

# Isothermal Amplification

RAPID NUCLEIC ACID DETECTION FOR MOLECULAR DIAGNOSTICS

**FREE**  
Next Day  
Delivery  
[www.neb.uk.com](http://www.neb.uk.com)



# 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](http://www.neb.com/isoamp) to find videos, protocols and recent publications, including a publication from NEB scientists describing pH-sensitive isothermal detection.

Did you know that many of these products can be purchased in larger volumes? Contact [custom.uk@neb.com](mailto:custom.uk@neb.com) to find out more.

## 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)

DOWNLOAD THE NEB AR APP\*

How is colorimetric LAMP used in point of care?



\*see back cover for details

# Featured products for isothermal a

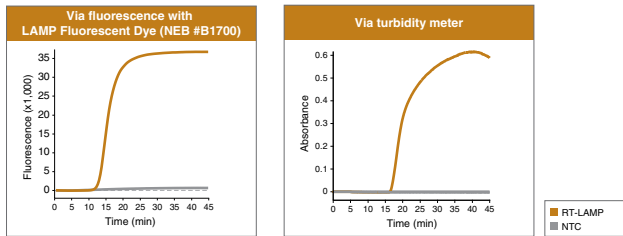
## WarmStart® Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) (NEB #M1708)

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

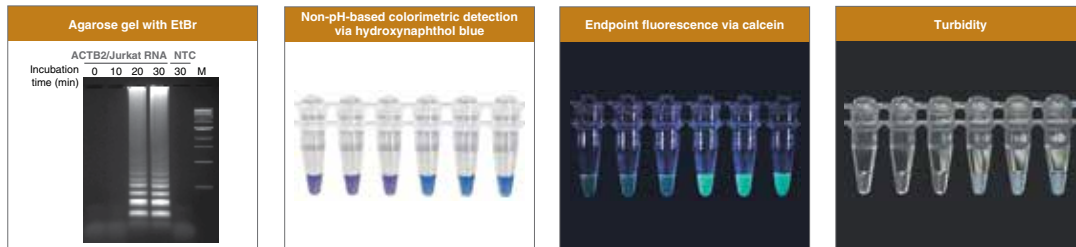
turbidity detection, real-time fluorescence detection, and end-point visualization such as colorimetric detection via a metal indicator (e.g., hydroxynaphthol blue). It features *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx Reverse Transcriptase, both *in silico*-designed enzymes for improved 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.



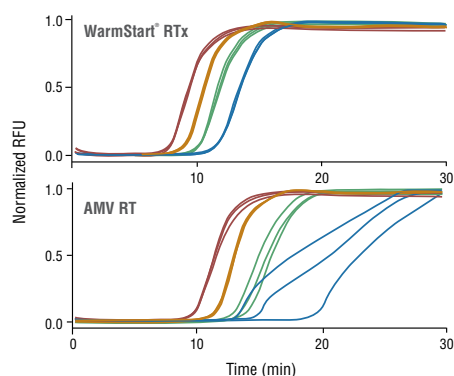
# Amplification

## 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

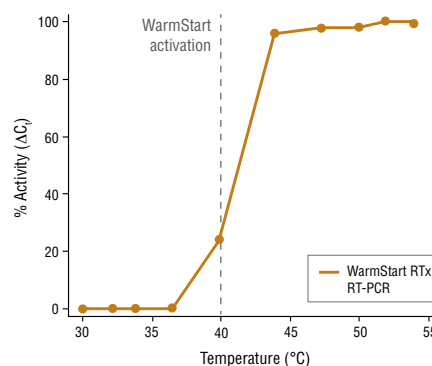
a template. RTx is a robust enzyme for RNA detection in amplification reactions and is particularly well-suited for use in LAMP. The WarmStart property enables high throughput applications, room temperature setup, and increases the consistency and specificity of amplification reactions. RTx contains 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 consistency 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 Ct's 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 ACTIVITY	INHIBITOR TOLERANCE	APPLICATIONS
<i>Bst</i> DNA Polymerase, Full Length	★★	N/A	N/A	N/A	★	<ul style="list-style-type: none"> <li>Nick translation reactions at elevated temperatures</li> </ul>
<i>Bst</i> DNA Polymerase, Large Fragment	N/A	★	N/A	★	★	<ul style="list-style-type: none"> <li>General strand-displacement reactions, original polymerase for LAMP and other diagnostic amplifications</li> </ul>
<i>Bst</i> 2.0 DNA Polymerase	N/A	★★	N/A	★★	★★	<ul style="list-style-type: none"> <li>Improved LAMP, SDA, and other amplification reactions</li> </ul>
<i>Bst</i> 2.0 WarmStart DNA Polymerase	N/A	★★	★★★	★★	★★	<ul style="list-style-type: none"> <li>Consistent, room-temperature, and high-throughput amplification assays</li> </ul>
<i>Bst</i> 3.0 DNA Polymerase	N/A	★★★	★★	★★★★	★★★★	<ul style="list-style-type: none"> <li>Engineered and fused to a novel nucleic acid binding domain</li> <li>Fastest, most robust LAMP and RT-LAMP reactions</li> <li>High reverse transcriptase activity up to 72°C</li> </ul>

- ★★★★ 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

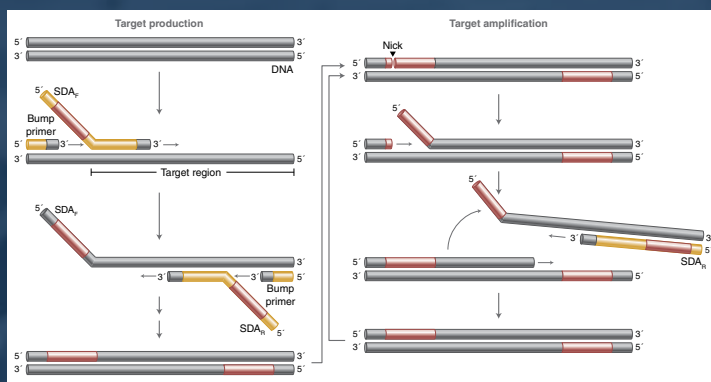


# Examples of isothermal technologies

## 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 concatemers. 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 SDA

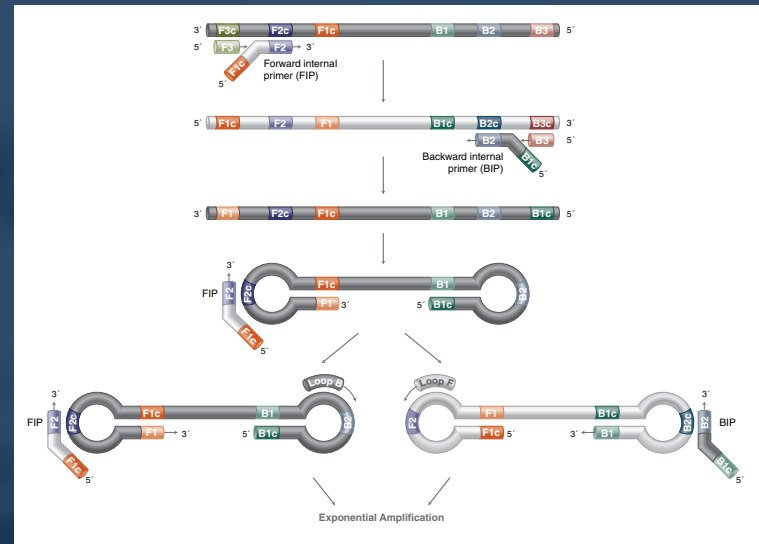


\* Target amplification, shown above for SDA<sub>p</sub>, will also occur simultaneously with SDA<sub>r</sub>.

## 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.

### Overview of LAMP



## 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.

View our  
loop mediated isothermal  
amplification tutorial.



# 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
<i>Bst</i> 3.0 DNA Polymerase	M0374S/L/M	1,600/8,000/8,000 units
<i>Bst</i> 2.0 WarmStart DNA Polymerase	M0538S/L/M	1,600/8,000/8,000 units
<i>Bst</i> 2.0 DNA Polymerase	M0537S/L/M	1,600/8,000/8,000 units
<i>Bst</i> DNA Polymerase, Large Fragment	M0275S/L/M	1,600/8,000/8,000 units
<i>Bst</i> DNA Polymerase, Full Length	M0328S	500 units
WarmStart RTx Reverse Transcriptase	M0380S/L	50/250 reactions
Nt.BstNBI	R0607S/L	1,000/5,000 units
<b>COMPANION PRODUCTS</b>		
<i>Tte</i> 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

One or more of these products are covered by patents, trademarks and/or copyrights owned or controlled by New England Biolabs, Inc. For more information, please email us at [busdev@neb.com](mailto:busdev@neb.com). The use of these products may require you to obtain additional third party intellectual property rights for certain applications.

Your purchase, acceptance, and/or payment of and for NEB's products is pursuant to NEB's Terms of Sale at <https://www.neb.uk.com/terms-conditions>. NEB does not agree to and is not bound by any other terms or conditions, unless those terms and conditions have been expressly agreed to in writing by a duly authorized officer of NEB.

Licensed under U.S. Patents Nos. U.S. 6,410,278; 6,974,670; 7,494,790; 8,017,357; 7,374,913; 7,851,186; and 7,846,695 including all foreign counterparts thereof. The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased product to perform loop-mediated isothermal amplification ("LAMP") for Research Use Only. The purchase of this product further conveys to the purchaser the limited, non-transferable right to use the purchased product to perform reverse transcription loop-mediated isothermal amplification ("RT-LAMP") for Research Use Only. LAMP is a patented technology belonging to Eiken Chemical Co., Ltd and any use other than research may require a license from Eiken Chemical Co., Ltd. Notice to Purchaser: Nucleic-acid based aptamers for use with thermophilic DNA polymerases are licensed exclusively by New England Biolabs, Inc. from Somalogic, Inc. New England Biolabs, Inc. gives the Buyer/User a non-exclusive license to use the aptamer-based RT-LAMP Master Mix for Research Purposes Only. Commercial use of the aptamer-based RT-LAMP Master Mix requires a license from New England Biolabs, Inc. Please contact [busdev@neb.com](mailto:busdev@neb.com) for more information.

The purchase of NEB *Bst* products conveys to the purchaser the limited, non-transferable right to use the purchased products to perform loop-mediated isothermal amplification ("LAMP") for research use only. LAMP is a patented technology belonging to Eiken Chemical Co., Ltd. and any use other than research may require a license from Eiken Chemical Co., Ltd.

The purchase of NEB RTx products conveys to the purchaser the limited, non-transferable right to use the purchased products to perform reverse transcription loop-mediated isothermal amplification ("RT-LAMP") for research use only. LAMP is a patented technology belonging to Eiken Chemical Co., Ltd. and any use other than research may require a license from Eiken Chemical Co., Ltd. This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

© Copyright 2021, New England Biolabs, Inc.; all rights reserved



Download the NEB AR App for iPhone® or iPad®. Scan the augmented reality butterfly icon located on the corner of the page to find videos, tutorials and immersive experiences.



## USA

New England Biolabs, Inc.  
Telephone (978) 927-5054  
Toll Free (USA Orders) 1-800-632-5227  
Toll Free (USA Tech) 1-800-632-7799  
Fax (978) 921-1350  
e-mail: [info@neb.com](mailto:info@neb.com)  
[www.neb.com](http://www.neb.com)

## New England Biolabs (UK) Ltd

75-77 Knowl Piece  
Wilbury Way  
Hitchin  
Herts  
SG4 0TY

Free Phone (General & Orders): 0800 318486  
Free Phone (Technical Support): 0800 6522890  
Free Phone (Accounts): 0800 6522891

Free Fax: 0800 435682

Email (General): [info.uk@neb.com](mailto:info.uk@neb.com)  
Email (Orders): [orders.uk@neb.com](mailto:orders.uk@neb.com)  
Email (Technical Support): [techsupport.uk@neb.com](mailto:techsupport.uk@neb.com)  
Email (Accounts): [accounts.uk@neb.com](mailto:accounts.uk@neb.com)

For UK prices visit:  
[www.neb.uk.com](http://www.neb.uk.com)



**Climate neutral**  
Print product

[ClimatePartner.com/12759-1804-1001](https://ClimatePartner.com/12759-1804-1001)

[www.neb.uk.com](http://www.neb.uk.com)



Printed on 100% recycled paper using vegetable based ink.

ISO\_AMP\_TRI – Version 3 – 08/21