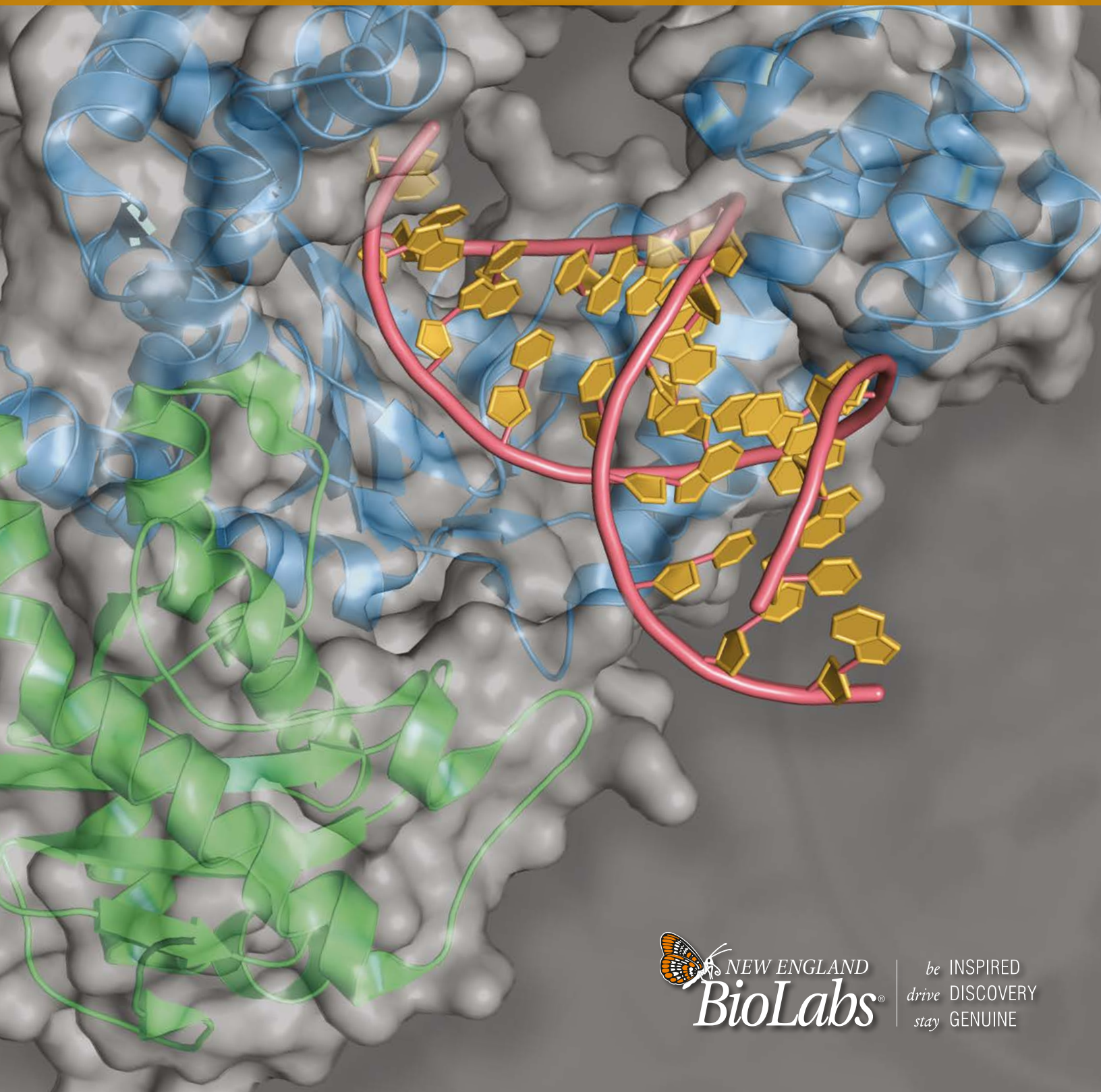
Isothermal Amplification

RAPID NUCLEIC ACID DETECTION FOR MOLECULAR DIAGNOSTICS



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What is isothermal amplification?

The Polymerase Chain Reaction (PCR) is a well-known approach for amplifying a specific DNA or RNA (RT-PCR) sequence. PCR involves the reiterative cycling of a reaction cocktail between different temperatures to achieve amplification. As routine as PCR is in the molecular biology and molecular diagnostics laboratory, there are other methods of sequence-specific nucleic acid amplification.

These alternative approaches often do not require changing the reaction temperature and are referred to as isothermal amplification protocols. Isothermal amplification protocols are varied and have different advantages. In general, isothermal techniques are extremely fast and do not require thermocyclers, making them particularly well suited for field applications and point-of-care molecular diagnostics assays.

Interested in learning how NEB scientists are using isothermal amplification?

Visit **www.neb.com/isoamp** to find videos, protocols and recent publications, including a publication from NEB scientists describing pH-sensitive isothermal detection.

Advantages

- Fast
- Minimal equipment required
- Robust reactions in the presence of inhibitors
- Options for simplified optical detection

Optimization tips for LAMP

- Use LAMP primer design software (e.g., NEB LAMP Primer Design Tool, **https://lamp.neb.com/**). Select 2–3 sets for each target and compare performance in a LAMP assay.
- Include loop primers for faster reactions
- Use high magnesium (6–8 mM) and dNTP (1–1.4 mM) concentrations for best reactions
- Omit betaine, unless it has a demonstrated benefit
- Optimize the reaction temperature (60–65°C for *Bst* LF and 63–70°C for *Bst* 2.0/3.0)
- To minimize contamination, use *Bst* 3.0 or master mixes containing dUTP and thermolabile UDG (NEB #M1708, #E1708, #M1804)

Did you know that many of these products can be purchased in larger volumes? Contact **custom@neb.com** to find out more.

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How is colorimetric LAMP used in point of care?

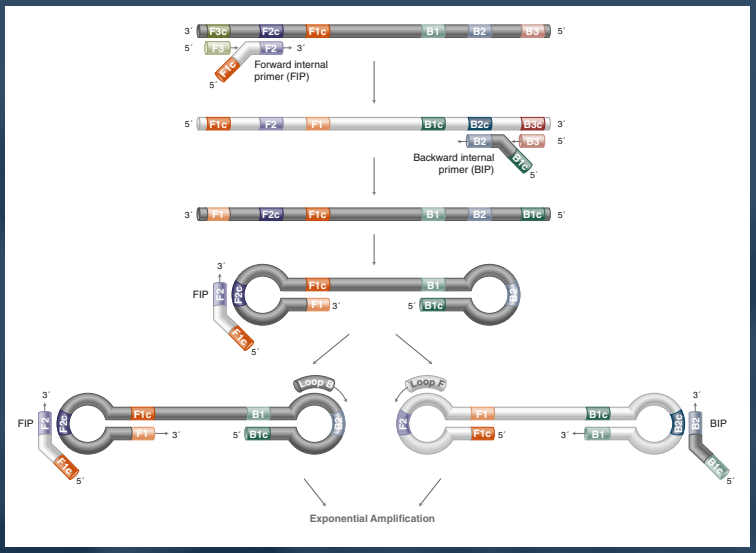
*see back cover for details

Examples of isothermal technologies

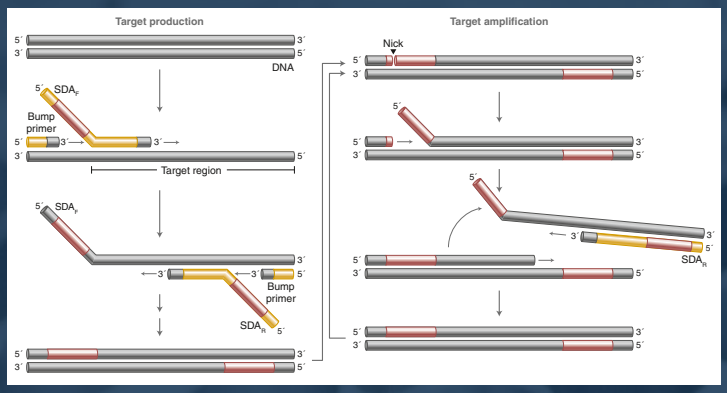
Loop-mediated Isothermal Amplification (LAMP & RT-LAMP)

LAMP uses 4–6 primers recognizing 6–8 distinct regions of target DNA for a highly specific amplification reaction. A strand-displacing DNA polymerase initiates synthesis and 2 specially designed primers form "loop" structures to facilitate subsequent rounds of amplification through extension on the loops and additional annealing of primers. DNA products are very long (> 20 kb) and formed from numerous repeats of the short (80–250 bp) target sequence, connected with single-stranded loop regions in long concatamers. These products are not typically appropriate for downstream manipulation, but target amplification is so extensive that numerous modes of detection are possible. Real-time fluorescence detection using intercalators or probes, lateral flow and agarose gel detection are all directly compatible with LAMP reactions. Instrumentation for LAMP typically requires consistent heating to the desired reaction temperature and, where needed, real-time fluorescence for quantitative measurements.

Overview of LAMP



Overview of SDA



** Target amplification, shown above for SDA_F, will also occur simultaneously with SDA_R.*

Strand Displacement Amplification (SDA)

SDA relies on a strand-displacing DNA polymerase, typically *Bst* DNA Polymerase, Large Fragment (NEB #M0275) or Klenow Fragment (3´→ 5´exo⁻) (NEB #M0212), to initiate amplification at nicks created by a nicking enzyme (e.g., Nt.BstNBI, NEB #R0607) at a site contained in a primer. The nicking site is regenerated with each polymerase displacement step, resulting in exponential amplification. SDA is typically used in clinical diagnostics.

Nucleic Acid Sequenced Based Amplification (NASBA)

NASBA and Transcription Mediated Amplification (TMA) are similar isothermal amplification techniques that proceed through RNA. Primers are designed to target a region of interest, but importantly, one primer includes the promoter sequence for T7 RNA polymerase at the 5´ end. This enables production of single-stranded RNA species, which are reverse transcribed to cDNA by a reverse transcriptase included in the reaction. The RNA in the DNA-RNA hybrids is destroyed by RNase H activity (from an exogenous protein in NASBA, or by an RNase H+ RT in TMA) and dsDNA is produced by the RT. This template then gets transcribed to RNA by T7 RNAP and exponential amplification results.

Nicking Enzyme Amplification Reaction (NEAR)

NEAR employs a strand-displacing DNA polymerase initiating amplification at a nick created by a nicking enzyme, rapidly producing many short nucleic acids from the target sequence. This process is extremely fast and sensitive, enabling detection of small target amounts in minutes. NEAR is commonly used for pathogen detection in clinical and biosafety applications.

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View our loop mediated isothermal amplification tutorial.

*see back cover for details

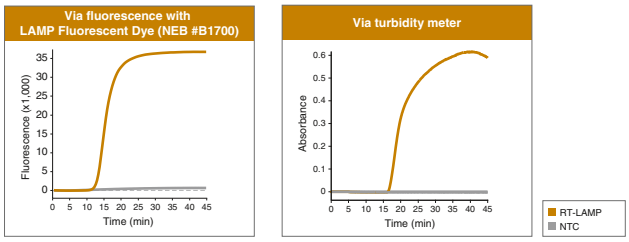
Featured products for isothermal a

WarmStart® Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) (NEB #M1708)

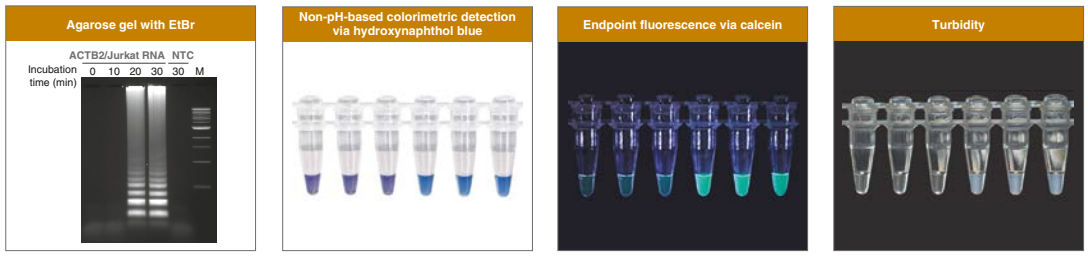
Loop Mediated Isothermal Amplification (LAMP) is a commonly-used technique for rapid nucleic acid detection. NEB's WarmStart LAMP products provide a simple, one-step solution for DNA or RNA targets. The WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) (NEB #M1708) is fully buffered and compatible with different sample types, enabling multiple detection methods including turbidity detection, real-time fluorescence detection, and end-point visualization such as colorimetric detection via a metal indicator (e.g., hydroxynapthol blue). It features *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx Reverse Transcriptase, both *in silico*-designed enzymes for improved performance in LAMP reactions. For real-time fluorescence detection, the master mix is available as a kit (NEB #E1708) that includes 50X LAMP Fluorescent Dye.

The WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) is compatible with multiple detection methods

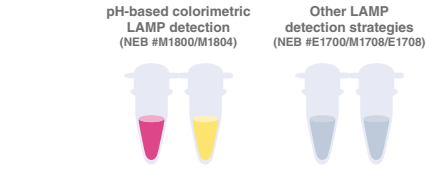
A. Real-time detection



B. Real-time detection



LAMP/RT-LAMP master mixes and sample type considerations



	pH-based colorimetric LAMP detection (NEB #M1800/M1804)	Other LAMP detection strategies (NEB #E1700/M1708/E1708)
Weakly buffered / neutral pH samples	✓	✓
Purified samples	✓	✓
Highly-buffered samples	✗	✓
Acidic samples	✗	✓

NEB's pH-based colorimetric LAMP master mixes with UDG (NEB #M1804) or without UDG (NEB# M1800) are weakly buffered to allow for visual detection of amplification using a pH-sensitive dye. However, the low buffering capacity required to generate the pink to yellow color change limits sample compatibility, as highly buffered sample inputs or acidic samples may impact the change. The WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix with UDG (NEB #M1708, NEB #E1708) or without UDG (NEB #E1700) is fully buffered and can more readily tolerate these types of sample inputs.

WarmStart Colorimetric LAMP 2X Master Mix with UDG

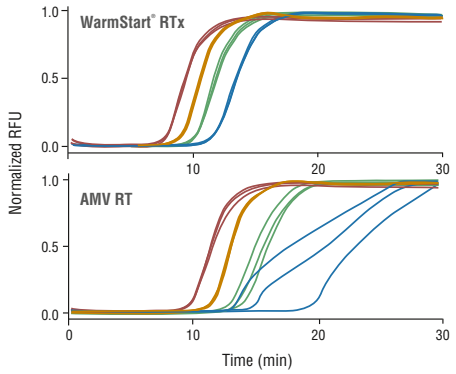
The WarmStart Colorimetric LAMP 2X Master Mix with UDG offers fast, clear, visible detection of amplification for either RNA or DNA targets.

mplification

WarmStart RTx Reverse Transcriptase

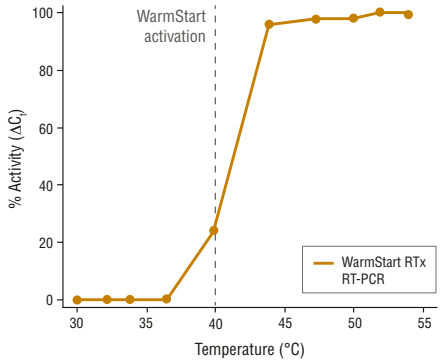
WarmStart RTx Reverse Transcriptase (NEB #M0380) is a unique *in silico*-designed, RNA-directed DNA polymerase coupled with a reversibly-bound aptamer that inhibits RTx activity below 40°C. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA (cDNA synthesis) or single-stranded DNA as a template. RTx is a robust enzyme for RNA detection in amplification reactions and is particularly well-suited for use in 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.

WarmStart improves speed and sensitivity in RT-LAMP



RT-LAMP reactions with Bst 2.0 WarmStart DNA Polymerase and the indicated reverse transcriptase were incubated at 65°C with 1 pg – 100 ng of Jurkat total RNA. Reactions were monitored with real-time fluorescence, and resulting curves are shown. WarmStart RTx provides faster reaction threshold times for improved consistancy and sensitivity with lower input RNA amounts. RT-LAMP reactions performed with AMV Reverse Transcriptase resulted in inconsistent detection, as indicated by wide variation at lower RNA input concentrations (blue curves).

WarmStart control of WarmStart RTx



cDNA synthesis was performed for 10 minutes, followed by qPCR analysis. Resulting Cts were normalized to a "no RT" control for 0% activity and fastest Ct for 100% activity. WarmStart RTx is inhibited by a reversibly bound aptamer at temperatures below 40°C, and is fully active at temperatures 42°C and higher.

Not sure which product will work best for your experiment?

NEB offers a selection of *Bst* DNA Polymerase-based products for isothermal amplification. Use this chart to determine which product will work best for your needs.

	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
***Bst* DNA Polymerase, Large Fragment**	N/A	★	N/A	★	★	• General strand-displacement reactions, original polymerase for LAMP and other diagnostic amplifications
***Bst* 2.0 DNA Polymerase**	N/A	★★	N/A	★★	★★	• Improved LAMP, SDA, and other amplification reactions
***Bst* 2.0 WarmStart DNA Polymerase**	N/A	★★	★★★	★★	★★	• Consistent, room-temperature, and high-throughput amplification assays
***Bst* 3.0 DNA Polymerase**	N/A	★★★	★★	★★★	★★★	• Engineered and fused to a novel nucleic acid binding domain • Fastest, most robust LAMP and RT-LAMP reactions • High reverse transcriptase activity up to 72°C

★★★ Optimal, recommended product for selected application
★★ Works well for selected application
★ Will perform selected application, but is not recommended
N/A Not applicable to this application

Choose from our selection of products

for your isothermal application.

PRODUCT	NEB #	SIZE
WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)	M1708S/L	100/500 reactions
WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG)	E1708S/L	100/500 reactions
WarmStart LAMP Kit (DNA & RNA)	E1700S/L	100/500 reactions
LAMP Fluorescent Dye	B1700S	0.25 ml
WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA)	M1800S/L	100/500 reactions
WarmStart Colorimetric LAMP 2X Master Mix with UDG	M1804S/L	100/500 reactions
SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit	E2019S	96 reactions
Bst 3.0 DNA Polymerase	M0374S/L/M	1,600/8,000/8,000 units
Bst 2.0 WarmStart DNA Polymerase	M0538S/L/M	1,600/8,000/8,000 units
Bst 2.0 DNA Polymerase	M0537S/L/M	1,600/8,000/8,000 units
Bst DNA Polymerase, Large Fragment	M0275S/L/M	1,600/8,000/8,000 units
Bst DNA Polymease, Full Length	M0328S	500 units
WarmStart RTx Reverse Transcriptase	M0380S/L	50/250 reactions
Nt.BstNBI	R0607S/L	1,000/5,000 units
COMPANION PRODUCTS		
Tte UvrD Helicase	M1202S	0.5 µg
AMV Reverse Transcriptase	M0277S/L	200/1,000 units
Deoxynucleotide (dNTP) Solution Mix	N0447S/L	8/40 µmol of each
Deoxynucleotide (dNTP) Solution Set	N0446S	25 µmol of each

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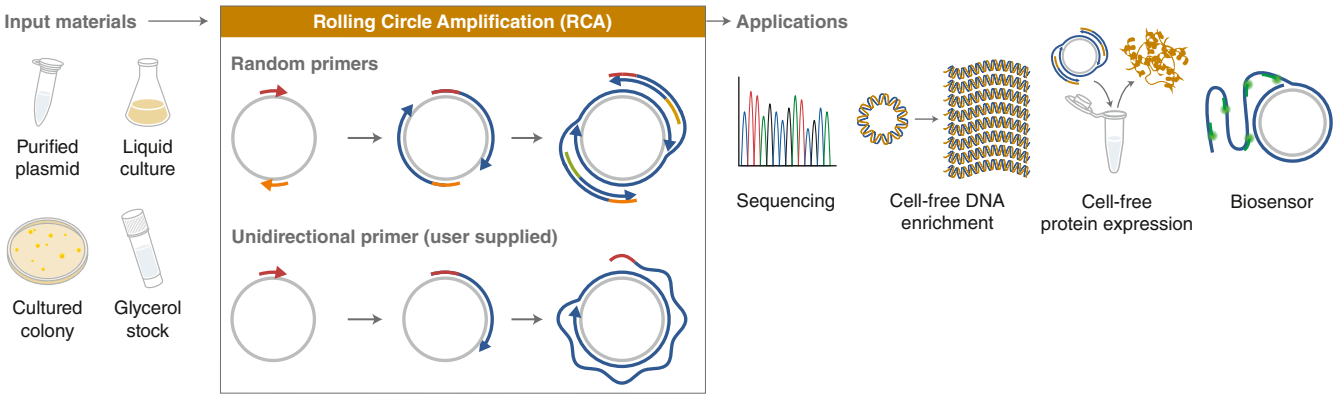
phi29-XT RCA Kit

Featuring phi29-XT DNA Polymerase, an engineered polymerase with improved thermostability, sensitivity, and capable of generating high product yield in a short reaction time, the phi29-XT RCA Kit includes everything needed for rolling circle amplification (RCA). Kit components include dNTPs and exonuclease-resistant random primers (containing phosphorothioate bonds) to universally amplify circular DNA sequences.

Overview of the phi29-XT RCA Kit



The phi29-XT RCA Kit (NEB #E1603) is a fast, simple to use and highly versatile kit containing all the required components for rolling circle amplification (RCA) using a random primer mix. The kit delivers high yields of DNA products from a variety of starting materials including purified circular DNA or bacterial cells. This kit is ideal for various DNA applications such as DNA sequencing, cell-free DNA enrichment, cell-free protein expression and DNA biosensors.

Benefits

- Amplify from as little as 1 fg of circular DNA input
- Flexible protocol offers compatibility with different types of input material
- Enables multiple downstream applications without further processing steps
- Kit includes dNTPs and exo-resistant random primers

View more technical data and recommended protocol at **www.neb.com/e1603**

03/23

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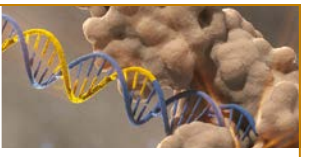
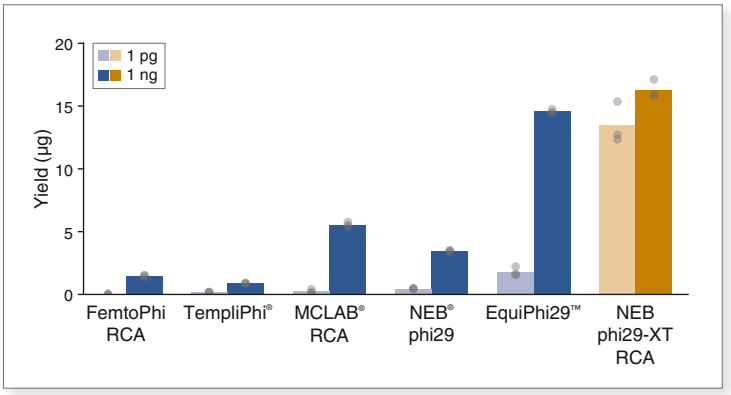
phi29-XT RCA Kit

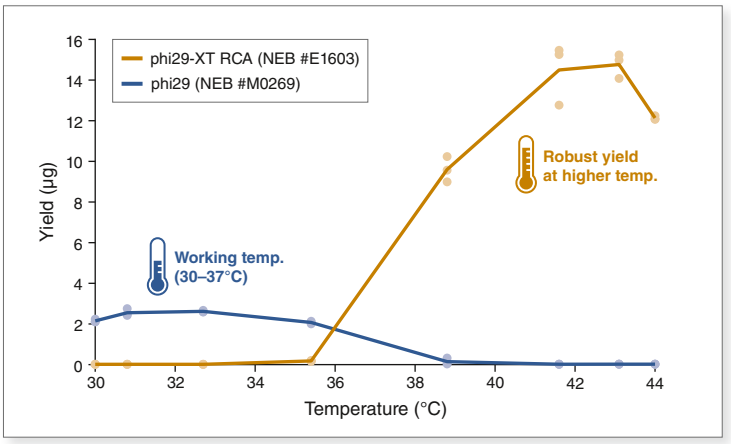


The phi29-XT RCA Kit offers exceptional sensitivity and product yield



Triplicate RCA reactions were carried out using commercially available phi29 DNA polymerases, according to manufacturers' protocols, for 2 hours with 1 pg or 1 ng pUC19 plasmid as the starting material. Reaction yields (dots) were quantified using Quant-iT® PicoGreen® dsDNA Reagent and averaged (bar). The phi29-XT RCA Kit (NEB #E1603) generates more product in less time than other commercially available products.

phi29-XT DNA Polymerase generates more product in less time than wild-type phi29 DNA Polymerase, at elevated temperatures



Triplicate RCA reactions were conducted with 0.1 pg pUC19 plasmid input (NEB #N3041) at the indicated temperatures for 2 hours, followed by heat inactivation at 65°C for 10 minutes. All reactions contained 1 mM dNTPs and 50 µM Exonuclease-Resistant Random Primers. Wild-type phi29 reactions were carried out with 10 units of phi29 DNA Polymerase (NEB #M0269) in 1X phi29 DNA Polymerase Reaction Buffer and 0.1 mg/mL Recombinant Albumin, and phi29-XT reactions were carried out with 1X phi29-XT DNA Polymerase and 1X phi29-XT Reaction Buffer. Reaction yields (dots) were quantified using Quant-iT PicoGreen dsDNA Reagent and were averaged (line) to determine the yield at each reaction temperature. While the working reaction temperature of wild-type phi29 DNA Polymerase is between 30°C and 37°C, phi29-XT DNA Polymerase generates robust product yields around 42°C.

Ordering Information

PRODUCT	NEB #	SIZE
phi29-XT RCA Kit	E1603S/L	100/500 reactions
COMPANION PRODUCTS		
NEBExpress® Cell-free *E. coli* Protein Synthesis System	E5360S/L	10/100 reactions
T7 Endonuclease I	M0302S/L	250/1,250 units
Monarch® DNA & PCR Cleanup Kit (5 µg)	T1030S/L	50/250 preps

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