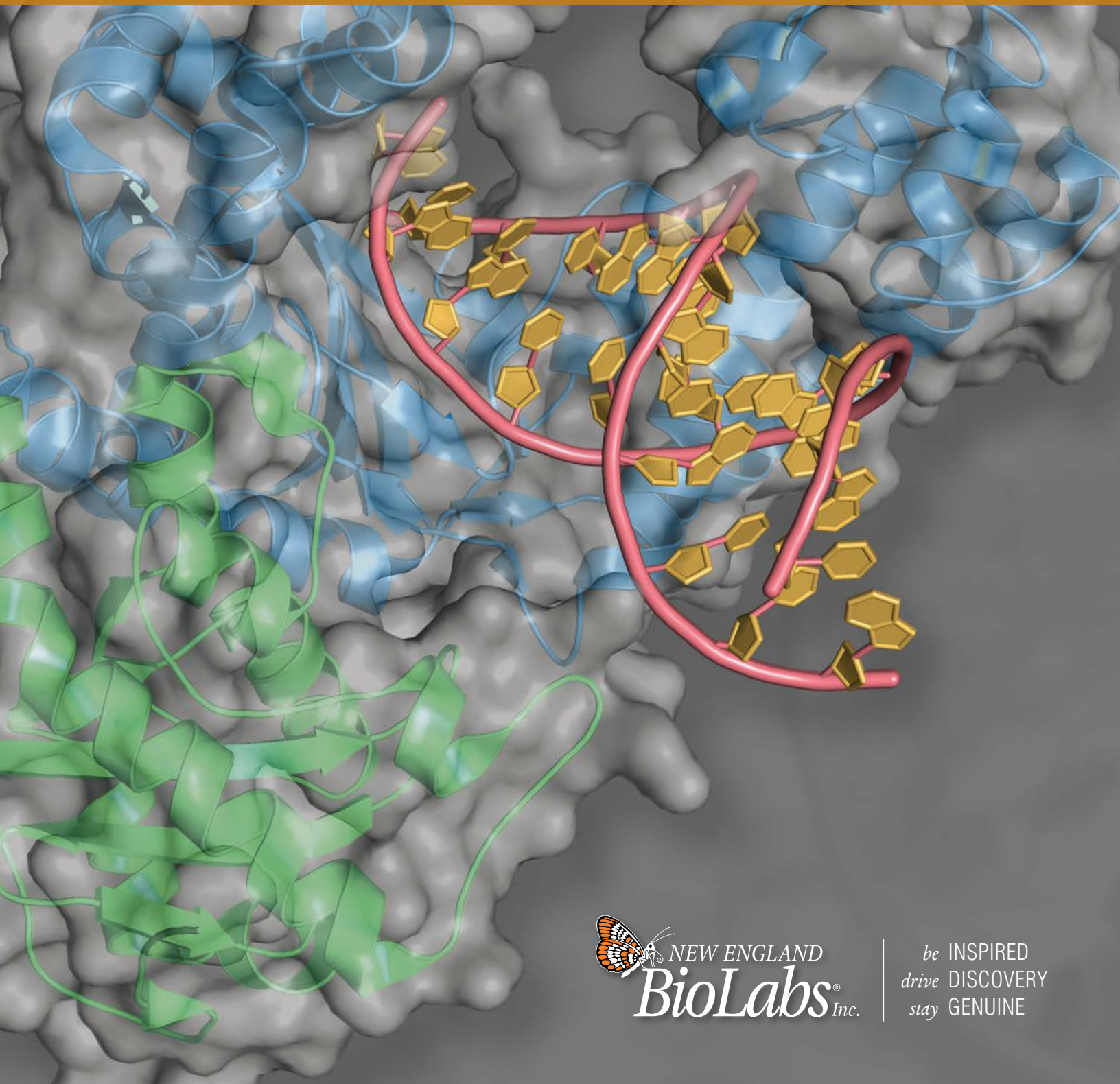


# Isothermal Amplification

RAPID NUCLEIC ACID DETECTION FOR MOLECULAR DIAGNOSTICS



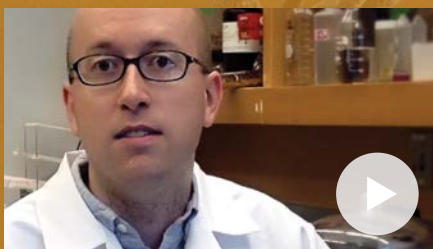
*be* INSPIRED  
*drive* DISCOVERY  
*stay* GENUINE



# 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 diagnostics 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.



## Interested in learning how NEB scientists are using isothermal amplification?

Visit [www.neb.com/IsothermalAmplification](http://www.neb.com/IsothermalAmplification) 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
- Simplified optical detection

## Optimization tips for LAMP

- Use LAMP primer design software (e.g., Primer Explorer – [primerexplorer.jp/e/](http://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



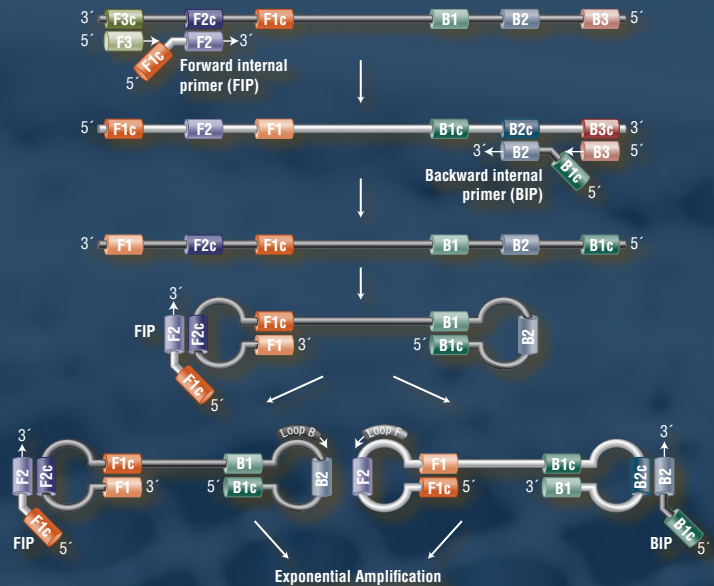
# Examples of isothermal technologies

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

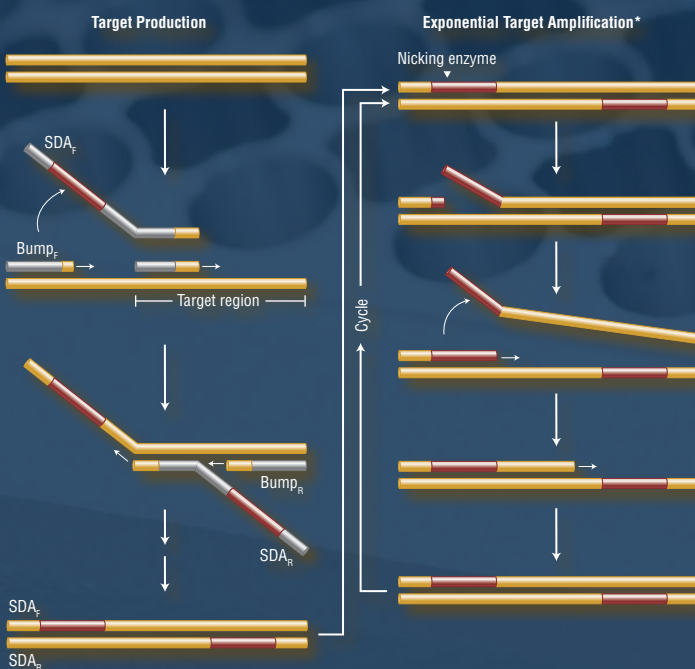
LAMP is designed to detect a target nucleic acid without sophisticated equipment. LAMP uses 4-6 primers recognizing 6-8 distinct regions of the target DNA. A strand-displacing DNA polymerase initiates synthesis and two of the primers form loop structures to facilitate subsequent rounds of amplification. LAMP provides high sensitivity (fg levels 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<sub>F</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.

## 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 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.

# Featured Products for Isothermal

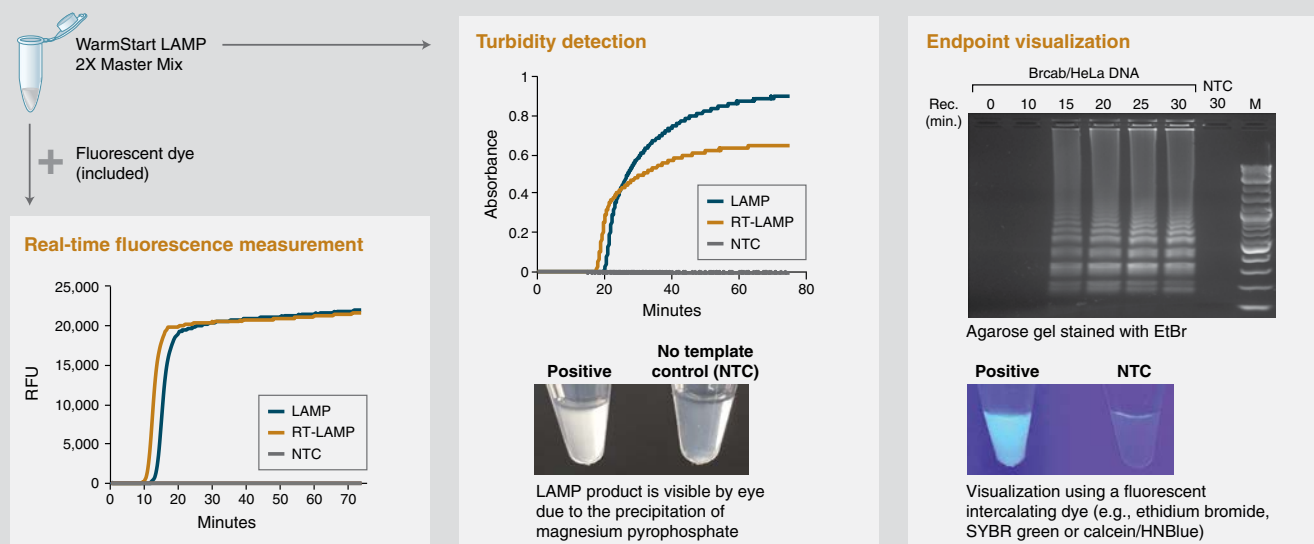
## WarmStart LAMP KIT (DNA & RNA)

✓ Validated for LAMP & RT-LAMP

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 master mix supplied with the WarmStart LAMP Kit contains the robust and rapid *Bst* 2.0 WarmStart DNA Polymerase and WarmStart

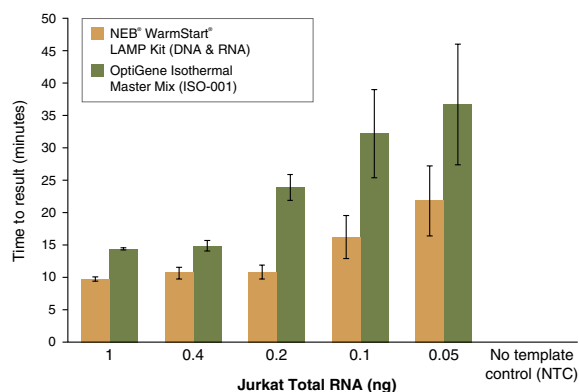
RTx Reverse Transcriptase, both *in silico*-designed enzymes for improved performance in LAMP reactions. The kit also includes a fluorescent dye to enable real-time fluorescence measurement of LAMP. The WarmStart LAMP Kit is compatible with multiple detection methods.

NEB's WarmStart LAMP Kit (DNA & RNA) is compatible with multiple detection methods\*



\* The NEB WarmStart LAMP Kit (DNA & RNA) includes separate fluorescent dye for real-time fluorescence measurement. Alternately, detection can be accomplished by turbidity detection or endpoint visualization.

### NEB's WarmStart LAMP Kit (DNA & RNA) offers speed and robust sensitivity



A RNA target (HMB2) was amplified from Jurkat total RNA using the WarmStart LAMP Kit and OptiGene Master Mix (ISO-001). Reactions were performed at 65°C for 74 minutes on a real-time thermocycler (Bio-Rad CFX96) in triplicate. Time to result is set as the time at which the fluorescence crossed a threshold of 10% of maximal fluorescence. NEB's WarmStart LAMP Kit resulted in faster and more sensitive detection as compared to the OptiGene Master Mix.

## WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA)

NEB's WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA) offers the same robust performance as the WarmStart LAMP Kit, but contains a colorimetric dye for best in class visual detection of your target.



# Amplification from NEB

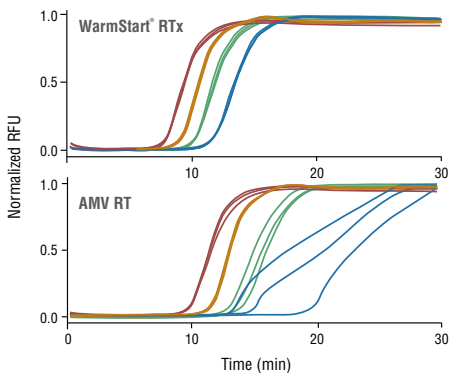
## WarmStart RTx Reverse Transcriptase

 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

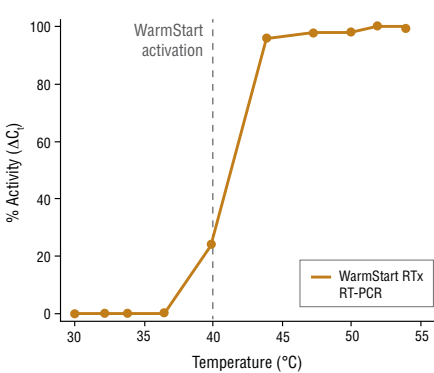
amplification reactions and is particularly well-suited for use in loop-mediated isothermal amplification (LAMP). The WarmStart property enables high throughput applications, room temperature setup, and increases the consistency and specificity of amplification reactions. RTx contains intact RNase H activity.

WarmStart 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 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 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 ACTIVITY	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	★★★	★★	★★★	★★★	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 LAMP KIT (DNA & RNA)	E1700S/L	100/500 reactions
WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA)	M1800S/L	100/500 reactions
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
COMPANION PRODUCTS		
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

ISOAMP® is a registered trademark of BioHelix Corporation. The IsoAmp® II Universal tHDA Kit was developed and produced by BioHelix Corporation, now a wholly owned subsidiary of Quidel Corporation.

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The purchase of NEB Bst products conveys to the purchaser the limited, nontransferable 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.

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## Interested in tips and tricks for PCR amplification?

Sign up for our PCR eNewsletter.



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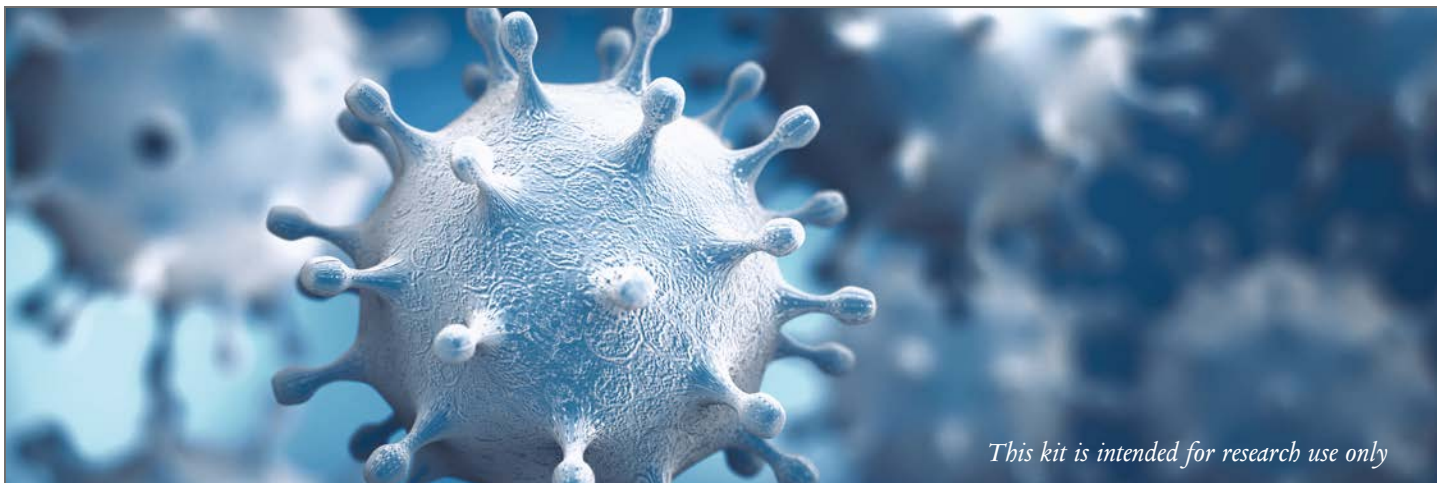
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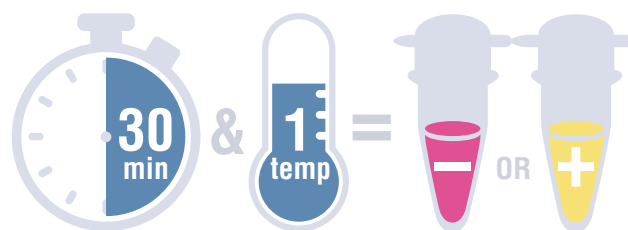
*This kit is intended for research use only*

# SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit

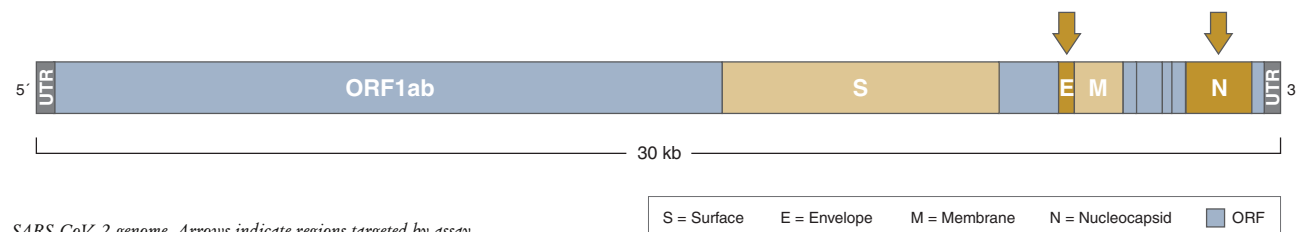
The SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit utilizes loop-mediated isothermal amplification for use in the analysis of SARS-CoV-2, the novel coronavirus that causes COVID-19.

## Highlights

- 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



The kit 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 shown to increase the speed and sensitivity of the RT-LAMP reaction and is also included.



For more details, visit [www.neb.com/E2019](http://www.neb.com/E2019)



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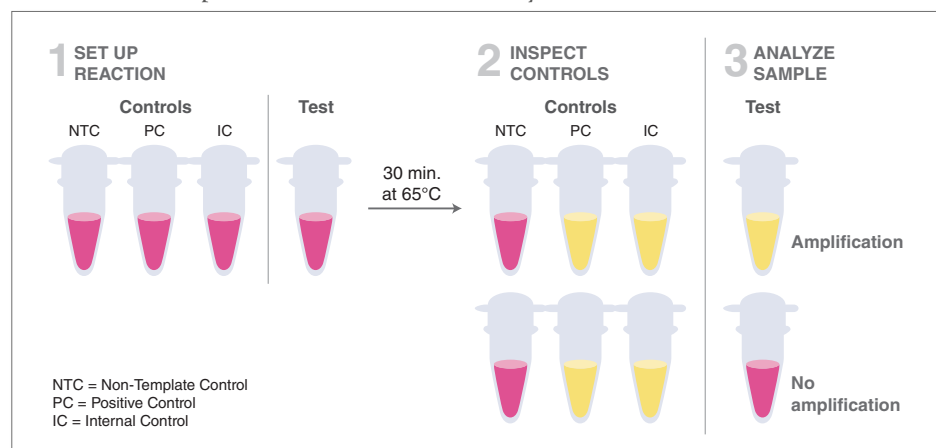
# SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit



WarmStart Colorimetric LAMP 2X Master Mix with UDG is an optimized formulation of *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx in a special low-buffer reaction solution containing a visible pH indicator for rapid and easy detection of LAMP and RT-LAMP reactions. The inclusion of dUTP and UDG in the master mix reduces the possibility of carryover contamination between reactions.

This system provides a fast, clear visual detection of amplification, based on the generation of protons and subsequent drop in pH that occurs from the extensive DNA polymerase activity in a LAMP reaction. The decrease in pH produces a color change from pink to yellow. The reaction requires only a heated chamber and samples, with a run time of 30 minutes.

## SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit workflow



## Ordering Information

PRODUCT	NEB #	SIZE	PRICE
SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit	E2019S	96 reactions	698 €
COMPONENT SOLD SEPARATELY			
WarmStart Colorimetric LAMP 2X Master Mix with UDG	M1804S/L	100/500 reactions	270 € / 1080 €

Learn more about how NEB is supporting COVID-19 research at [www.neb.com/COVID19](http://www.neb.com/COVID19)

This product is not available for sale in China (including Hong Kong and Macau), Japan and Taiwan. For questions, please contact [busdev@neb.com](mailto:busdev@neb.com).

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Nucleic acid-based aptamers for use with thermophilic DNA polymerases and Reverse Transcriptase are licensed exclusively by New England Biolabs, Inc. from SomaLogic, Inc. (US Patent Nos. 7,947,447; 8,404,830; 8,975,388). New England Biolabs, Inc. gives the purchaser a non-exclusive license to the aptamer-based WarmStart® Colorimetric LAMP 2X Master Mix with UDG for research and in vitro diagnostic tests for the detection of severe acute respiratory syndrome coronavirus, SARS-CoV-2.

**This product is not approved by the US Food and Drug Administration, or any foreign equivalent, for diagnostic use. It is the purchaser's responsibility to ensure it has appropriate authorization for its particular use.**

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