FEB 21, 2021

LAMP/RT-LAMP Reaction protocol

In 1 collection

Tamara Matute¹, Isaac Núñez², Fernan Federici¹

¹Laboratorio Tecnologías Libres; ²SynBioUC

Laboratorio de Tecnologías Libres

Reclone.org (The Reagent Collaboration Network)

[1 more workspace](#) ↓

Tamara Matute

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.bsefnbbn

Protocol Citation: Tamara Matute, Isaac Núñez, Fernan Federici 2021. LAMP/RT-LAMP Reaction protocol.

protocols.io
<https://dx.doi.org/10.17504/protocols.io.bsefnbbn>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Feb 14, 2021

Last Modified: Feb 21, 2021

PROTOCOL integer ID:
47271

Keywords: LAMP, RT-LAMP, Homemade reagents, Homebrew, Low-cost, Free technology, RNA detection, DNA detection, viral detection, ReClone

ABSTRACT

This protocol describes how to perform **LAMP / RT-LAMP reactions with home-made reagents**, which means home-made buffers and positive controls, together with home-brewed BstLF and MMLV enzymes.

Home-brewed enzymes information:

BstLF polymerase:

expression and [purification protocol](#).

expression [plasmid sequence](#).

M-MLV reverse transcriptase:

expression and [purification protocol](#).

expression [plasmid sequence](#).

Positive control information

[Sample preparation protocol here](#).

Testing

We test this protocol in LAMP or RT-LAMP reactions with [SARS-CoV-2 synthetic dsDNA or ssRNA](#) respectively. As shown in **Figure 1**, the general performance of reactions with homemade reagents seems appropriate compared with the commercial standard option. No amplification was observed in non-template controls (NTC).

Figure 1. RT-LAMP Amplification results. 10.000 copies of synthetic SARS-Cov-2 N gene were added as the positive sample and N2 primers set [1] was used to carry the RT-LAMP reaction. Commercial enzymes reactions were carried out in NEB Isothermal Amplification Buffer (IAB) and the rest in Home-made IAB. Polymerase concentrations are indicated per uL of reaction. BstLF: home-brewed polymerase enzyme. Bst2.0: NEB Bst2.0 Warmstart. MMLV: M-MLV reverse transcriptase at 62,5 pg/uL reaction. RTx: NEB RTx Warmstart. NTC: Non-Template Control.

[1] [Zhang, Y. et al. Enhancing colorimetric loop-mediated isothermal amplification speed and sensitivity with guanidine chloride. Biotechniques69, \(2020\).](#)

IMAGE ATTRIBUTION

Tamara Matute & Isaac Núñez. GNU General Public License v3.0.

MATERIALS

- Pipettes
- Ice
- tube grids
- 0.2 uL and 1.5 uL tubes
- Pipette tips

Equipments

- Reactions heater device: it could be from a water bath to a Real-Time machine
- Device to register the fluorescence: from transilluminator and filters to Real-Time machine

Primers Stock

20m

- 1 Prepare stocks (100µM) of each primer in nuclease-free water and store at –20°C for up to 2 years.

10m

- 2 Prepare 10X LAMP Primer Mix stock according to the following table:

10m

	10X Concentration (Stock)	Volume (µL)
FIP	16 µM	16
BIP	16 µM	16
F3	2 µM	2
B3	2 µM	2
LOOP F	4 µM	4
LOOP B	4 µM	4
H ₂ O		56
<i>Total Volume</i>		<i>100</i>

Volumes are listed to prepare 100ul of standard 10X LAMP Primer Mix stock, but you can amplify the volumes according to your requirements or modify the proportions to any other specific primer mix formulation.

Reaction

1h

- 3 Prepare reaction mix as described in the following table:

🧊 On ice Always keep all components and reaction mix on ice.

Note

All the protocols for the preparation of the buffers are available here:

Protocol

NAME

LAMP/RT-LAMP Buffer protocol

CREATED BY

Tamara Matute

PREVIEW

Step 3 includes a Step case.

LAMP Reaction

RT-LAMP Reaction

step case

LAMP Reaction

Component	Volume (µL)
Isothermal Amplification Buffer 10X	2,50
dNTPs 10mM	3,50
MgSO4 100mM	1,50
LAMP Primer Mix 10X	2,50
Evagreen 20X	1,25
BstLF [0,41 mg/mL]	1,00
H ₂ O	11,75
Sample	1,00
Total Volume	25,00

To adjust the concentration of the BstLF enzyme use **1x Storage Buffer**.

If higher sample volumes are needed, adjust the volume of H₂O. For non-template reactions add equivalent volume of H₂O.

- Optionally 2.5 µL of 100mM DTT can be added by replacing the corresponding volume of H₂O. You can add any other enhancer this way.
- This formulation recommends *Evagreen* 20X as the fluorescent dye but you can replace it with any other of your choice by following the fabrication indications.

4 Vortex and spin reaction mix.

On ice

Note

If you need to add different samples; pipet 24 µL per reaction into desired reaction vessels and add 1 µL sample. Vortex and spin reaction mix. Always keep On ice to reduce spurious amplifications risk or sample degradation.

5 Incubate at 65 °C for 01:00:00

1h

Note

- Time can be between 20 - 60 minutes depending on the specific assay.
- Specific temperature can vary according to the optimal temperature of the primer set used.
- The reaction can be carried out in any heater device from a water bath to real-time machines but the performance of the reactions can vary with respect to the temperature control.