

LAMP/RT-LAMP Reaction protocol

 In 1 collection

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 Works for me dx.doi.org/10.17504/protocols.io.bsefnbbn

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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).

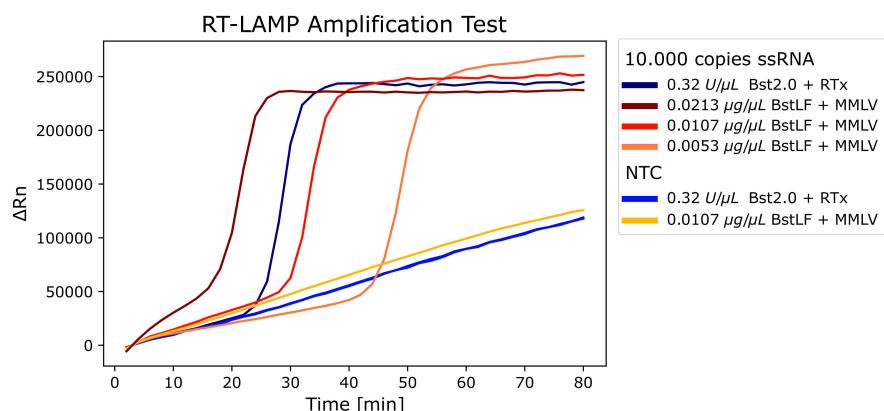


Figure 1. RT-LAMP Amplification results. 10.000 copies of synthetic SARS-CoV-2 N gene were added as the positive sample and N2 primers set [1] was used to carry the RT-LAMP reaction. Commercial enzymes reactions were carried out in NEB Isothermal Amplification Buffer (IAB) and the rest in Home-made IAB. Polymerase concentrations are indicated per μ L of reaction. BstLF: home-brewed polymerase enzyme. Bst2.0: NEB Bst2.0 Warmstart. MMLV: M-MLV reverse transcriptase at 62,5 μ g/ μ L 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, \(2020\)](#).

DOI

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PROTOCOL CITATION

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COLLECTIONS



Low Cost LAMP and RT-LAMP

KEYWORDS

LAMP, RT-LAMP, Homemade reagents, Home-brew, Low-cost, Free technology, RNA detection, DNA detection, viral detection, ReClone

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IMAGE ATTRIBUTION

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PARENT PROTOCOLS

Part of collection

[Low Cost LAMP and RT-LAMP](#)

MATERIALS TEXT

- 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

10m

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

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

10m

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

Volumes are listed to prepare 100 μ l 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..

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



LAMP/RT-LAMP Buffer protocol
by Tamara Matute

PREVIEW

RUN

Step 3 includes a Step case.

LAMP Reaction

RT-LAMP Reaction

step case

LAMP Reaction

Component	Volume (μ L)
Isothermal Amplification Buffer 10X	2,50
dNTPs 10mM	3,50
MgSO ₄ 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
<i>Total Volume</i>	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 μ L 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**

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

- 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.