Amplification Reagents for Molecular Diagnostics Applications

Nathan A. Tanner, Andrew N. Gray, Nicole M. Nichols
New England Biolabs, Ipswich, MA 01938, USA

INTRODUCTION

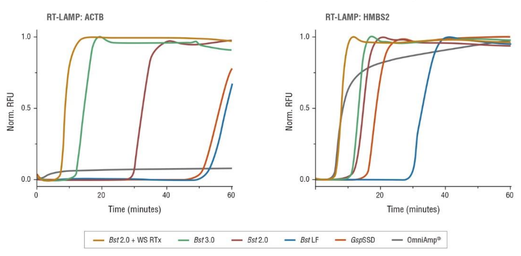
Molecular diagnostic methods to detect DNA and RNA targets represent a significant and growing focus for point-of-care and rapid testing. Though varied in application, design, and even mechanism, these methods all rely on enzymes to carry out the reactions. NEB has long provided reagents and enzymes to enable molecular biology research, including providing reliable and novel materials for molecular diagnostic applications. Isothermal amplification methods have emerged as a promising option for reliable point-of-care diagnostics and our research and development on these techniques have produced novel and more versatile isothermal DNA polymerases for faster and robust amplification; WarmStart® technology to permit room-temperature reaction set-up and consistent performance; and colorimetric LAMP detection for field and point-of-care applications. For customization of methods and use, reagents are tested for stability in glycerol-free formats and compatibility with lyophilization. And to enable a wide range of intended applications, we have developed reagents that support the next generation of tests in isothermal (SDA, NASBA, LAMP) and PCR or qPCR methods. Developing better reagents for molecular diagnostics is essential to broader adoption and reach of these powerful tools.

ISOTHERMAL AMPLIFICATION REAGENTS

Isothermal Polymerases

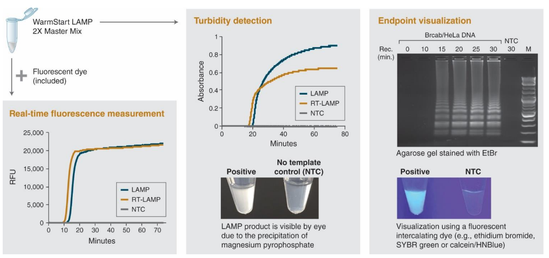
	5′ → 3′ EXO ACTIVITY	AMPLIFICATION SPEED	ROOM TEMPERATURE SETUP	REVERSE TRANSCRIPTASE ACTIVITY	INHIBITOR TOLERANCE	APPLICATIONS
Bst DNA Polymease, Full Length	**	N/A	N/A	N/A	*	Nick translation reactions at elevated temperatures
Bst DNA Polymerase, Large Fragment	N/A	*	N/A	*	*	General strand-displacement 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

Properties of available Bst DNA polymerase variants. The full-length form is not commonly used for isothermal amplification, but truncation to remove 5′-3′ exonuclease confers the desired strand displacement activity.

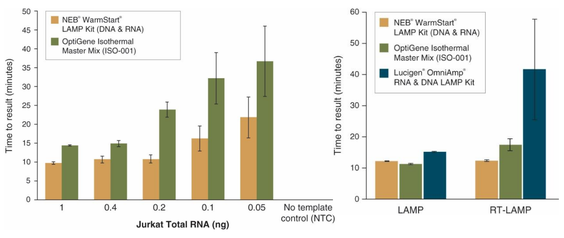


RT-LAMP was performed using indicated DNA polymerase, Jurkat RNA and primers. Fastest results were observed with a 2-enzyme system (Bst 2.0 + RTx), but robust amplification was also observed using Bst 3.0 alone. Bst LF, Bst 2.0, and competitor enzymes showed highly variable amplification performance.

WarmStart LAMP Kit



The WarmStart LAMP Kit (DNA & RNA) is designed to provide a simple, one-step solution for LAMP with DNA or RNA targets. This kit is supplied with WarmStart LAMP 2X Master Mix, containing a blend of Bst 2.0 WarmStart DNA Polymerase and WarmStart RTx Reverse Transcriptase, and a fluorescent dye if desired for real-time detection of amplification. The WarmStart LAMP Kit is compatible with multiple detections methods, including turbidity detection, real-time fluorescence, and end-point methods.



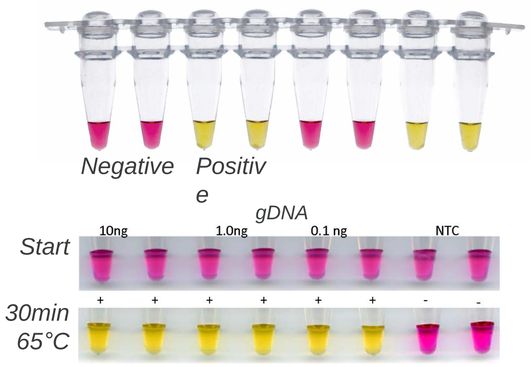
LAMP and RT-LAMP reactions were conducted using DNA (BRCA, Jurkat gDNA) or RNA (HMBS, Jurkat total RNA) targets, measured using real-time fluorescence detection. The WarmStart LAMP Kit provides a fast, sensitive LAMP detection for both DNA and RNA targets and offers similar reaction speed for either DNA or RNA inputs.

The key enzyme that drives isothermal amplification reactions is the Large Fragment of *Bst* DNA Polymerase I, offering the requisite strand displacement activity for separating double-stranded DNA and high activity at 65°C. NEB has developed engineered *Bst* variants to offer improved performance. *Bst* 2.0 provides faster, more robust isothermal reactions while *Bst* 3.0 again increases reaction speed with high reverse transcriptase activity and inhibitor tolerance.

Companion enzymes

- For reactions at lower temperatures (25–42°C), enzymes such as *Bsu* DNA polymerase, large fragment and Klenow exo- are typically the best options.
- WarmStart RTx for RNA targets
- Nt.BstNBI and other nicking enzymes for nicking-based applications (SDA, NEAR)
- Antarctic Thermolabile UDG for carryover contamination in LAMP (with *Bst* 2.0)

WarmStart Colorimetric LAMP Master Mix

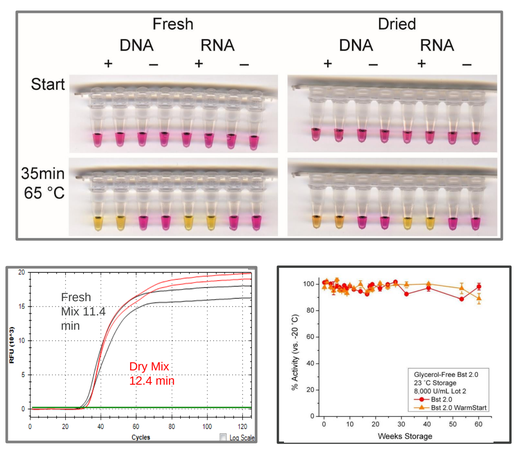


The WarmStart Colorimetric LAMP Master Mix is an optimized formulation of Bst 2.0 WarmStart and WarmStart RTx in a special low-buffer reaction solution containing a pH indicator for rapid and easy detection of LAMP and RT-LAMP reactions. This system is designed to provide a fast, clear visual detection 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. This detection requires only LAMP primers, sample, and heating to 65°C with the readout of positive amplification judged by eye in 15–40 minutes.

- Fast, sensitive DNA and RNA detection
- Dual WarmStart formulation for room-temperature setup and consistent detection
- Clear visual detection for field, point-of-care applications
- Available in glycerol-free, lyophilization-compatible formats

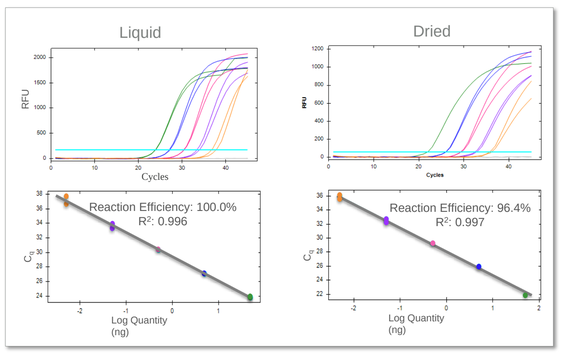
GLYCEROL-FREE AND LYOPHILIZATION

Isothermal Reagents



Isothermal and LAMP polymerases (*Bst* 2.0, *Bst* 3.0, WarmStart RTx) are stable and available in glycerol-free format. Both LAMP Master Mix formulations can be prepared glycerol-free and are compatible with lyophilization.

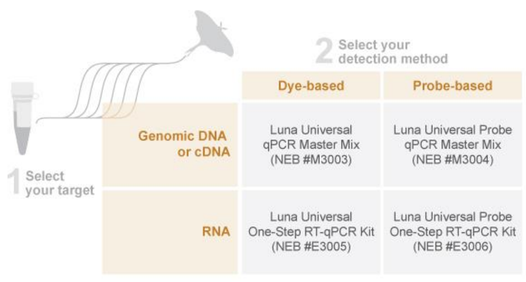
Luna qPCR

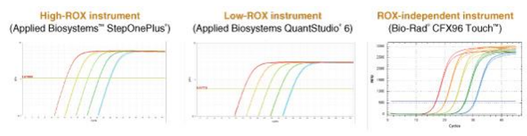


We have prepared lyophilization-compatible versions of the Luna qPCR Master Mix. Additives in the standard versions of the mix provide for maximum performance and efficiency, but for applications requiring freeze-drying a lyo-friendly version of the HotStart *Taq*-containing Master Mix can be made and successfully lyophilized without sacrificing performance.

Luna® UNIVERSAL qPCR REAGENTS

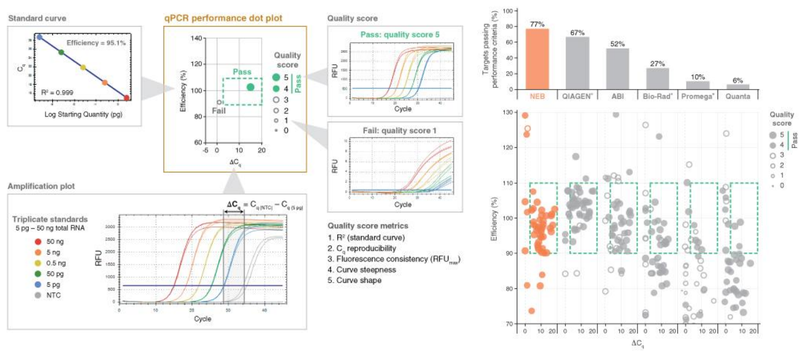
Streamlined Portfolio





- Universal reference dye provides instrument compatibility
- DNA or RNA, Dye or Probe: 4 formulations to support all qPCR applications
- Inert blue dye for easy visualization of plate loading

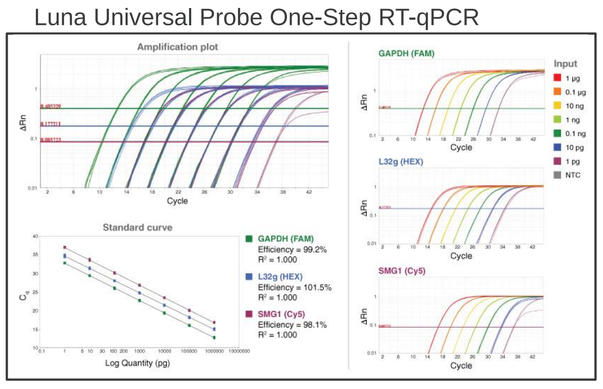
Best-in-class Performance



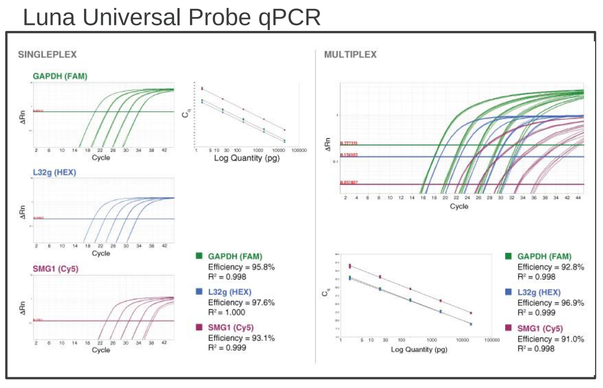
- Quantitative, high-throughput testing used to analyze and optimize performance
- Comprehensive comparisons to ensure consistency, sensitivity
- Tested using large target database, variety of sample types and sources

Robust Multiplexing

Luna Universal Probe One-Step RT-qPCR



Luna Universal Probe qPCR

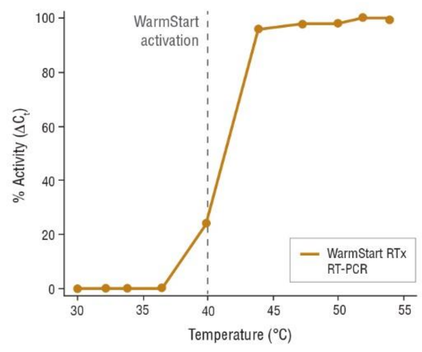


- Maintain performance of individual (singleplex) amplicon in multiplex format
- High efficiency with 3–, 4–plex reactions and standard fluorophore probes
- Can optimize primer/probe concentrations for significant copy number differences

WarmStart® ENZYME CONTROL

Standard HotStart modifications used for PCR reagents require activation at high temperature, making them incompatible with mesophilic enzymes (e.g. reverse transcriptases) and isothermal amplification. NEB has built the capacity for selection and development of modified nucleic acid SOMAmer® temperature-sensitive reversible inhibitors. Through selection temperature and SOMAmer modifications we can control the inhibition and activation temperatures for an enzyme of interest to enable WarmStart control at moderate temperature ranges (37–55°C).

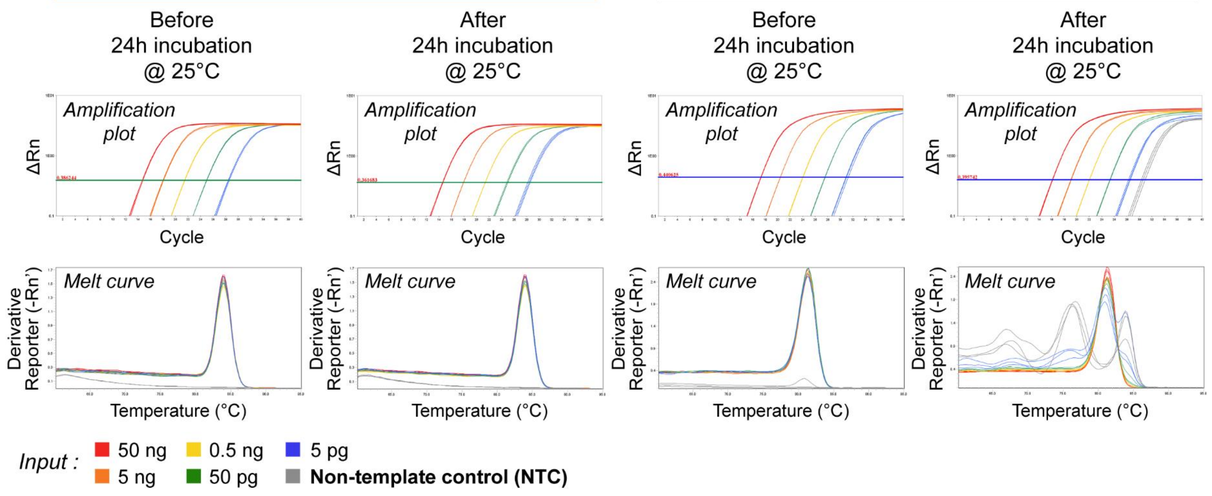
WarmStart RTx Temperature Profile



Better Control, Better Results: Luna WarmStart Reverse Transcriptase

NEB Luna Universal One-Step RT-qPCR Kit *featuring Luna WarmStart Reverse Transcriptase*

ABI Power SYBR Green 1-Step Kit *featuring ArrayScript™ UP Reverse Transcriptase*



Use of a dual-WarmStart/HotStart enzyme formulation (Luna WarmStart RT, HotStart *Taq*) provides maximum control of enzyme activity and avoidance of spurious amplification. Unique to NEB reagents, WarmStart control of reverse transcriptase and mesophilic polymerases brings the diagnostic specificity and consistency of HotStart enzymes to RNA detection and isothermal amplification, enabling room-temperature setup and high-throughput applications for a wider range of amplification methods.

WarmStart Enzymes

- *Bst* 2.0 WarmStart
- WarmStart RTx
- WarmStart LAMP Kit, Colorimetric LAMP Master Mix
- Luna WarmStart Reverse Transcriptase
- Luna Universal One-Step RT-qPCR

Aptamer-Controlled HotStart Enzymes

- Hot Start *Taq* DNA Polymerase
- OneTaq® Hot Start
- LongAmp® Hot Start
- Q5® Hot Start DNA Polymerase
- Luna Universal qPCR

SUMMARY

- Engineering of fast, robust diagnostic amplification reagents
- Variety of reagents to support Isothermal and PCR/qPCR methods
- Glycerol-free formats for lyophilization
- Unique WarmStart chemistry for maximum control and performance
- *Contact NEB for custom formulations and products, GMP capabilities*