+	<+	+	<+	<+
BsrFI-v2	<+	–	–	–	–
BsrGI	<+	+	+++	<+	+++
BsrI	+++	<+	+++	++	+++
BssHII	+++	<+	+	+++	+++
BssSI-v2	+++	–	+	+++	+++
BstAPI	+++	<+	++	+++	+++
BstBI	+++	++	+++	+++	+++
BstEII	+++	<+	<+	+++	+++
BstEII-HF	+++	<+	<+	++	++
BstNI	+++	<+	<+	<+	<+
BstUI	+++	<+	<+	+++	+
BstXI	<+++	+	+	+	<+
BstYI	+++	<+	<+	++	+
BstZ17I-HF	+++	–	+	+++	+++
Bsu36I	<+	<+	<+	<+	+
BtgI	+++	<+	+	<+	<+
BtgZI	+++	+	++	++	++
BtsI-v2	+++	–	+	+++	+++
BtsCI	+++	<+	<+	+++	+++
Cac8I	+++	<+	<+	+++	++
ClaI	++	<+	<+	<+	++
CspCI	<+	–	+	<+	<+
CviAII	+++	<+	+	+++	+++
CviKI-1	+++	<+	++	+++	+++
CviQI	+++	+	+++	++	+++
DdeI	+++	++	+	+++	+++
DpnI	+++	++	+++	++	++
DpnII	+++	++	+++	+++	++
DraI	+++	<+	+++	+++	+++
DraIII-HF	++	++	+++	++	++
DrdI	+++	<+	+++	+++	+++

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
EaeI	+++	<+	–	<+	<+
EagI	<+	+++	+++	+++	+++
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	<++	<+	<+	<+	<+
HpyAV	+++	–	++	+	++
HpyCH4III	<++	<+	+	<++	<++
HpyCH4IV	+++	<+	<+	+++	+++
HpyCH4V	+++	<+	<+	+++	+++
Hpy99I	+++	–	+	<+	<+
Hpy188I	+++	<+	+	++	++
Hpy166II	+++	+	++	+++	+++
Hpy188III	+	<+	<+	+	<+
KasI	+++	<+	+++	+++	–
KpnI	+++	++	+	++	<+
KpnI-HF	++	–	++	<+	<+
MboI	+++	<+	+++	+++	+++
MboII	+++	+	++	+	+
MfeI	+++	<+	<+	+++	+
MfeI-HF	+	–	–	+++	<+
MluCI	+	<+	<+	++	+
MluI	+++	++	++	++	++
MluI-HF	++	–	++	++	++
MlyI	+++	+	++	<+	+
MmeI	<+	–	++	<+	<+
MnlI	+++	+	+	+	+
MscI	<+	<+	+	<+	<+
MseI	<+	<+	<+	<+	<+
MslI	+++	<+	+	+++	++
MspA1I	+++	<+	+++	++	+++
MspI	+++	<+	+++	++	+++
MwoI	+++	+++	+++	++	+++
NaeI	<+	<+	+	<+	<+
NarI	–	<+	++	+++	+++
NciI	+++	<+	<+	+	<+
NcoI	+++	<+	+	++	++
NcoI-HF	+++	<+	–	++	+
NdeI	<++	++	+++	++	<+
NgoMIV	–	<+	+	<+	<+
NheI	+++	<+	<+	+++	+++
NheI-HF	+++	<+	–	++	++
NlaIII	<+	<+	+	++	<+
NlaIV	+++	<+	+++	+++	+++
NmeAIII	<+	–	+++	<+	<+
NotI	++	<+	+	<+	<+
NotI-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
NruI	++	+	+	++	++
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	<+	<+	+++	–	<+
PvuI-HF	+++	<+	+++	++	+++
PvuII	+++	<+	+	+++	+++
PvuII-HF	+	–	–	<+	<+
RsaI	+++	<+	++	+++	+++
RsrII	<++	–	–	<+	<+
SacI	+++	<+	+	++	++
SacI-HF	+++	<+	<+	<+	++
SacII	+++	<+	+++	++	+
SalI	<+	+	++	–	–
SalI-HF	+	<+	+++	+	+++
SapI	<++	<+	++	++	++
Sau3AI	+++	<+	<+	<+	<+
Sau96I	<++	+	+	+++	+++
SbfI	<++	<+	+	<+	+++
SbfI-HF	+	–	–	<+	<+
ScaI-HF	+	<+	<+	–	–
ScrFI	+++	+++	+++	+++	+++
SexAI	+++	<+	+++	+++	+++
SfaNI	–	<+	++	<++	<++
SfcI	+++	<+	<+	+	+
SfiI	+++	–	–	+++	+++
SfoI	+++	<+	+++	+	+++
SgrAI	<++	<+	++	+	+++
SmaI	+++	<+	++	+++	+++
SmlI	<+	<+	+	+	+
SnaBI	<+	<+	<+	+++	+++
SpeI	+++	+	<+	+++	+++
SpeI-HF	+++	–	<+	+++	+++
SphI	+++	+	++	<+	<+
SphI-HF	+++	<+	+	+++	+++
SrfI	<+	<+	+++	+	++
SspI-HF	++	<+	+	+++	+++
StuI	+++	<+	<+	+++	+++
StyD4I	<++	<+	+	<+	<+
StyI	<+	+	<+	<+	<+
StyI-HF	+	<+	<+	++	+++
SwaI	<+	<+	<+	<+	+++
TaqI-v2	+++	<+	+	+++	+++
TfiI	<++	<+	<+	++	++
TseI	+++	+++	+++	+++	+++
Tsp45I	+++	–	–	+	<+
TspMI	+++	<+	+	+++	+++
TspRI	+	<+	<+	+++	+++
Tth111I	+++	<+	++	<+	+
XbaI	+++	–	<+	++	++
XcmI	+++	<+	+	+++	+++
XhoI	<+	<+	+++	++	+++
XmaI	+++	<+	+	–	–
XmnI	+++	<+	<+	++	+++
ZraI	+++	<+	<+	++	+

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 Plasmid Miniprep Kit, NEB #T1010 for DNA plasmids, Monarch PCR & DNA Cleanup Kit, NEB #T1030 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 20–50 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 PCR & DNA Cleanup Kit, NEB #T1030). 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 PCR & DNA Cleanup Kit, NEB #T1030). 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 DNA Gel Extraction Kit, NEB #T1020) 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.

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

TIPS FOR OPTIMIZATION

Looking to assemble multiple DNA fragments in a single reaction? Here are some tips to keep in mind when planning your Golden Gate Assembly experiment.

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 mutagenesis. The Q5® Site-Directed Mutagenesis Kit (NEB #E0554) and the NEB web tool NEBaseChanger work well for this purpose. Alternately, a junction point can be created at the internal site's recognition 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 Golden Gate Assembly Kit manuals 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 new destination construct is included in all 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 or 1.1 for Bsa-HFv2, NEBuffer r2.1 or 2.1 for BsmBI-v2 & rCutSmart or CutSmart Buffer for PaqCI, if these buffers are supplemented with 1 mM ATP and 5-10 mM DTT.

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 used in conjunction with the NEB Golden Gate Assembly Kits (BsaI-HFv2 or BsmBI-v2) to achieve high efficiencies and accurate complex assemblies. Please use the free NEB 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. When working with complex assemblies (> 20 fragments), refer to the ligase fidelity tools on the NEBeta Tools site.

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?

Optimization Tips for NEBuilder HiFi DNA Assembly and NEB Gibson Assembly

TIPS FOR OPTIMIZATION

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 #T1030) is preferable to gel extraction (NEB #T1020).

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

  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® DNA Gel Extraction Kit, NEB #T1020, Monarch PCR & DNA Cleanup Kit, NEB #T1030)
- 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
10X NEBuffer r2.1 or 2.1	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 the Quick CIP

DNA	1 pmol of DNA ends
10X rCutSmart or CutSmart 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 min for RE-digested DNA; 30 min for sheared/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 #T1030), phenol extraction/ethanol precipitation, or gel extraction (NEB #T1020).*

Traditional Cloning Quick Guide (continued)

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 DNA Gel Extraction Kit or PCR & DNA Cleanup Kit (NEB #T1020 or T1030)
- 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 (365 nM) to minimize UV exposure that may cause DNA damage

LIGATION OF VECTOR AND INSERT

- Use a molar ratio of 1:3 vector to insert. 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.

Ligation with the Quick Ligation Kit

Vector DNA (3 kb)	50 ng
Insert DNA (1 kb)	To 50 ng
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

Ligation with Instant Sticky-end Ligase Master Mix

Vector DNA (3 kb)	50 ng
Insert DNA (1 kb)	50 ng
Master Mix	5 µl
Nuclease-free Water	To 10 µl
Incubation	None

Ligation with Blunt/TA Ligase Master Mix

Vector DNA (3 kb)	50 ng
Insert DNA (1 kb)	50 ng
Master Mix	5 µl
Nuclease-free Water	To 10 µl
Incubation	Room temperature for 15 minutes

TRANSFORMATION

- To obtain transformants in 8 hrs., use NEB Turbo Competent *E. coli* (NEB #C2984)
- 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 5-alpha (NEB #C2989) or 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 PCR & DNA Cleanup Kit (NEB #T1030) • Try NEB's ElectroLigase (NEB #M0369)
	If using electrocompetent cells, arcing was observed or no voltage was registered	• Clean up the DNA prior to ligation with the Monarch PCR & DNA Cleanup Kit (NEB T1030) • 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*
	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 PCR & DNA Cleanup Kit (5 µg) (NEB #T1030) • 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)
	Inefficient phosphorylation	• Purify the DNA prior to phosphorylation with Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030). 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 (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 PCR & DNA Cleanup Kit (NEB #T1030) • 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 PCR & DNA Cleanup Kit (NEB #T1030). 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 PCR & DNA Cleanup Kit (NEB #T1030). • 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 10-beta or NEB Stable Competent *E. coli*
	Incorrect PCR amplicon was used during cloning	• Optimize the PCR conditions • Gel purify the correct PCR fragment. NEB recommends the Monarch DNA Gel Extraction Kit (NEB #T1020).
	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*)
	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 PCR & DNA Cleanup Kit (NEB #T1030).
	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 PCR & DNA Cleanup Kit (NEB #T1030). • 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, Quick Ligation Kit or concentrated T4 DNA Ligase
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 the Monarch PCR & DNA Cleanup Kit (NEB #T1030).

PROBLEM	CAUSE	SOLUTION
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, which some enzymes are sensitive to • If the enzyme is inhibited by Dam or Dcm methylation, grow the plasmid in a dam-/dcm- strain (NEB #C2925) or use PCR DNA • 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 prior to digestion. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. Monarch kits (NEB #T1010, #T1020, #T1030) 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 the Monarch PCR & DNA Cleanup Kit (NEB #T1030).
	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 PCR & DNA Cleanup Kit (NEB #T1030). • Clean DNA with a spin column, we recommend the Monarch PCR & DNA Cleanup Kit (NEB #T1030), 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 or 3.1) may be salt sensitive. Make sure to clean up the DNA prior to digestion. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. Monarch kits (NEB #T1010, #T1020, #T1030) 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 the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • Use the recommended buffer supplied with the restriction enzyme • Use at least 3–5 units of enzyme per µg 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 on pages 306 and 322, respectively.

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 PCR & DNA Cleanup Kit (NEB #T1030).
- 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 inactivable, then a clean-up step prior to blunting is not needed. Alternatively, if the restriction enzyme(s) used are not heat inactivable, 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 PCR & DNA Cleanup Kit, NEB #T1030) 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 PCR & DNA Cleanup Kit (NEB #T1030)].

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 PCR & DNA Cleanup Kit (NEB T1030). 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 PCR & DNA Cleanup Kit, NEB #T1030) or Phenol/EtOH purification

DNA

- Heat inactivate (AP, SAP, Quick Dephosphorylation Kit) 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

- Up to 10 µl of DNA from a ligation mix can be used with only a 2-fold loss of efficiency

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 PCR & DNA Cleanup Kit) or phenol/chloroform extract and ethanol precipitate

Troubleshooting Guide for DNA Cleanup & Plasmid Purification using Monarch® Kits

PROBLEM	PRODUCT	POSSIBLE CAUSE	SOLUTION
No DNA purified	Monarch Plasmid Miniprep Kit (NEB #T1010)	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 Plasmid Wash Buffer 2
		Plasmid loss during culture growth	• Ensure proper antibiotic and concentration was used to maintain selection during culture growth
	Monarch DNA Gel Extraction Kit (NEB #T1020)	Ethanol not added to wash buffer	• Ensure the proper amount of ethanol was added to Monarch DNA Wash Buffer
	Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)		
Low DNA yield	Monarch Plasmid Miniprep Kit (NEB #T1010)	Incomplete lysis	• Pellet must be completely resuspended before addition of Plasmid 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 or consider using two columns.
		Too many cells used	• The amount of cells used should not exceed 15 O.D. units
		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
		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 DNA Gel Extraction Kit (NEB #T1020)	Buffers 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 Monarch 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 PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)	Buffers 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 Plasmid Miniprep Kit (NEB #T1010)	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 Plasmid Miniprep Kit (NEB #T1010)	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 1 step.
	Monarch DNA Gel Extraction Kit (NEB #T1020)	Guanidine carryover from Gel Dissolving Buffer	• Use smaller agarose plugs, which will require less Gel Dissolving Buffer • Use 3 or 3.5 volumes of Gel Dissolving Buffer instead of 4
		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 PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)	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 Genomic Purification 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 Genomic DNA Purification Kit. 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.

Troubleshooting Guide for Genomic DNA Purification using the Monarch 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
	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 Thiocyanate salt from the binding buffer was carried over into the eluate	• 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.
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 Total RNA Miniprep Kit

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 Total RNA Miniprep Kit. It is very important not to overload the column when extracting and purifying RNA, as yields, purity and integrity may suffer.

SAMPLE TYPE [1]		INPUT	AVERAGE YIELD (µg)	OBSERVED RIN	MAXIMUM STARTING MATERIAL
CULTURED CELLS					
HeLa		1 x 10^6 cells	12–15	9-10	1 x 10^7 cells
HEK 293		1 x 10^6 cells	12–14	9-10	1 x 10^7 cells
NIH3T3		1 x 10^6 cells	8–12	9-10	1 x 10^7 cells
MAMMALIAN BLOOD [2]					
Human	Fresh	200 µl	0.5–1.0	7-8	3 ml
	Frozen	200 µl	0.5–1.0	7-8	3 ml
	Stabilized	200 µl	0.5–1.0	7-8	3 ml
Rat	Frozen	100 µl	5.6	9	1 ml*
BLOOD CELLS					
PBMC (isolated from 5 ml whole blood)		5 ml	3	7	1 x 10^7 cells
TISSUE					
Rat liver	Frozen pulverized	10 mg	25	8–9	20 mg
	Stabilized solid	10 mg	50–60	8–9	20 mg
Rat spleen (stabilized solid with bead homogenizer)		10 mg	40–50	9	20 mg
Rat kidney (frozen pulverized)		10 mg	7–10	9	50 mg
Rat brain	Frozen pulverized	10 mg	2–3	8–9	50 mg
	Stabilized solid	10 mg	0.5–1.5	8–9	50 mg
	Stabilized solid with bead homogenizer	10 mg	5–8	8–9	50 mg
Rat muscle (frozen pulverized)		10 mg	2–3	8–9	50 mg
Mouse muscle	Frozen pulverized	10 mg	3	8–9	50 mg
	Powder with bead homogenizer	10 mg	5	7–8	50 mg
	Stabilized solid with bead homogenizer	10 mg	8–10	9	50 mg
Mouse heart (stabilized solid w/bead homogenizer)		10 mg	5–6	8–9	50 mg
YEAST					
S. cerevisiae	Frozen with bead homogenizer	1 x 10^7 cells	50	9–10**	5 x 10^7 cells
	Fresh with Zymolyase®	1 x 10^7 cells	60	9**	5 x 10^7 cells
BACTERIA					
E. coli	Frozen	1 x 10^9 cells	5	10	1 x 10^9 cells
	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 lysozyme	1 x 10^8 cells	20–30	9	1 x 10^9 cells
	Frozen with bead homogenizer	1 x 10^8 cells	8	9–10	1 x 10^9 cells
PLANT					
Corn leaf (frozen pulverized with bead homogenizer)		100 mg	45	8	100 mg
Tomato leaf (frozen pulverized with bead homogenizer)		100 mg	30	8	100 mg

[1] RNA for other blood samples, including drosophila, zebrafish embryos/larvae, plasma, serum, saliva, buccal swabs and nucleated blood have been successfully purified with this kit; protocols are available in the product manual.

[2] A protocol for nucleated blood (e.g., birds, reptiles) is also available.

* Mouse blood also has a maximum input of 1 ml.

** *S.cerevisiae* total RNA was run on an Agilent® Nano 6000 Chip using plant assay.

Find tips for RNA purification using Monarch.

Troubleshooting Guide for Total RNA Extraction & Purification Using Monarch Kits

PROBLEM	CAUSE	SOLUTION
Clogged column	Insufficient sample disruption or homogenization	• Increase time of sample digestion or homogenization • Centrifuge sample after Proteinase K digestion or homogenization to pellet debris and use only supernatant for next steps • Use larger volume of DNA/RNA Protection Reagent (NEB #T2011) and/or RNA Lysis Buffer (NEB #T2012) for sample disruption and homogenization. See sample-specific protocols in the product manual.
	Too much sample	• 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 on page 344.
Low RNA yield	Incomplete elution	• After addition of Nuclease-free Water (NEB #B1500) to column matrix, incubate 5-10 min at room temperature and then centrifuge to elute • Perform a second elution (note: this will dilute sample)
	Sample is degraded	• Store input sample at -80°C prior to use • Use Monarch DNA/RNA Protection Reagent (NEB #T2011) to maintain RNA integrity during storage
	Insufficient disruption or homogenization	• Increase time of sample digestion or homogenization • Centrifuge sample after Proteinase K digestion or homogenization to pellet debris and use only supernatant for next steps • Use larger volume of DNA/RNA Reagent (NEB #T2011) and/or RNA Lysis Buffer (NEB #T2012) for sample disruption and homogenization. See sample specific protocol in the product manual. • For Proteinase K treated samples, doubling Proteinase K (from 5% to 10%) may lead to an increase in RNA yield
	Too much sample	• 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 on page 344.
RNA degradation	Starting material not handled/stored properly	• Store input sample at -80°C prior to use. Degradation of RNA may occur if sample is not flash frozen or protected by a preservation reagent. Use Monarch DNA/RNA Protection Reagent (NEB #T2011) to maintain RNA integrity during storage.
	Deviation from the stated protocol may expose RNA to unwanted RNase activities	• Refer to the General Guidelines for working with RNA in the product manual
	RNase contamination of eluted materials or kit buffers may have occurred	• See General Guidelines for working with RNA in the product manual for advice on reducing risks of contamination
Low OD ratios	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.
	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. Use care to ensure the tip of the column does not contact the flow-through after the final wash. If unsure, please repeat centrifugation. When reusing collection tubes, blot rim of tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer.
DNA contamination	Genomic DNA not removed by column	• 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
	Too much sample	• 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 on page 344.
Low performance of RNA in downstream steps	Salt and/or ethanol carryover has occurred	• Use care to ensure the tip of the RNA Purification Column does not contact the flow-through after the final wash. If unsure, please repeat centrifugation. • Be sure to spin the RNA Purification Column for 2 minutes following the final wash with RNA Wash Buffer • 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 30 µl of nuclease-free water • Increase amount of starting material (within kit specifications). See Guidelines for Choosing Sample Input Amounts on page 344.
	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

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. 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?
					A260/280	A260/230		
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?
						A260/280	A260/230		
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) per 5 µl	PURITY RATIOS		RNA CONTENT	VALIDATED FOR ONT SEQUENCING?
						A260/280	A260/230		
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 10^6 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. 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?
						A260/280	A260/230		
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 λ 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.

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). ASM Press.

(3) Raleigh, E.A. et al. (1991) *J. Bacteriol.,* 173, 2707–2709.

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, DNA that does not contain methylation

hsdS 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.

lacIq 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.

Genetic Markers (continued)

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.

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 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.

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).

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.

Carole has been with NEB for over 33 years and serves as Director of International Business. Carole works closely with our subsidiary offices and distributors to make sure that our global network runs smoothly. In past years, Carole spent much of her time traveling to various locations around the world – now she is spending more time in her home office, which her dog Bonnie much appreciates!

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

Find tips for successful transformation.

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
- Up to 10 µl of DNA from a ligation mix can be used with only a 2-fold loss of efficiency
- 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 5-alpha (NEB #C2989) and NEB 10-beta (NEB #C3020) Competent *E. coli* Strains are 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.

Protein Expression with T7 Express Strains

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 *Lac*I^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)

Ron has worked at NEB for 13 years as an Automation Engineer. He is responsible for the implementation of many of the processes needed to package our products. In his spare time, Ron enjoys the outdoors and riding his motorcycle.

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
Low Library Yield	Input DNA is damaged	• Shear input DNA on a Covaris® instrument in 1X TE Buffer, and/ or use the NEBNext FFPE DNA Repair Mix (NEB #M6630) after shearing and prior to library prep
	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 view our video
	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 view our video
	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 view our video
	Sample storage after A-tailing	• Avoid prolonged storage of 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.

Guidelines for NGS Library Prep

DNA SAMPLE INPUT GUIDELINES

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.

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.

RNA SAMPLE INPUT GUIDELINES

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 Total RNA Miniprep Kit) 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.

LIBRARY QUANTITATION

- Library quantity and quality can be determined using the Agilent TapeStation or similar instrumentation, or qPCR-based methods.

The NEBNext Library Quant Kit for Illumina is an ideal method for highly accurate library quantitation, that is especially important for PCR-free workflows.

BEAD-BASED CLEAN-UPS 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.

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.
- 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	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 O^6 -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

48,502 base pairs
GenBank Accession #: NC_001416
See page 128 for ordering information.

There are no restriction sites for the following enzymes: AbsI(x), AsiSI, FseI, MauBI(x), MreI(x), NotI, PacI, SfiI, SpeI, SrfI, SwaI

(x) = enzyme not available from NEB

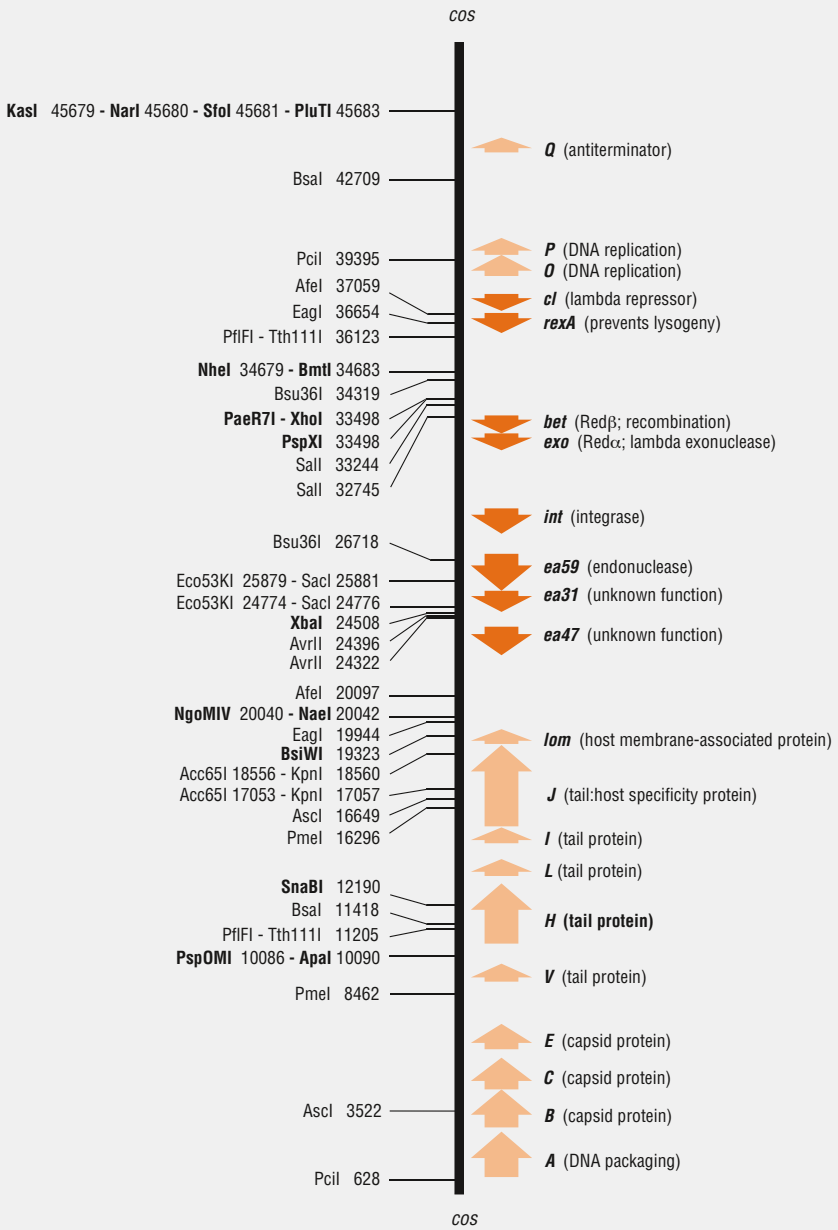
We recommend NEBcutter at NEBcutter.com to identify the restriction sites within your DNA sequence. NEBcutter indicates cut frequency and methylation-state sensitivity.

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 *ind*1 mutation in the *c*I 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 (bottom of diagram below) 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. In previous catalogs, coordinates referred to the position of the 5´ most base on the top strand, please make note of new numbering system.**



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(2) Szybalski, E.H. and Szybalski, W. (1979) *Gene,* 7, 217–270
(3) Daniels, D.L., de Wet, J.R. and Blattner, F.R. (1980) *J. Virol.,* 33, 390–400.
(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

GenBank Accession #: X02513
Revised sequence file available at www.neb.com.
See page 128 for ordering information.

There are no restriction sites for the following enzymes: 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

We recommend NEBcutter at NEBcutter.com to identify the restriction sites within your DNA sequence. NEBcutter indicates cut frequency and methylation-state sensitivity.

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).

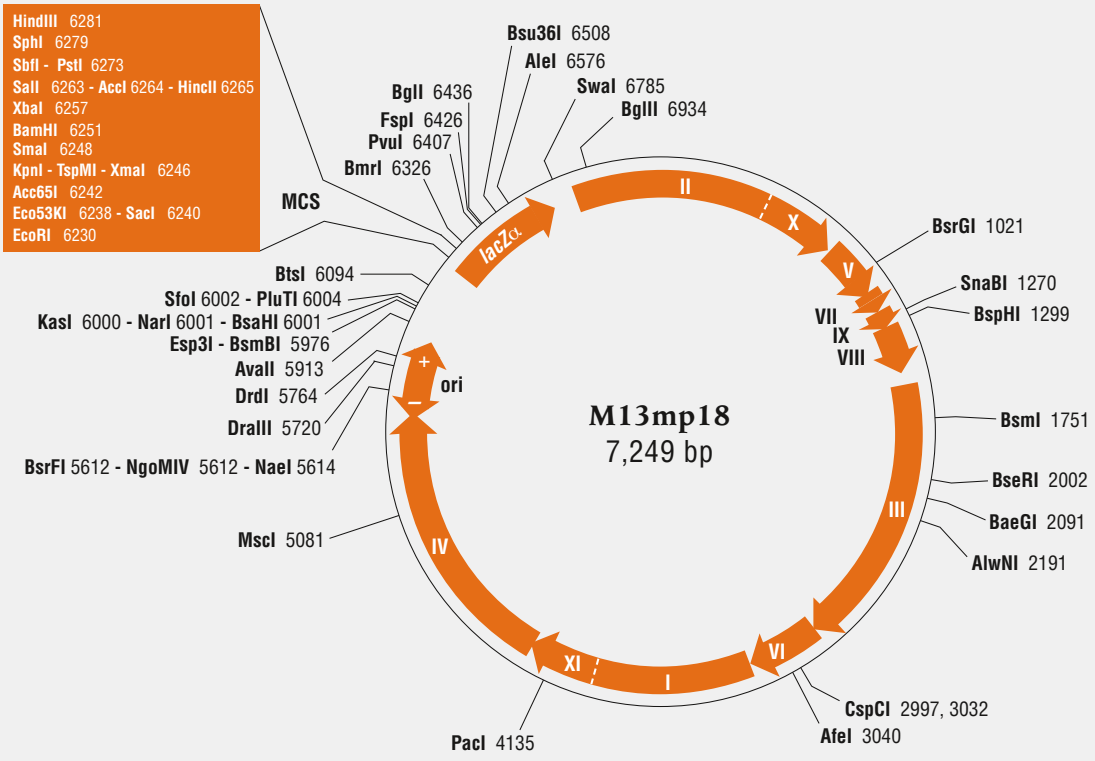
Enzymes with unique restriction sites are shown in **bold** type.
Coordinates indicate position of cutsite on the top strand. In previous catalogs, coordinates referred to the position of the 5´ most base on the top strand, please make note of new numbering system.

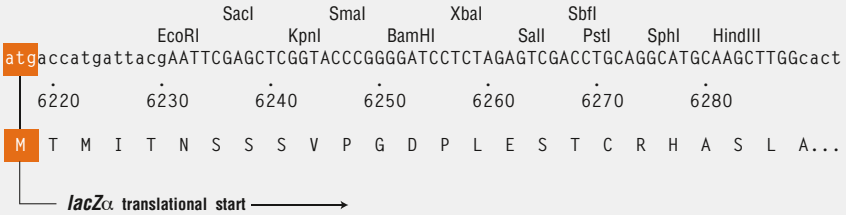
Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

M13 origin of replication arrows indicate the direction of synthesis of both the (+) and (–) strands.

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





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(1) Stewart, F.J. (2002) unpublished observations.
(2) Messing, J. et al. (1977) *Proc. Natl. Acad. Sci.* USA, 74, 3652–3646.
(3) Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene,* 33, 103–119.

pBR322

GenBank Accession #: J01749
See page 128 for ordering information.

There are no restriction sites for the following enzymes: 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

We recommend NEBcutter at NEBcutter.com to identify the restriction sites within your DNA sequence. NEBcutter indicates cut frequency and methylation-state sensitivity.

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).

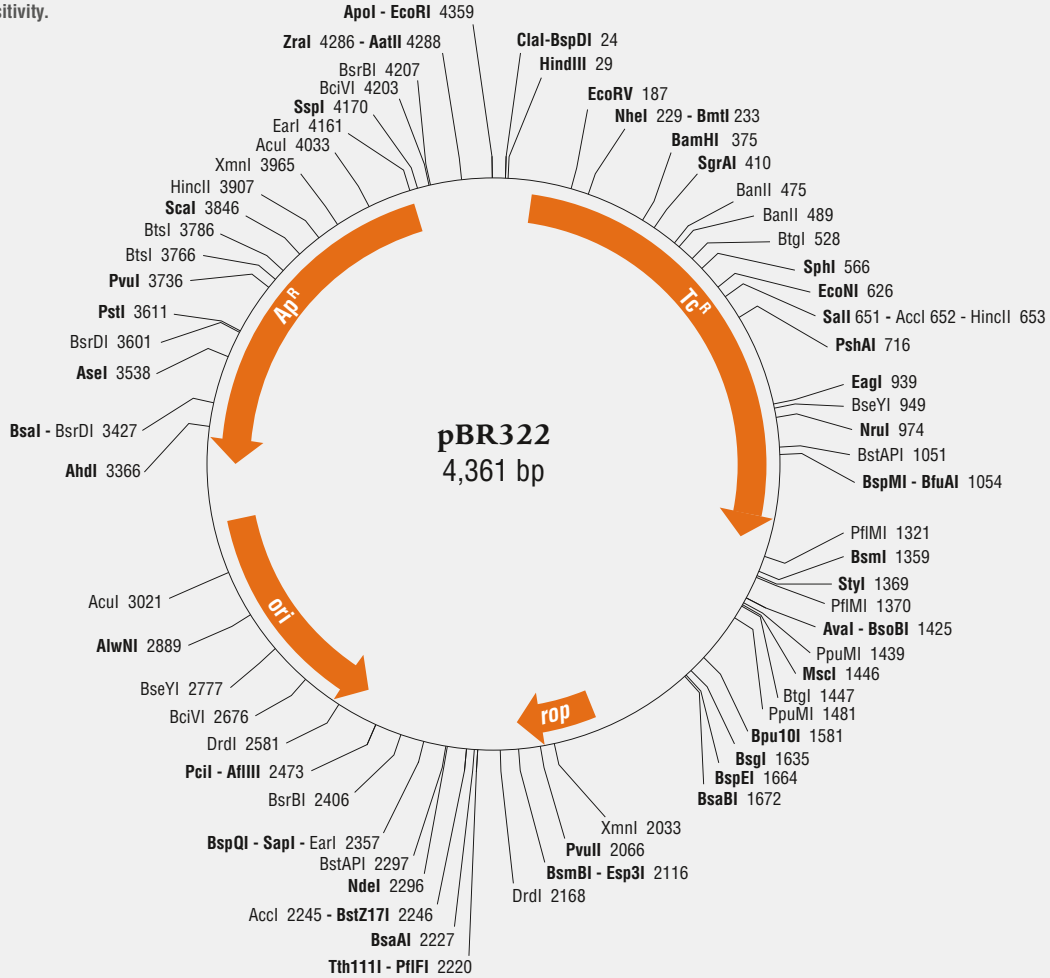
Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand. In previous catalogs, coordinates referred to the position of the 5´ most base on the top strand, please make note of new numbering system.**

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.

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



References

(1) Bolivar, F. et al. (1977) *Gene,* 2, 95–113.
(2) Sutcliffe, J.G. (1979) *Cold Spring Harb. Symp. Quant. Biol.,* 43, 77–90.
(3) Watson, N. (1988) *Gene,* 70, 399–403.
(4) Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual,* (2nd ed.), Cold Spring Harbor, Cold Spring Harbor Laboratory Press.

pKLAC2

GenBank Accession #: EU196354
See page 231 for ordering information.

There are no restriction sites for the following enzymes: 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

We recommend NEBcutter at NEBcutter.com to identify the restriction sites within your DNA sequence. NEBcutter indicates cut frequency and methylation-state sensitivity.

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).

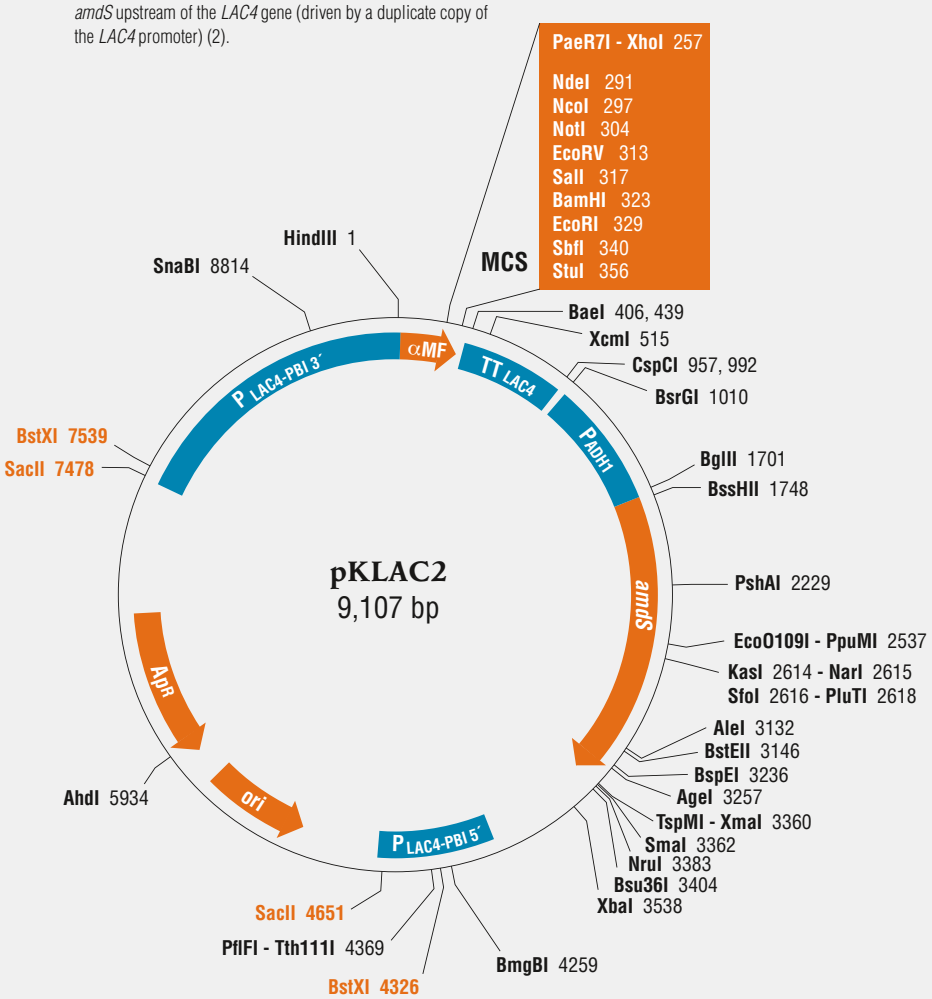
Enzymes with unique restriction sites are shown in **bold** type and selected enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand. In previous catalogs, coordinates referred to the position of the 5´ most base on the top strand, please make note of new numbering system.**

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.

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



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

Sequence file available at www.neb.com.
See page 229 for ordering information.

Feature	Coordinates	Source
lacI^{q}	80-1162	*E. coli*
P_{tac}	1405-1432	–
expression ORF	1527-2761	–
malE	1527-2721	*E. coli*
MCS	2722-2761	–
bla (Ap^{R})	3101-3961	*Tn3*
origin	4049-4637	pMB1
rop	5007-5198	pMB1

There are no restriction sites for the following enzymes: AatII, AbsI(x), Acc65I, AflII, AgeI, AjuI(x), AleI, ArsI(x), AscI, AsiSI, AvrII, BaeI, BarI(x), BbvCI, BmtI, BpiI(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

We recommend NEBcutter at NEBcutter.com to identify the restriction sites within your DNA sequence. NEBcutter indicates cut frequency and methylation-state sensitivity.

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.

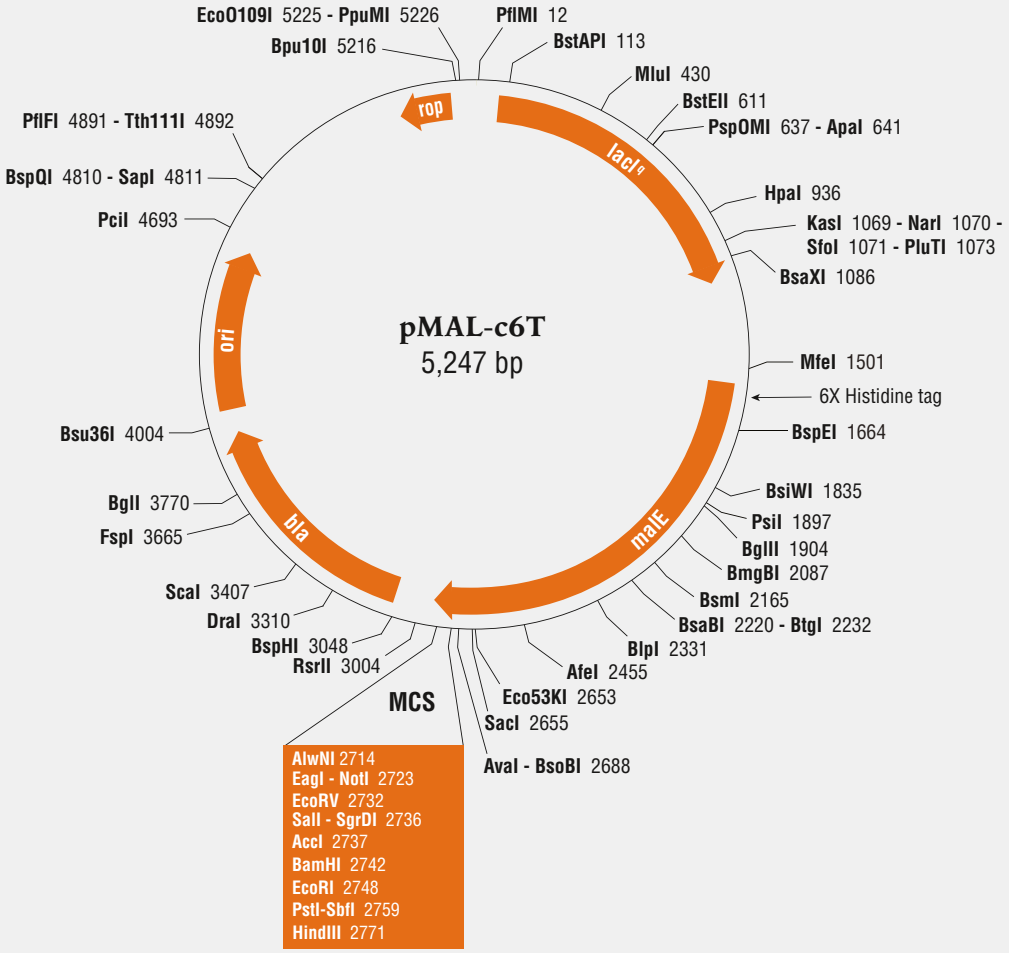
The multiple cloning site (MCS) is positioned to allow translational fusion of the *E. coli* maltose binding protein (MBP, encoded by the *malE* gene lacking its secretory signal sequence) to the *N*-terminus of the cloned target protein, thus resulting in an MBP-fusion protein localized in the cytoplasm. 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. In previous catalogs, coordinates referred to the position of the 5´ most base on the top strand, please make note of new numbering system.**

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     PstI/SbfI
5´ malE...ATG CTG ATG GGC GGC CGC GAT ATC GTC GAC GGA TCC GAA TTC CCT GCA GGT AAT
                    HindIII
TAA 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

Sequence available at www.neb.com
See page 97 for more information.

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

There are no restriction sites for the following enzymes: 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, FaII(x), FseI, FspAI(x), HincII, HindIII, HpaI, KasI, KfII(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

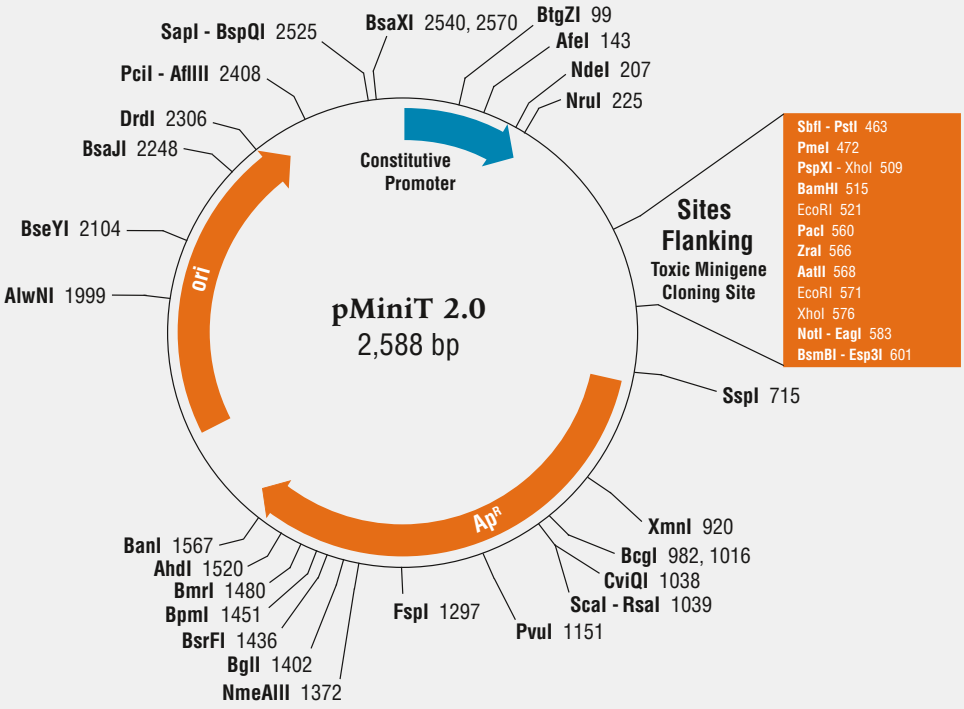
(x) = enzyme not available from NEB

We recommend NEBcutter at NEBcutter.com to identify the restriction sites within your DNA sequence. NEBcutter indicates cut frequency and methylation-state sensitivity.

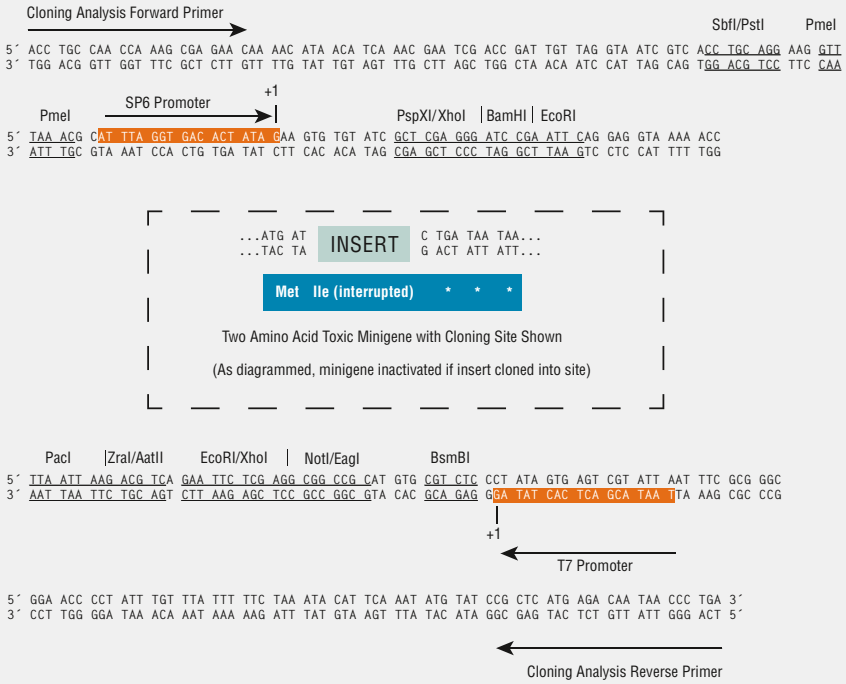
pMiniT 2.0 is an *E. coli* plasmid cloning vector designed for cloning blunt-ended or single-base overhang PCR products, or amplicons, using the NEB PCR Cloning Kit (NEB #E1202, #E1203). The pMiniT2.0 also enables *in vitro* transcription using SP6 and T7 promoters. It is compatible with Golden Gate 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* (ApR) 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. In previous catalogs, coordinates referred to the position of the 5´ most base on the top strand, please make note of new numbering system.**



Features within Sequence Flanking the Toxic Minigene/Cloning Site:



pNEB206A

Sequence file available at www.neb.com.

There are no restriction sites for the following enzymes: 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

We recommend NEBcutter at NEBcutter.com to identify the restriction sites within your DNA sequence. NEBcutter indicates cut frequency and methylation-state sensitivity.

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 *lacZ*α 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.

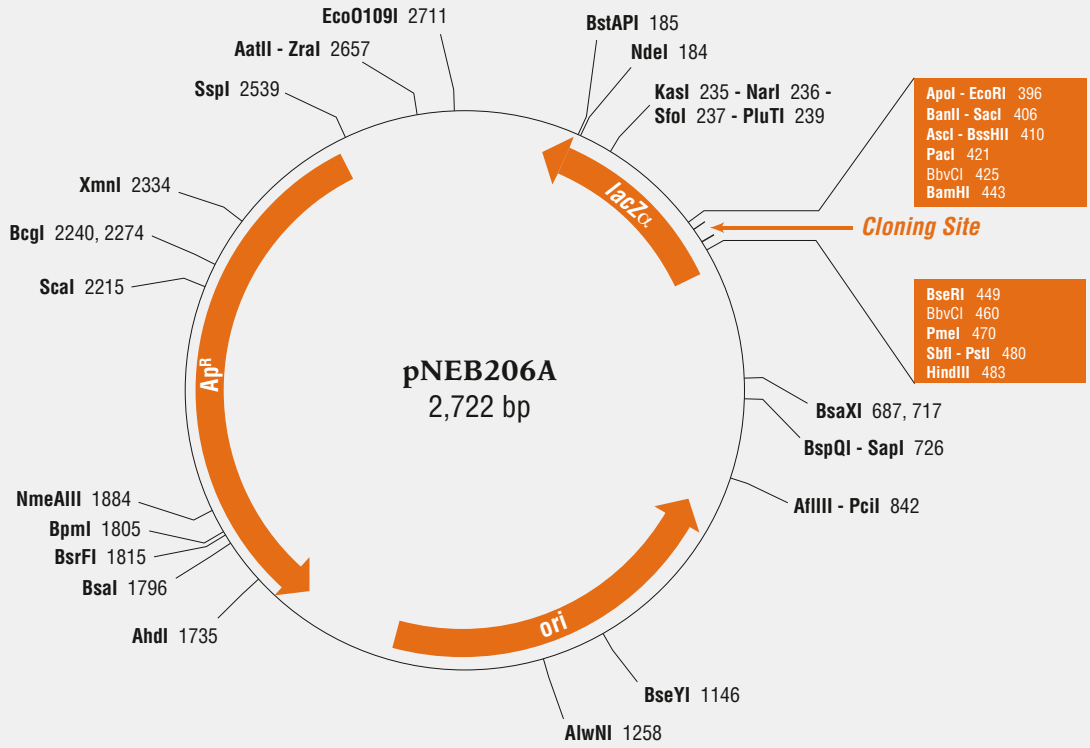
Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand. In previous catalogs, coordinates referred to the position of the 5´ most base on the top strand, please make note of new numbering system.**

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.

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 (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
```

*lacZ*α translational start

References

(1) Bitinaite, J. and Vaiskunaite, R. (2003) unpublished observations.
(2) Yanisch-Perron, C. et al. (1985) *Gene,* 33, 103–119.

pSNAP$_f$

Sequence file available at www.neb.com.
See page 298 for ordering information.

There are no restriction sites for the following enzymes: 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), KfiI(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

We recommend NEBcutter at NEBcutter.com to identify the restriction sites within your DNA sequence. NEBcutter indicates cut frequency and methylation-state sensitivity.

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. SNAP$_f$ 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. Further details are provided with the SNAP-Cell Starter Kit (NEB #E9100) and SNAP-Surface Starter Kit (NEB #E9120).

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.

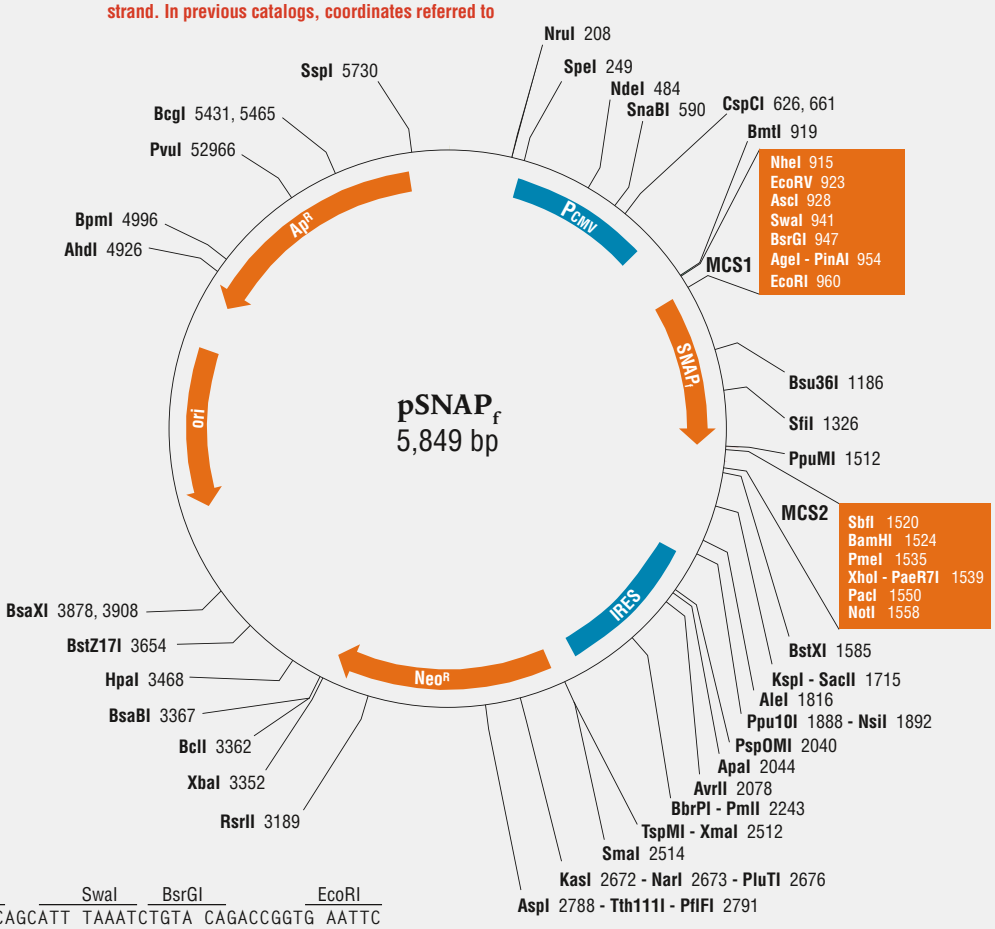
Enzymes with unique restriction sites are shown in **bold** type. **Coordinates indicate position of cutsite on the top strand. In previous catalogs, coordinates referred to the position of the 5´ most base on the top strand, please make note of new numbering system.**

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.

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



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

Sequence file available at www.neb.com.
See page 230 for ordering information.

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

There are no restriction sites for the following enzymes: 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

We recommend NEBcutter at NEBcutter.com to identify the restriction sites within your DNA sequence. NEBcutter indicates cut frequency and methylation-state sensitivity.

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.

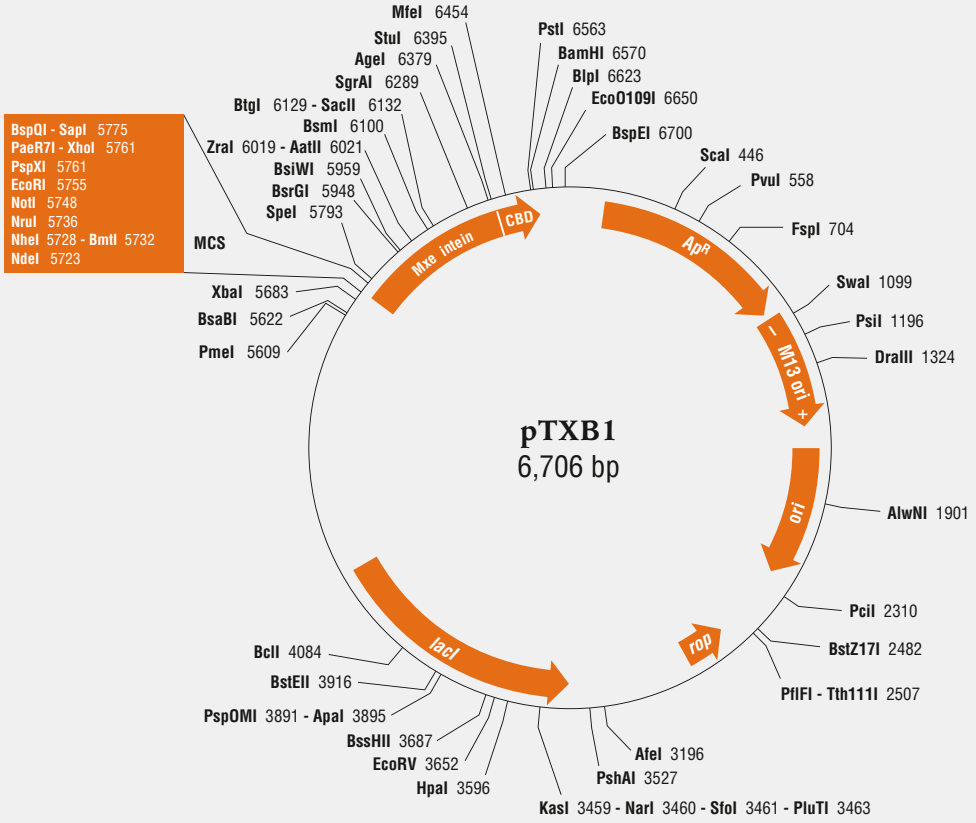
Transcription of the gene fusion is controlled by the inducible T7 promoter, requiring *E. coli* strains containing integrated copies of the T7 RNA polymerase gene [e.g., C2566 or BL21(DE3)] for expression. Basal expression from the T7 promoter is minimized by the binding of the Lac repressor, encoded 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).

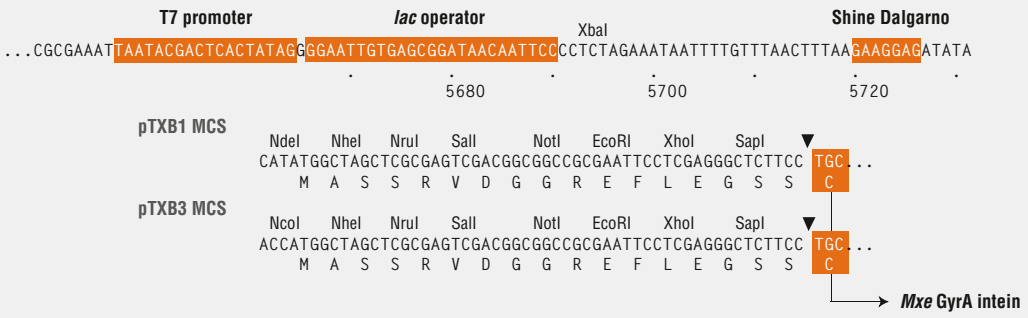
pTXB1 and pTXB3 are identical except for the MCS regions: pTXB1 contains an NdeI site, and pTXB3 an NcoI site, overlapping the initiating methionine codon of the intein fusion gene. The N-terminal cysteine residue ("Cys$_1$") of the intein is shaded.

Enzymes with unique restriction sites are shown in **bold** type. **Coordinates indicate position of cutsite on the top strand. In previous catalogs, coordinates referred to the position of the 5´ most base on the top strand, please make note of new numbering system.**

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.

Sequence file available at www.neb.com.
See page 230 for ordering information.

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

There are no restriction sites for the following enzymes: AatII, AbsI(x), AflII, AgeI, AjuI(x), AscI, AsiSI, AvrII, BbvCI, BmgBI, BplI(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

We recommend NEBcutter at NEBcutter.com to identify the restriction sites within your DNA sequence. NEBcutter indicates cut frequency and methylation-state sensitivity.

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.

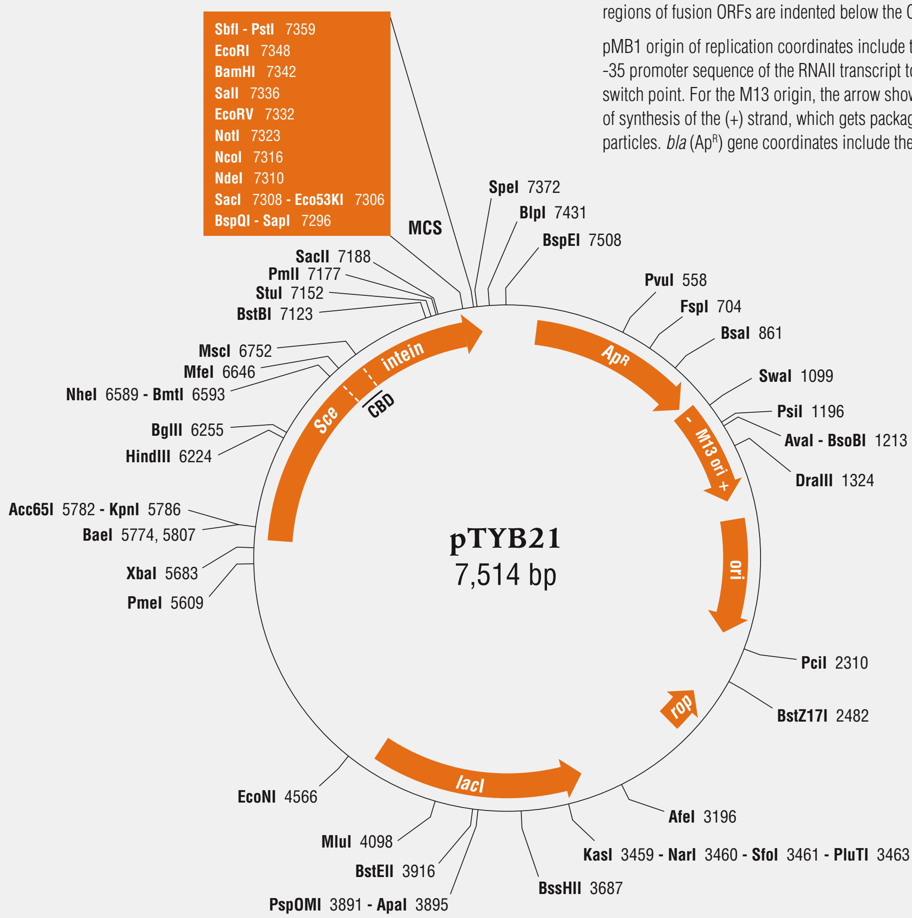
Transcription of the gene fusion is controlled by the inducible T7 promoter, requiring *E. coli* strains containing integrated copies of the T7 RNA polymerase gene [e.g., C2566 or BL21(DE3)] for expression. Basal expression from the T7 promoter is minimized by the binding of the Lac repressor, encoded 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. In previous catalogs, coordinates referred to the position of the 5´ most base on the top strand, please make note of new numbering system.**

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                                                             SbfI
                   SapI        NdeI      NcoI     NotI                SalI      BamHI  EcoRI     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 ──┐
                 │                                                                     SbfI
            BsmI ▼          NdeI      NcoI     NotI                SalI      BamHI  EcoRI     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.

APPENDIX

pUC19

GenBank Accession #: L09137
See page 128 for ordering information.

Feature	Coordinates	Source
*lacZ*α	469-146	–
origin	1455-867	pMB1 (mutant)
bla (Ap^R)	2486-1626	*Tn3*

ori = origin of replication
Ap = ampicillin

There are no restriction sites for the following enzymes: 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, BpiI(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, FaiI(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

We recommend NEBcutter at NEBcutter.com to identify the restriction sites within your DNA sequence. NEBcutter indicates cut frequency and methylation-state sensitivity.

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.

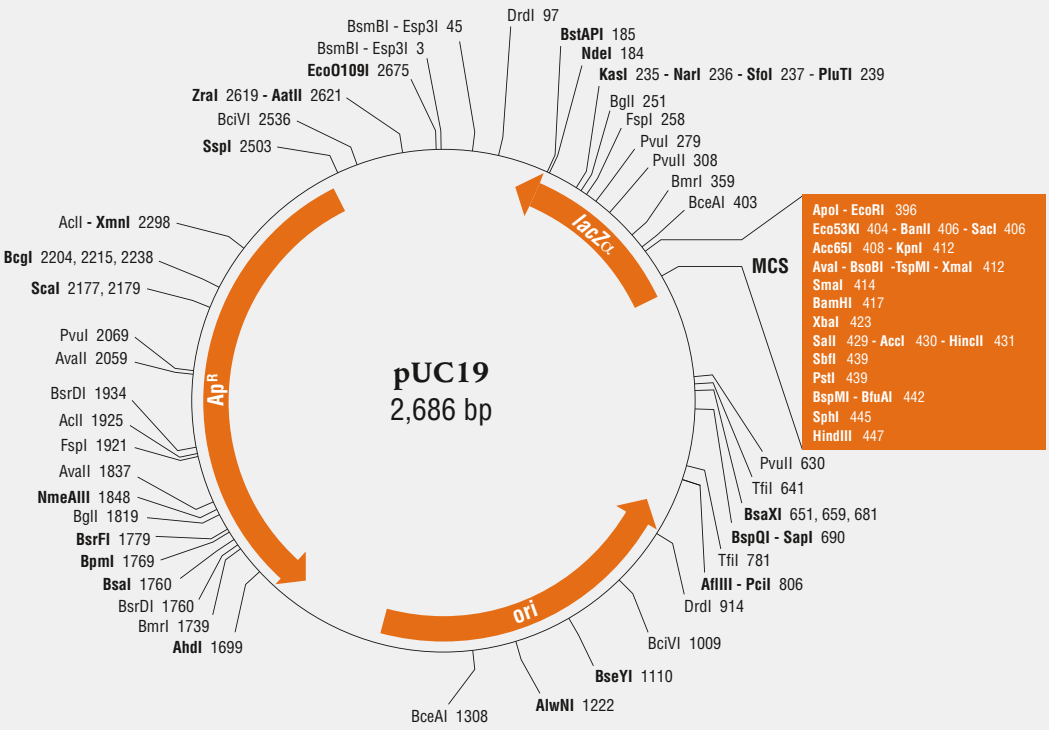
Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand. In previous catalogs, coordinates referred to the position of the 5´ most base on the top strand, please make note of new numbering system.**

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.

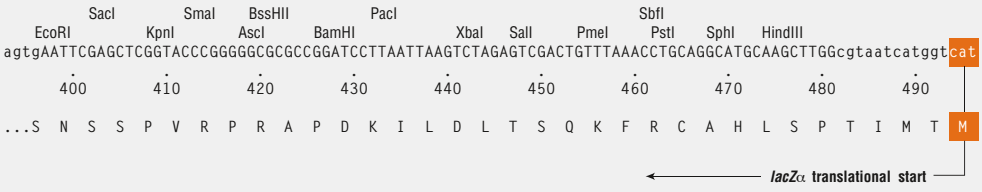
References

(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.



pUC19 MCS

pNEB193 MCS



The Genetic Code

	A Ala	R Arg	N Asn	D Asp	C Cys	Q Gln	E Glu	G Gly	H His	I Ile	L Leu	K Lys	M Met	F Phe	P Pro	S Ser	T Thr	W Trp	Y Tyr	V 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 Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA Stop UAG Stop	UGU Cys UGC Cys UGA Stop UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G
A	AUU Ile AUC Ile AUA Ile AUG Met	ACU Thr ACC Thr ACA Thr ACG Thr	AAU Asn AAC Asn AAA Lys AAG Lys	AGU Ser AGC Ser AGA Arg AGG Arg	U C A G
G	GUU Val GUC Val GUA Val GUG Val	GCU Ala GCC Ala GCA Ala GCG Ala	GAU Asp GAC Asp GAA Glu GAG Glu	GGU Gly GGC Gly GGA Gly GGG Gly	U C A G

Termination Signals

UAA (Ochre)
UAG (Amber)
UGA (Opal)

Single Letter Code

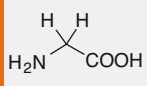
A = adenosine
C = cytidine
G = guanosine
T = thymidine
U = uridine

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

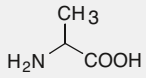
Amino Acid Structures

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

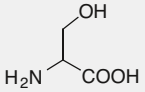


Glycine (Gly, G)
MW: 75.07

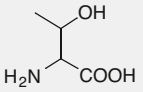


Alanine (Ala, A)
MW: 89.09

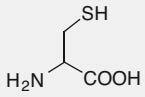
NUCLEOPHILIC



Serine (Ser, S)
MW: 105.09, pK_a ~ 16

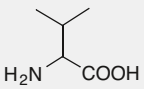


Threonine (Thr, T)
MW: 119.1, pK_a ~ 16

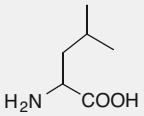


Cysteine (Cys, C)
MW: 121.2, pK_a = 8.18

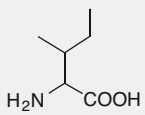
HYDROPHOBIC



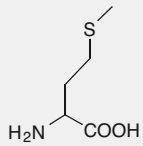
Valine (Val, V)
MW: 117.1



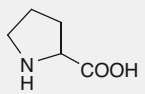
Leucine (Leu, L)
MW: 131.2



Isoleucine (Ile, I)
MW: 131.2

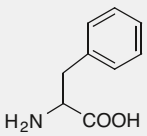


Methionine (Met, M)
MW: 149.2

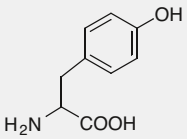


Proline (Pro, P)
MW: 115.1

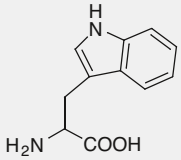
AROMATIC



Phenylalanine (Phe, F)
MW: 165.2

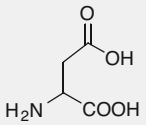


Tyrosine (Tyr, Y)
MW: 181.2, pK_a = 10.46

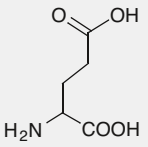


Tryptophan (Trp, W)
MW: 204.2

ACIDIC

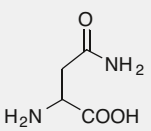


Aspartic Acid (Asp, D)
MW: 133.1, pK_a = 3.9

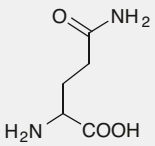


Glutamic Acid (Glu, E)
MW: 147.1, pK_a = 4.07

AMIDE

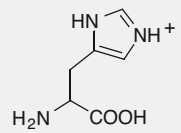


Asparagine (Asn, N)
MW: 132.1

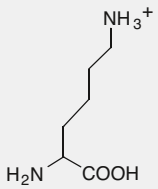


Glutamine (Gln, Q)
MW: 146.1

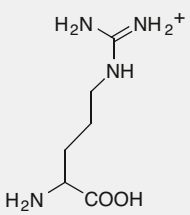
BASIC



Histidine (His, H)
MW: 155.2, pK_a = 6.04



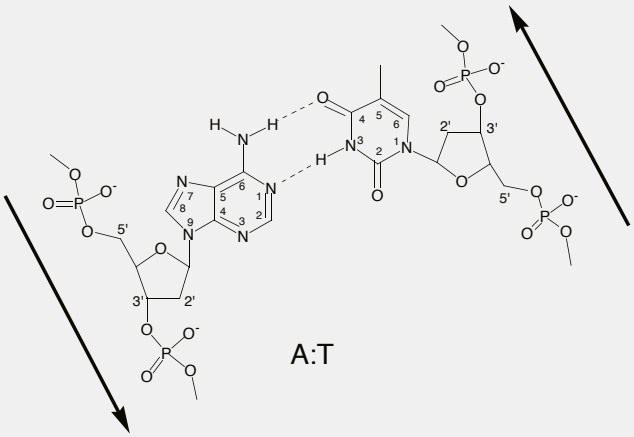
Lysine (Lys, K)
MW: 146.2, pK_a = 10.79

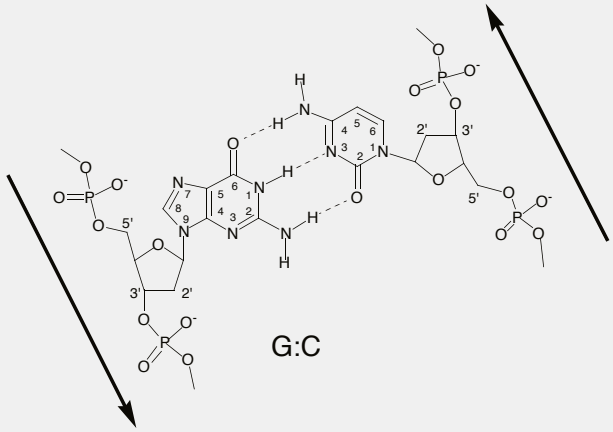


Arginine (Arg, R)
MW: 174.2, pK_a = 12.48

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 = 9.1×10^{11} molecules
1 µg of pUC18/19 DNA (2686 bp) = 0.57 pmol = 3.4×10^{11} molecules
1 µg of pBR322 DNA (4361 bp) = 0.35 pmol = 2.1×10^{11} molecules
1 µg of M13mp18/19 DNA (7249 bp) = 0.21 pmol = 1.3×10^{11} molecules
1 µg of λ DNA (48502 bp) = 0.03 pmol = 1.8×10^{10} 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

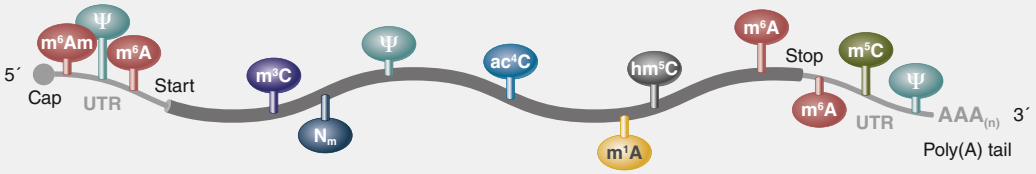
1.0 kb DNA = coding capacity for 333 amino acids ≈ 37,000 dalton protein
10,000 dalton protein ≈ 270 bp DNA
50,000 dalton protein ≈ 1.35 kb DNA

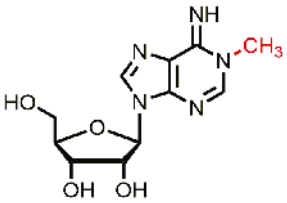
Isotope Data

Isotope	Particle Emitted	Half Life	
^{14}C	β	5,730 years	1 Ci = 1,000 mCi
^{3}H	β	12.3 years	1 mCi = 1,000 µCi
^{125}I	γ	60 days	1 µCi = 2.2×10^{6} disintegrations/minute
^{32}P	β	14.3 days	1 Becquerel = 1 disintegration/second
^{33}P	β	25 days	1 µCi = 3.7×10^{4} Becquerels
^{35}S	β	87.4 days	1 Becquerel = 2.7×10^{-5} µCi

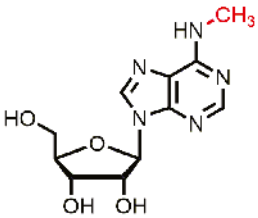
Messenger RNA Modifications

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

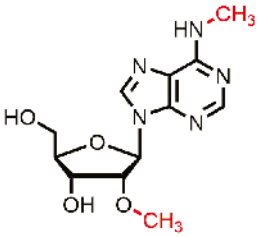




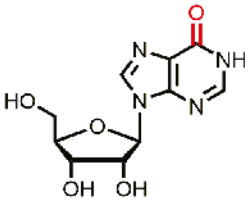
1-methyladenosine (m^1A) [1A]



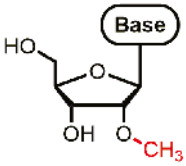
N^6-methyladenosine (m^6A) [6A]



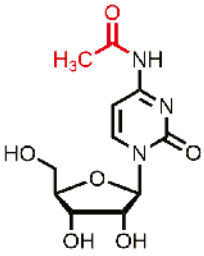
N^6,2´-*O*-dimethyladenosine (m^6Am) [06A]



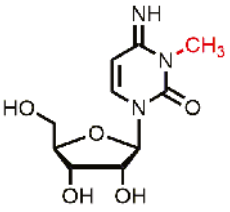
inosine (I) [9A]



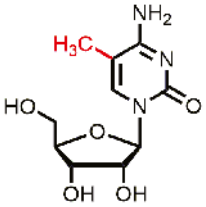
2´-*O*-methylnucleoside (Nm) [ON]



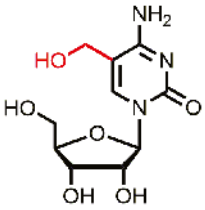
N^4-acetylcytidine (ac^4C) [42C]



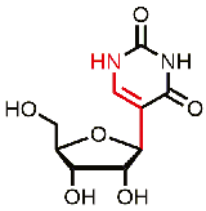
3-methylcytidine (m^3C) [3C]



5-methylcytidine (m^5C) [5C]



5-hydroxymethylcytidine (hm^5C) [51C]



pseudouridine (Ψ) (9U)

Key:

Nucleoside Name (short name)
[new nomenclature]

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	H_3PO_4	98.0	1.70	85	18.1
Sulfuric acid	H_2SO_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

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

Bacterial Cells: *E. coli* or *Salmonella typhimurium*

Cell Data	per cell	per liter at 10^9 cells per 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		

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

pH vs Temperature for Tris Buffer

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

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For over 45 years, the New England Biolabs catalog has been a resource for scientists around the world. Each edition contains a collection of mini-reviews that addresses various scientific, environmental and/or humanitarian topics. This year, we are dedicating the Catalog to our Founder, Donald G. Comb, and sharing some of the values that he was passionate about. As part of this effort, NEB will offer support to an organization working to conserve biodiversity throughout the world.

The Marine Conservation Action Fund

The New England Aquarium's Marine Conservation Action Fund (MCAF) builds hope for the future of the ocean by supporting conservation leaders who tackle critical marine research and conservation needs. From funding for urgent, community-based initiatives to mentorship, networking, and professional development, MCAF has supported more than 170 projects in over 60 countries since it was founded in 1999. The program focuses its support on country-national scientists and grassroots leaders in low-and middle-income countries – areas that host much of the world's biodiversity but have fewer financial resources for conservation efforts.

MCAF, which is housed in the Aquarium's Anderson Cabot Center for Ocean Life and is a grantee of the New England Biolabs Foundation, has supported community leaders in bringing about landmark conservation victories through pioneering scientific research, outreach and collaboration with local stakeholders. These landmark successes include full legal protection of giant manta rays in Peru; the launch of the first marine mammal stranding network in Iran; the rescue of hundreds of entangled sea turtles in collaboration with local fishers in Ghana; and, the designation or strengthening of marine protected areas (MPA's) in countries such as Costa Rica, Bangladesh, the Philippines, Indonesia and Panama.

MCAF builds enduring two-way relationships with conservation leaders, connects them to a supportive global community of their peers and helps to ensure their long-term conservation impact. Together we are striving towards our vision of a healthy and vibrant ocean stewarded by local leaders and their communities for generations to come.

To learn more, please visit: **www.neaq.org/mcaf**

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Planeta Océano

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The NEB Catalog & Technical Reference is printed with sustainability in mind.

- This catalog is printed on certified paper from forests that are managed in an environmentally and socially responsible way.
- At least 70% recycled post-consumer fiber (or equivalent) is used.
- The printing facility is also certified "environmental-friendly" by renowned conservation organizations.
- The facility uses environmental-friendly inks and coatings.

As with previous catalogs and other print productions, also this catalog is a certified "Carbon-neutral" product. To achieve this, we collaborate with "ClimatePartner" a world-leading provider of climate action strategies including carbon reduction solutions. The unavoidable CO_2 generated by the production of this catalog has been reduced to net zero through verified carbon offset projects. These offsets will be used in support of the project Nr. 1072 "Hydropower for the habitat of mountain gorillas" in Virunga, DR Congo.

More information about the project Nr. 1072 supported by this NEB catalog can be found at:
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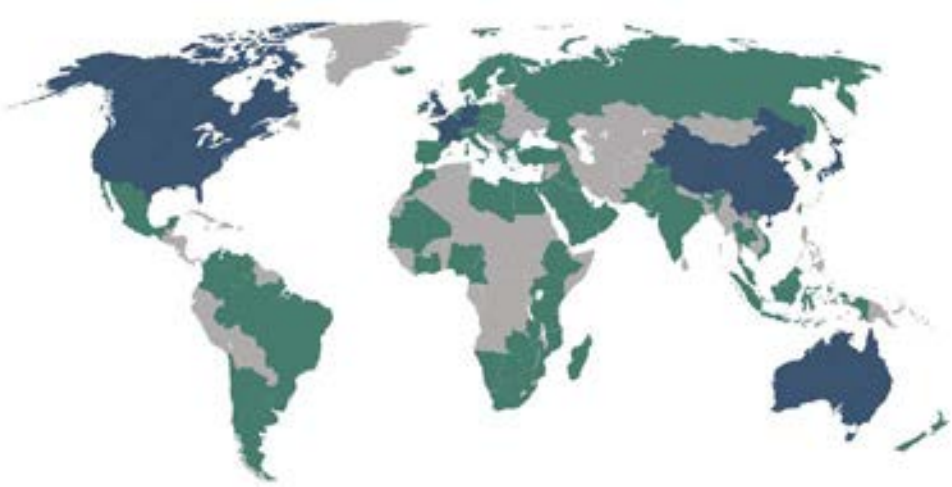

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