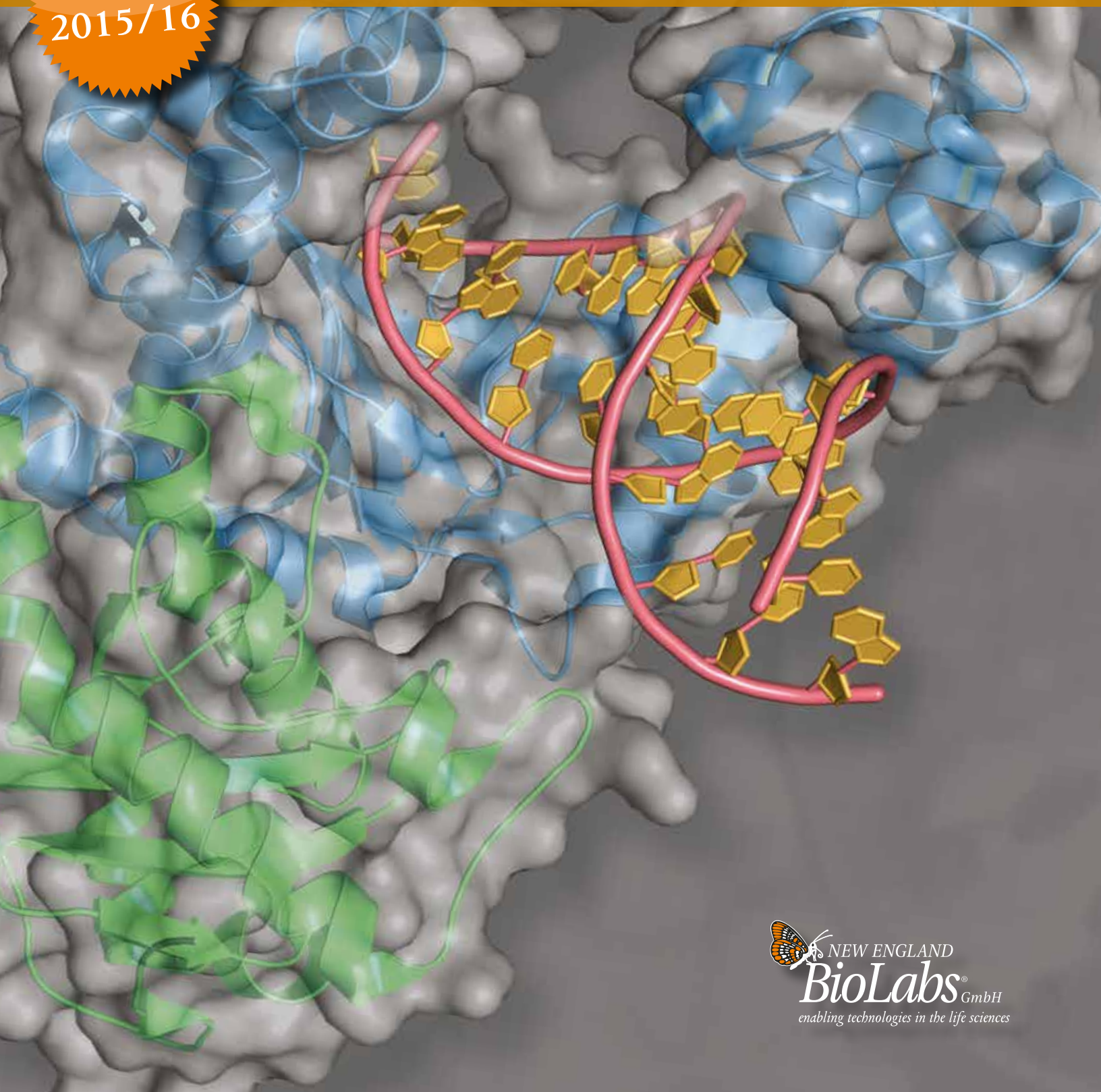
Isothermal DNA Amplification

ROBUST TECHNOLOGIES FOR RAPID NUCLEIC ACID DETECTION

Update 2015/16



NEW ENGLAND BioLabs® GmbH
enabling technologies in the life sciences

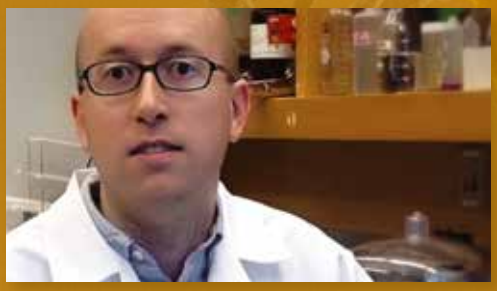
What is isothermal DNA amplification?

The Polymerase Chain Reaction (PCR) is a well-known approach for amplifying a specific DNA 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 diagnostic laboratory, there are other methods of sequence-specific DNA amplification.

These alternative approaches often do not require changing the reaction temperature and are, therefore, often 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.

Advantages

- Fast
- Minimal equipment required
- Robust reactions in the presence of inhibitors
- Simplified optical detection



Interested in learning how NEB scientists are using isothermal amplification in their research?

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

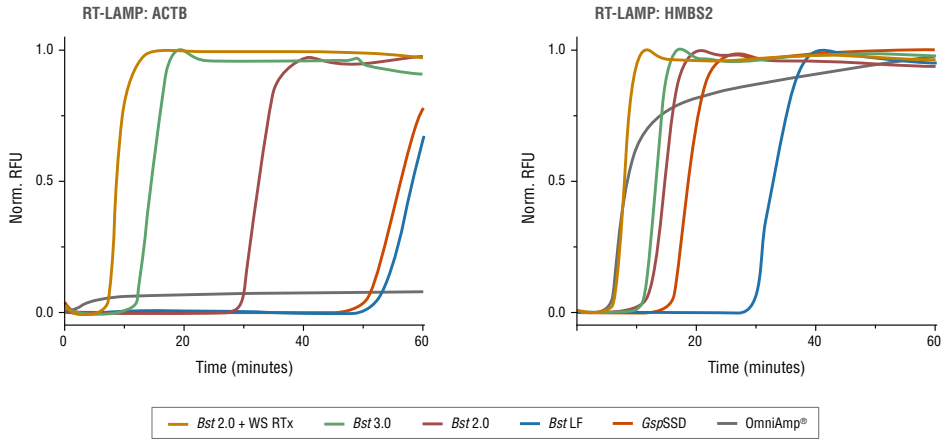
Featured Products for Isothermal

Bst 3.0 DNA Polymerase

Validated for LAMP

Bst 3.0 DNA Polymerase (NEB #M0374) is an *in silico* designed homolog of *Bacillus stearothermophilus* DNA Polymerase I, Large Fragment, (NEB #M0275) engineered for improved isothermal amplification performance and increased reverse transcription activity. *Bst* 3.0 contains 5´→ 3´ DNA polymerase activity with either DNA or RNA templates and strong strand displacement activity, but lacks 5´→ 3´ and 3´ → 5´ exonuclease activity. *Bst* 3.0 demonstrates robust performance even in high concentrations of amplification inhibitors and features significantly increased reverse transcriptase activity compared to *Bst* DNA Polymerase.

Fast, single-enzyme RT-LAMP can be performed using *Bst* 3.0



RT-LAMP was performed using indicated DNA polymerase and Jurkat total RNA and primers for two genes (ACTB, left; HMBS2, right). Fastest results were observed with a 2-enzyme system, Bst 2.0 and WarmStart RTx, but robust amplification was also observed using Bst 3.0 without additional RT. Bst LF, Bst 2.0 and competitor enzymes showed highly variable performance, with slow threshold times or reaction failure on one of the two targets.

Not sure which product will work best for your experiment?

NEB offers a selection of *Bst* DNA Polymerase-based products for isothermal DNA 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 Polymease, 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	★★★	★★	★★★	★★★	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

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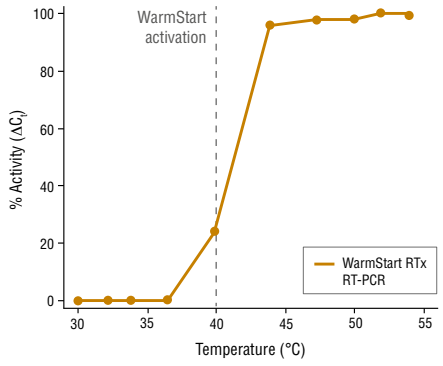
DNA Amplification from NEB

WarmStart RTx Reverse Transcriptase

☑ Validated for RT-LAMP

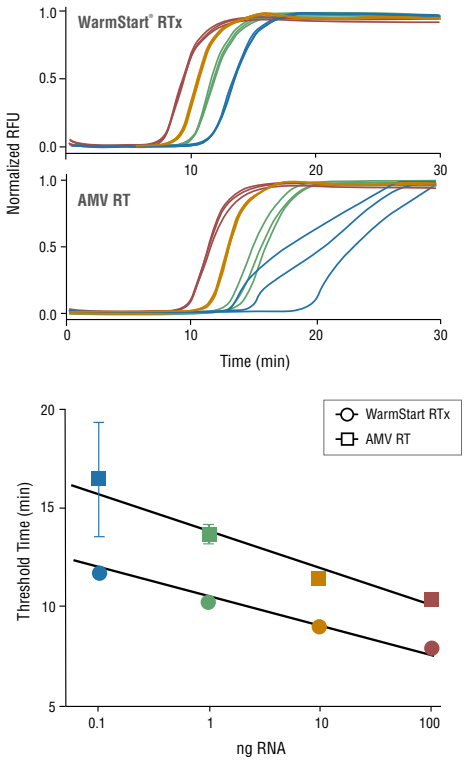
WarmStart RTx Reverse Transcriptase (NEB #M0380) is a unique *in silico* designed RNA-directed DNA polymerase coupled with a reversibly-bound aptamer that inhibits RTx activity below 40°C. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA (cDNA synthesis) or single-stranded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

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.

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 the resulting curves are shown on left, with the corresponding threshold times on right. 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).

What is WarmStart?

"WarmStart" is the term we use to describe a mesophilic enzyme that is inactive at room temperature, and activated when the reaction is warmed above approximately 40°C. NEB currently supplies two enzymes, *Bst* 2.0 DNA Polymerase (NEB #M0537) and WarmStart RTx Reverse Transcriptase (NEB #M0380), that have this property.

For room temperature reaction setup, such as in high-throughput workflows, we recommend pairing *Bst* 2.0 WarmStart with WarmStart RTx for RT-LAMP. The double WarmStart mixture ensures reproducibility.

Optimization tips for LAMP

- Use LAMP primer design software (e.g., Primer Explorer – primerexplorer.jp/e/). 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 prevent contamination, use *Bst* 3.0 or Antarctic Thermolabile UDG (NEB #M0372), which denatures rapidly

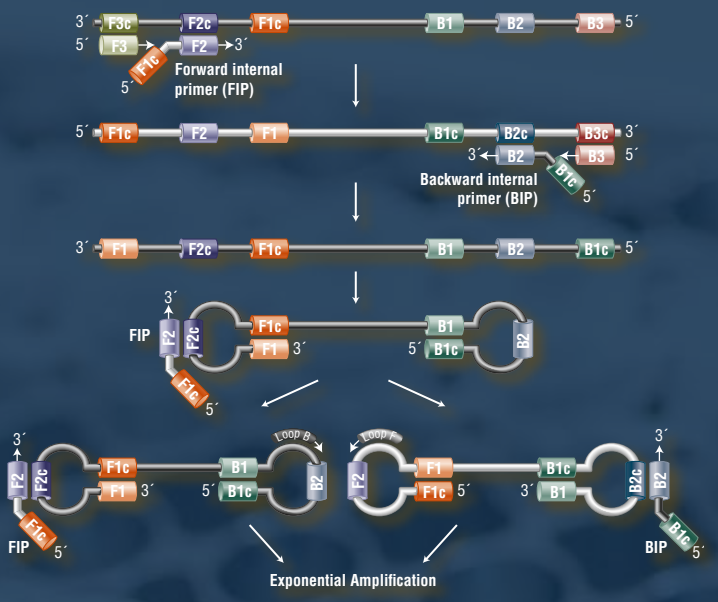
Examples of isothermal technologies

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

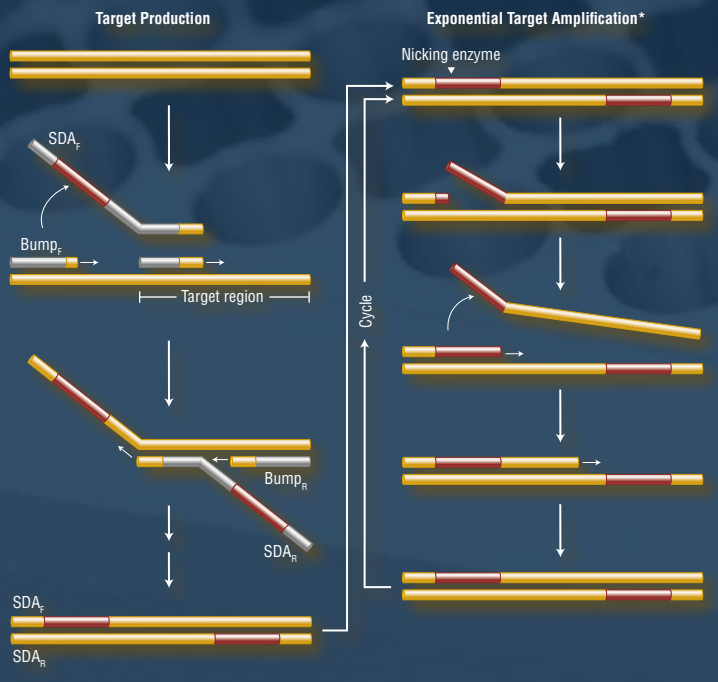
LAMP is designed to detect a target nucleic acid without sophisticated equipment. LAMP uses 4-6 primers recognizing 6-8 distinct regions of the target DNA. A strand-displacing DNA polymerase initiates synthesis and two of the primers form loop structures to facilitate subsequent rounds of amplification. LAMP provides high sensitivity (fg or <10 copies of target), and reactions can be performed in as little as 5–10 minutes. Additionally, reactions can be performed with limited resources (e.g., using a water bath for incubation and detection of results by eye), or with real-time measurement and high-throughput instruments.

Detection of RNA targets is accomplished by simple addition of a reverse transcriptase to the LAMP reaction (e.g., WarmStart® RTx Reverse Transcriptase), with RT-LAMP performed as a true one-step, isothermal workflow.

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 at nicks created by a strand-limited restriction endonuclease or 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.

Helicase-dependent Amplification (HDA)

HDA employs the double-stranded DNA unwinding activity of a helicase to separate strands, enabling primer annealing and extension by a strand-displacing DNA polymerase. Like PCR, this system requires only two primers. HDA has been employed in several diagnostic devices and FDA-approved tests.

Nicking Enzyme Amplification Reaction (NEAR)

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

Choose from our selection of products

For your isothermal DNA application.

PRODUCT	NEB #	SIZE
Bst 3.0 DNA Polymerase	M0374S/L/M	1,600/8,000/8,000 units
Bst 2.0 WarmStart DNA Polymerase	M0538S/M/L	1,600/8,000 units
Bst 2.0 DNA Polymerase	M0537S/M/L	1,600/8,000 units
Bst DNA Polymerase, Large Fragment	M0275S/M/L	1,600/8,000 units
Bst DNA Polymease, Full Length	M0328S/L	500/2,500 units
WarmStart RTx Reverse Transcriptase	M0380S/L	50/250 reactions
Nt.BstNBI	R0607S/L	1,000/5,000 units
IsoAmp® II Universal tHDA Kit	H0110S	50 reactions
AMV Reverse Transcriptase	M0277S/T/L	200/500/1,000 units
Antarctic Thermolabile UDG	M0372S/L	100/500 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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