INSTRUCTION MANUAL

WarmStart® LAMP Kit (DNA & RNA)

NEB #E1700S/L

100/500 reactions

Version 4.0_10/20

Table of Contents

Kit Components:

The volumes provided are sufficient for preparation of up to 100 reactions (NEB #E1700S) or 500 reactions (NEB #E1700L). All components should be stored at -20°C.

WarmStart LAMP 2X Master Mix

LAMP Fluorescent Dye (50X)

Required Materials Not Included

Target Nucleic Acid Samples

Molecular Biology Grade H_2O

Heat block, water bath, real-time turbidimeter or thermocycler
(with real-time fluorescence measurement if desired) and instrument-appropriate reaction vessels

Introduction

The WarmStart LAMP Kit (DNA & RNA) is designed to provide a simple, one-step solution for Loop-Mediated Isothermal Amplification (LAMP) of DNA or RNA (RT-LAMP) targets. LAMP and RT-LAMP are commonly used isothermal amplification techniques that provides rapid detection of a target nucleic acid using LAMP-specific primers (supplied by the user) and a strand-displacing DNA polymerase. This kit is supplied with the WarmStart LAMP 2X Master Mix, which contains a blend of *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx Reverse Transcriptase in an optimized LAMP buffer solution. Both *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx Reverse Transcriptase have been engineered for improved performance in LAMP and RT-LAMP reactions. A fluorescent dye is also supplied to enable real-time fluorescence measurement of LAMP.

The WarmStart LAMP Kit is compatible with multiple detection methods, including turbidity detection, real-time fluorescence detection (when used with LAMP fluorescent dye) and end-point visualization.

Reaction Setup: *For simplicity in setting up reactions, we recommend making stocks of the LAMP primers at a usable concentration. For example, we suggest a 10X Primer Mix containing all 6 LAMP primers.*

A 10X LAMP Primer Mix contains:

LAMP PRIMERS	10X CONCENTRATION	1X CONCENTRATION
FIP	16 µM	1.6 µM
BIP	16 µM	1.6 µM
F3	2 µM	0.2 µM
B3	2 µM	0.2 µM
Loop F	4 µM	0.4 µM
Loop B	4 µM	0.4 µM

Prepare primer stocks in nuclease-free water and store at –20°C for up to 2 years.

1. Thaw all components to be used at room temperature and place on ice. Vortex briefly to mix and centrifuge to collect material.
2. Prepare reaction mix as described below. Volumes are listed for a 25 µl LAMP reaction, but other volumes (10, 20, 50 µl etc.) are all effective; if desired, adjust volumes accordingly. A 1 µl target DNA volume is shown; if higher sample volumes are needed, adjust volume of H_2O. For non-template reactions add equivalent volume of H_2O or sample storage buffer.

COMPONENT	DNA TARGET DETECTION	RNA TARGET DETECTION	NO TEMPLATE CONTROL (NTC)
WarmStart LAMP 2X Master Mix	12.5 µl	12.5 µl	12.5 µl
Fluorescent dye (50X)	0.5 µl	0.5 µl	0.5 µl
LAMP Primer Mix (10X)	2.5 µl	2.5 µl	2.5 µl
Target DNA	1 µl	–	–
Target RNA	–	1 µl	–
dH_2O	8.5 µl	8.5 µl	9.5 µl
Total Volume	25 µl	25 µl	25 µl

3. Vortex reaction mix and centrifuge to collect material.
4. Pipet 24 µl per reaction into desired reaction vessels and add sample. Mix by vortexing and centrifuge to collect, or by pipetting if using a plate or other vessel.
5. Seal reaction vessel.
6. Incubate at 65°C for 30 minutes. Time can be extended as necessary for very low copy targets, challenging sample types, or reactions known to be produce slower amplification times.
7. If reaction products will be manipulated or analyzed after LAMP is complete, *Bst* 2.0 and RTx can be inactivated by heating at > 80°C for 5 minutes.

Experimental Considerations

- LAMP is an extremely sensitive detection method, and accordingly care should be taken to avoid any contamination of new reactions with products of previous LAMP reactions. Reaction vessels do not need to be opened after a reaction is completed when using a real-time or in situ detection of amplification, reducing the risk of potential carryover contamination. But if agarose gel or other post-reaction analysis is desired, vessels should be opened and handled in a secondary location with separate equipment. Regular decontamination of setup locations and equipment using chlorine bleach is recommended to avoid potential carryover contamination.
- For reactions detecting RNA targets in RT-LAMP, standard RNase prevention protocols are recommended, including use and frequent changing of gloves, RNase-free water and plasticware, and periodic decontamination of surfaces and equipment.
- LAMP primers can be challenging to design manually, and software programs are strongly recommended for both ease of design and likelihood of reaction success (we recommend using PrimerExplorer for design of optimal LAMP Primers; PrimerExplorer V5 is

recommended, https://primerexplorer.jp, though requiring browser translation from Japanese; V4 in English accessible via http://primerexplorer.jp/e). As performance and levels of nontemplate amplification can vary even with in silico design, we recommend evaluating 2–4 complete sets of LAMP primers for optimal sensitivity and specificity before choosing a final set.

- Use of Loop primers is strongly recommended for maximum amplification speed.
- Primers can be ordered with any level of desired purity; PAGE or HPLC purification of LAMP primers is not required, standard desalting is sufficient.
- For real-time fluorescence detection with the included fluorescent dye, data should be collected using the SYBR® Green I or FAM channel of any real-time instrument.
- Add the fluorescent dye fresh when preparing reactions, do not store the combined dye and master mix.
- Melt curve analysis can be performed using real-time fluorescent instruments. LAMP products, though long and varied in size and structure, are concatamers of a defined 150–400 bp amplicon and, when evaluated using melt analysis, tend to give a single species. Non-template amplification products can thus be discriminated from positive reactions using differences in their respective melt curves if desired.

Negative Controls and Non-template Amplification

The most common problem with LAMP reactions is amplification in negative or no-template controls. This result can occur due to carryover contamination of amplification products or non-template amplification of LAMP primers.

Carryover:

LAMP is an extremely sensitive detection method, and care should be taken to avoid any potential contamination of setup areas and equipment with products of completed reactions. If reaction vessels are to be opened for analysis or processing of products, this should be done in a secondary laboratory space and with separate, or thoroughly cleaned, equipment.

Signs of Carryover Contamination:

- Change in reaction performance. Reactions with previously acceptable performance and discrimination between positive and negative samples may display variation in which the non-template or negative controls give much faster amplification. This indicates potential carryover contamination from LAMP products, particularly where the same reaction is performed routinely.
- Extremely poor sensitivity vs. negative controls, with NTC overlapping with moderate template inputs (> 1000 copies). This problem can occur with poor primers (see below), but a decontamination cleaning is recommended.
- Melt curve signature. If using real-time fluorescence, a melt or denaturation curve can be included after the LAMP incubation. When NTC signal is a result of carryover contamination, the melt profiles of reactions with and without template will be identical.

Mitigation Strategies:

- Secondary laboratory areas and equipment if LAMP reaction tubes or plates will be opened
- Periodic cleaning of setup space and equipment using a 10% chlorine bleach solution
- Frequent replacement of all reagents, primer stocks, water, etc.
- If desired, 700 µM dUTP and Antarctic Thermolabile UDG (NEB #M0372) can be added to the reaction setup for carryover contamination prevention. Note that it is important to use Antarctic Thermolabile UDG, as the isothermal temperature (65°C) of the LAMP reaction is insufficient to inactivate *E. coli* UDG, and use of the *E. coli* form can result in inhibition of LAMP. Simply add 0.02 units/µl Antarctic Thermolabile UDG and set up reactions at room temperature to destroy contaminating LAMP products.

Nonspecific Amplification:

Due to the LAMP reaction conditions (high concentrations of Mg and dNTP) and the high concentration and nature of LAMP primers, amplification can occur from secondary structure and terminal transferase-like activity of the DNA polymerases used in LAMP. This activity is hard to predict from sequence, and even when using primer design software parameters it is not easily eliminated. One source of this nonspecific amplification is the activity of the LAMP polymerases at room or setup temperature, but the activity control by the WarmStart aptamers in the WarmStart LAMP Kit removes this source of nonspecific activity and enables reaction setup without ice. However, a significant level of non-template amplification can occur at elevated reaction temperatures (65°C) for many primer sets.

Signs of Nonspecific Amplification:

- Positive amplification in negative or non-template control reactions. Threshold times can be variable, and can overlap with low input (< 1000 copy) samples but will likely be slower.

- Carryover contamination prevention measures (reagent replacement, bleach decontamination) show no effect on NTC amplification.
- Melt curve signature. If using real-time fluorescence, a melt or denaturation curve can be included after the LAMP incubation. When NTC signal is a result of nonspecific amplification, the melt profiles of reactions with and without template will likely be different.

Mitigation Strategies:

- Design multiple (2–4) primer sets for each target, selecting entire distinct sets from Primer Explorer or other software. Each set can be ordered (desalting of oligos is sufficient) and should be tested with and without target. Evaluate performance based on speed and successful amplification of positive and discrimination of positive from negative sample. The optimal set will display little to no non-template amplification in the desired time frame (60 minutes or less).
- Change reaction temperature. A 65°C incubation is recommended for general LAMP, but the temperature can be increased to eliminate non-template amplification. Reactions showing NTC should be tested at 65–70°C.

Troubleshooting Guide

PROBLEM	POSSIBLE CAUSE(S)	SOLUTION(S)
Positive reactions do not show amplification	Reaction has not amplified sufficient LAMP product	• Increase incubation time • Check primers are added at: 1.6 µM FIP/BIP, 0.2 µM F3/B3, 0.4 µM LoopF/LoopB • If not using loop primers, add to increase reaction speed
	No amplification occurred, reaction failed	• Remake primer stocks, Check primers are added at: 1.6 µM FIP/BIP, 0.2 µM F3/B3, 0.4 µM LoopF/LoopB • Purify nucleic acid target from sample if high inhibitor concentrations are present • Add positive control reaction using validated standard target material • For real-time detection, check that a 1X amount of the fluorescent dye was added fresh to the reaction and that the instrument is collecting in the SYBR or FAM channel • Some precipitation of the master mix can occur, thoroughly mix the reagents by votexing before use • For RNA targets RNase contamination could prevent amplification, ensure RNase-free water and other materials are used, or add RNase Inhibitor

PROBLEM	POSSIBLE CAUSE(S)	SOLUTION(S)
Negative reactions show amplification *(see Experimental Considerations for more detail)*	Carryover contamination of previous reaction product	• Avoid opening reaction vessels after amplification • Use secondary preparation area and equipment if post-reaction processing is necessary • Clean equipment and areas with 10% chlorine bleach solution • Replace reagent stocks with new materials • Add 700 µM dUTP and 0.2 units/µl Antarctic Thermolabile UDG to prevent carryover contamination in future reactions
	Non-template amplification from LAMP primers	• Ensure the qPCR plate is properly sealed before inserting into the qPCR thermal cycler. • Exclude problematic trace(s) from data analysis

References

1. Notomi, T., et al. (2000) *Nucleic Acids Res*., 28, E63.
2. Nagamine, K., Watanabe, K., Ohtsuka, K., Hase, T., Notomi, T. (2001) *Clin. Chem.,* 47, 1742–1743.
3. Nagamine, K., Hase, T., Notomi, T. (2002) *Mol. Cell Probes*, 16, 223–229.
4. Mori, Y., Nagamine, K., Tomita, N., Notomi, T. (2001) *Biochem. Biophys. Res. Commun.,* 289, 150–154.
5. Mori, Y., Kitao, M., Tomita, N., Notomi, T. (2004) *J. Biochem. Biophys. Methods*, 59, 145–157.
6. Tomita, N., Mori, Y., Kanda, H., Notomi, T. (2008) *Nat. Protoc.,* 3, 877–882.
7. Tanner, N.A. and Evans, T.C. Jr. (2014) *Curr. Protoc. Mol. Biol.,* 105, Unit 15.14.
8. Hsieh et al. (2014) *Chem. Comm.* 50(28), 3747–3749.
9. Tanner, N.A., Zhang, Y., and Evans, T.C. Jr. (2015) *Biotechniques* 58(2), 59–68.

Ordering Information

NEB #	PRODUCT	SIZE
E1700S/L	WarmStart LAMP Kit (DNA & RNA)	100/500 reactions
KIT COMPONENTS SOLD SEPARATELY		
M0538S/L	*Bst* 2.0 WarmStart DNA Polymerase	1,600/8,000 units
M0380S/L	WarmStart RTx Reverse Transcriptase	50/250 reactions
B0537S	Isothermal Amplification Buffer Pack	6 ml
N0447S/L	Deoxynucleotide (dNTP) Solution Mix	8/40 µmol

COMPANION PRODUCTS

NEB #	PRODUCT	SIZE
M1800S/L	WarmStart Colorimetric LAMP Master Mix	100/500 units
M0374S/L/M	*Bst* 3.0 DNA Polymerase	1,600/8,000/8,000 units

Revision History

REVISION #	DESCRIPTION	DATE
1.0		9/16
2.0	Update to new manual format	1/20
3.0	Update legal text	3/20
4.0	Update legal text	10/20

ISO 9001 Registered Quality Management | ISO 14001 Registered Environmental Management | ISO 13485 Registered Medical Devices

www.neb.com

NEW ENGLAND BioLabs Inc.

be INSPIRED drive DISCOVERY stay GENUINE

New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938-2723 Telephone: (978) 927-5054 Toll Free: (USA Orders) 1-800-632-5227 (USA Tech) 1-800-632-7799 Fax: (978) 921-1350 e-mail: info@neb.com