

## WarmStart® Fluorescent LAMP/RT-LAMP Kit (with UDG)

NEB #E1708S/L

100/500 reactions

Version 2.0\_11/21

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### Kit Components:

*The volumes provided are sufficient for preparation of up to 100 reactions (NEB #E1708S) or 500 reactions (NEB #E1708L). All components should be stored at -20°C.*

WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)

LAMP Fluorescent Dye (50X)

### Required Materials Not Included

Target Nucleic Acid Samples

Molecular Biology Grade H<sub>2</sub>O

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

### Introduction

The WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG) 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 provide rapid detection of a target nucleic acids using LAMP-specific primers (supplied by the user) and a strand-displacing DNA polymerase. This kit is supplied with the WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG), 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 inclusion of dUTP and Antarctic Thermolabile UDG in the master mix reduces the possibility of carryover contamination between reactions.

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

## WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG) Protocols

**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 (STOCK)	1X CONCENTRATION (FINAL)
FIP	16 $\mu$ M	1.6 $\mu$ M
BIP	16 $\mu$ M	1.6 $\mu$ M
F3	2 $\mu$ M	0.2 $\mu$ M
B3	2 $\mu$ M	0.2 $\mu$ M
Loop F	4 $\mu$ M	0.4 $\mu$ M
Loop B	4 $\mu$ M	0.4 $\mu$ M

Prepare primer stocks in nuclease-free water and store at  $-20^{\circ}\text{C}$  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. An overview of the setup for one reaction is described in the table below. Volumes are listed for a 25  $\mu$ l LAMP reaction, but other volumes (10, 20, 50  $\mu$ l etc.) are all possible; if desired, adjust volumes accordingly. A 2  $\mu$ l sample input (DNA or RNA) volume is shown; if higher sample volumes are needed, adjust volume of H<sub>2</sub>O. For no-template reactions, add equivalent volume of H<sub>2</sub>O or sample storage buffer. We recommend creating a reaction mix that lacks template (23  $\mu$ l per reaction) such that the input sample can be added last. Room temperature set up of the reactions will enable carryover prevention.

COMPONENT	DNA TARGET DETECTION	RNA TARGET DETECTION	NO TEMPLATE CONTROL (NTC)
WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)	12.5 $\mu$ l	12.5 $\mu$ l	12.5 $\mu$ l
Fluorescent dye (50X)*	0.5 $\mu$ l	0.5 $\mu$ l	0.5 $\mu$ l
LAMP Primer Mix (10X)	2.5 $\mu$ l	2.5 $\mu$ l	2.5 $\mu$ l
Target DNA	2 $\mu$ l	–	–
Target RNA	–	2 $\mu$ l	–
dH <sub>2</sub> O	7.5 $\mu$ l	7.5 $\mu$ l	9.5 $\mu$ l
Total Volume	25 $\mu$ l	25 $\mu$ l	25 $\mu$ l

\*A 1X concentration of fluorescent dye is recommend for most real-time PCR instruments. Lower concentrations of dye (e.g., 0.1X) may be necessary on some instruments to avoid a saturated fluorescence signal.

3. Vortex reaction mix and centrifuge to collect material.
4. Pipet 23  $\mu$ l 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  $65^{\circ}\text{C}$  for 30 minutes. Time can be extended as necessary for very low copy targets, challenging sample types, or reactions known to 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  $> 80^{\circ}\text{C}$  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 even when using a master mix with dUTP/UDG. 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 the NEB LAMP Primer Design Tool, <https://lamp.neb.com/>). As performance and levels of no-template 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; although some LAMP primer sets may benefit from PAGE or HPLC purification, particularly for the FIP and BIP primers.
- For real-time fluorescence detection with the included fluorescent dye, data should be collected using the SYBR<sup>®</sup> 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. No-template amplification products can thus be discriminated from positive reactions using differences in their respective melt curves if desired.

## Negative Controls and No-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 no-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. The inclusion of dUTP and Antarctic Thermolabile UDG in the master mix reduces the possibility of carryover contamination between subsequent reactions but may not eliminate it entirely.

### 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 no-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.
- Carryover contamination can be minimized by incorporating dUTP during amplification, which allows contaminating, dU-containing LAMP products from previous reactions to be eliminated during an initial incubation step with UDG. This product includes dUTP and Antarctic Thermolabile UDG. Antarctic Thermolabile UDG is active during setup and very short up-front incubations at 4°C and/or room temperature but is completely inactivated during the amplification step at 65°C.

### 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 the secondary structure and terminal transferase-like activity of the DNA polymerases used in LAMP. This activity is hard to predict from the 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 this kit remove this source of nonspecific activity and enable reaction setup without ice. However, a significant level of no-template amplification can occur at elevated reaction temperatures (65°C) for many primer sets.

### Signs of Nonspecific Amplification:

- Positive amplification in negative or no-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 the LAMP Primer Design Tool or other software. Test each set with and without the target. Evaluate performance based on speed and successful amplification of the positive sample, and discrimination of positive from negative sample. The optimal set will display little to no no-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 no-template amplification. Reactions showing NTC should be tested at 65–70°C.
- Change reaction volume. Nonspecific amplification can be caused by non-optimal reaction volumes (e.g., 25 µl in 384-well plate) for some primer sets.

## Troubleshooting Guide

PROBLEM	POSSIBLE CAUSE(S)	SOLUTION(S)
Positive reactions do not show amplification	Reaction has not amplified sufficient LAMP product	<ul style="list-style-type: none"><li>• Increase incubation time</li><li>• Check primers are added at: 1.6 µM FIP/BIP, 0.2 µM F3/B3, 0.4 µM LoopF/LoopB</li><li>• If not using loop primers, add to increase reaction speed</li></ul>
	No amplification occurred, reaction failed	<ul style="list-style-type: none"><li>• Remake primer stocks, Check primers are added at: 1.6 µM FIP/BIP, 0.2 µM F3/B3, 0.4 µM LoopF/LoopB</li><li>• Purify nucleic acid target from sample if high inhibitor concentrations are present</li><li>• Add positive control reaction using validated standard target material</li><li>• 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</li><li>• Some precipitation of the master mix can occur, thoroughly mix the reagents by vortexing before use</li><li>• For RNA targets RNase contamination could prevent amplification, ensure RNase-free water and other materials are used, or add RNase Inhibitor</li></ul>

PROBLEM	POSSIBLE CAUSE(S)	SOLUTION(S)
Negative reactions show amplification <i>(see Experimental Considerations for more detail)</i>	Carryover contamination of previous reaction product	<ul style="list-style-type: none"> <li>• Avoid opening reaction vessels after amplification</li> <li>• Use secondary preparation area and equipment if post-reaction processing is necessary</li> <li>• Clean equipment and areas with 10% chlorine bleach solution</li> <li>• Replace reagent stocks with new materials</li> </ul>
	No-template amplification from LAMP primers	<ul style="list-style-type: none"> <li>• Evaluate multiple primer sets for each target</li> <li>• Optimize reaction temperature and/or reaction volume</li> <li>• Ensure the qPCR plate is properly sealed before inserting into the qPCR thermal cycler</li> <li>• Exclude problematic trace(s) from data analysis</li> </ul>

## References

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## Ordering Information

NEB #	PRODUCT	SIZE
E1708S/L	WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG)	100/500 reactions
KIT COMPONENTS SOLD SEPARATELY		
M0538S/L	<i>Bst</i> 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 $\mu$ mol
M1708S/L	WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG)	100/500 reactions
B1700S	LAMP Fluorescent Dye	0.25 ml

### COMPANION PRODUCTS

NEB #	PRODUCT	SIZE
M1804S/L	WarmStart Colorimetric LAMP 2X Master Mix with UDG	100/500 units
M0374S/L/M	<i>Bst</i> 3.0 DNA Polymerase	1,600/8,000/8,000 units
E2019S	SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit	96 reactions

## Revision History

REVISION #	DESCRIPTION	DATE
1.0	N/A	07/21
2.0	Update legal text	11/21

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