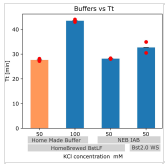

Feb 21, 2021

LAMP/RT-LAMP Buffer protocol

In 1 collection

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[1]LabTecLibres; [2]SynBioUC

1 Works for me dx.doi.org/10.17504/protocols.io.bsehnbb6

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SUBMIT TO PLOS ONE

ABSTRACT

This protocol describes the preparation of **reaction buffer** for homemade LAMP / RT-LAMP reactions which involves the use of home-brewed BstLF and MMLV enzymes. It also contains the instructions to prepare the enzyme **storage buffers**.

Home-brewed enzymes information:

BstLF polymerase**:**
expression and purification protocol.
expression plasmid sequence.

M-MLV reverse transcriptase:
expression and purification protocol.
expression plasmid sequence.

Testing
We test the buffers on SARS-CoV-2 LAMP or RT-LAMP reactions with synthetic dsDNA or ssRNA respectively. The general performance of Homemade buffer seems appropriate and no amplification was obtained for non-template controls (NTC).

To properly formulate the buffer we tested different KCl concentrations which is known to be a critical factor in the buffer.
For the home-brewed BstLF, we found that 50 mM KCl is the best concentration, displaying equal or better performance than the commercial buffer. No amplification was observed for 150 mM KCl formulation.

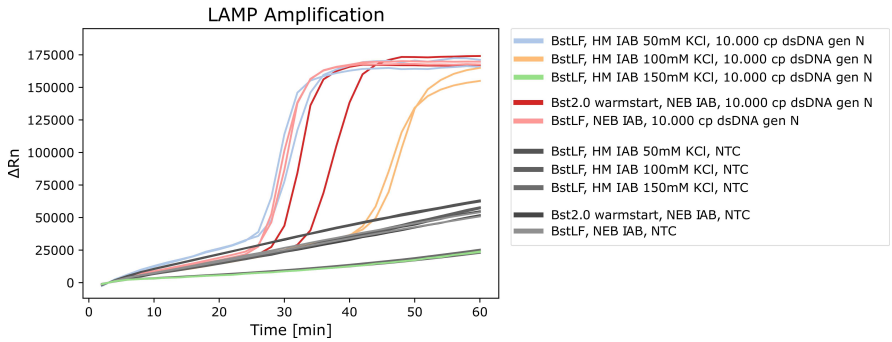

Figure 1. LAMP Amplification results. 10.000 copies of plasmid synthetic sample with SARS-Cov2 gen N were added as positive sample and N2 primers set [1] was used to carry the LAMP reaction. BstLF corresponds to the home-brewed enzyme and Bst2.0 warmstart to the commercial reference used. NEB IAB: NEB Isothermal Amplification Buffer. HM IAB: Homemade Isothermal Amplification Buffer. NTC: non Template control.

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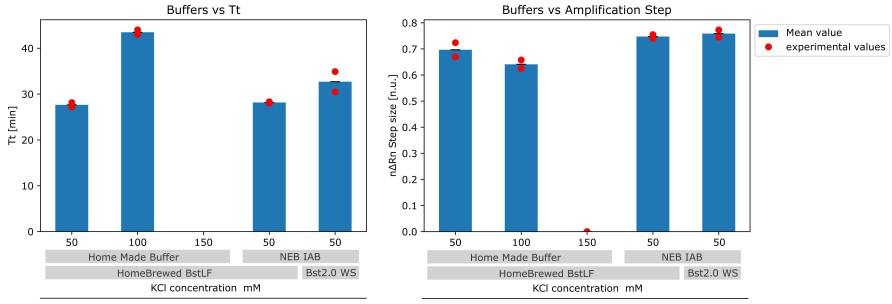

Figure 2. Buffer formulations effect on Threshold time (Tt) and Amplification Step size of positive LAMP reactions. Commercial NEB Isothermal Amplification Buffer (IAB) and Bst2.0 Warmstart Polymerase (Bst2.0 WS) was used to benchmark. KCl indicated concentrations correspond to 1X buffer.

[1] Zhang, Y. et al. Enhancing colorimetric loop-mediated isothermal amplification speed and sensitivity with guanidine chloride. Biotechniques**69**, (2020).

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PROTOCOL CITATION

Tamara Matute, Isaac Núñez, Fernan Federici 2021. LAMP/RT-LAMP Buffer protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bsehnbb6

COLLECTIONS

Low Cost LAMP and RT-LAMP

KEYWORDS

LAMP, RT-LAMP, Isothermal Amplification Buffer, IAB, Storage Buffer, ReClone, BstLF, MMLV, RNA detection, Viral detection, Home-made reactions, Enzyme storage buffer, open recipes

IMAGE ATTRIBUTION

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PROTOCOL INTEGER ID

47273

PARENT PROTOCOLS

Part of collection
Low Cost LAMP and RT-LAMP

MATERIALS TEXT

- pH-meter
- Magnetic Stirrer
- Precision Scale
- 0.22 uM filters
- Syringe
- Autoclave
- Shott Bottles
- 15 and 50 ml conical tubes

BstLF and MMLV storage buffer

1 Enzyme storage buffer was made based on NEB indicated Enzymes Storage Conditions.

Component	10X Storage Buffer	1x Storage Buffer
Tris-HCl (pH 7.1 @ 25°C)	100 mM	10 mM
KCl	500 mM	50 mM
EDTA	1 mM	0.1 mM
Triton® X-100	1%	0.1%
Glycerol	-	50%
DTT	-	1 mM

DTT should be added as fresh as possible. Add DTT and Glycerol at the moment of assembling the 1X Buffer. Freeze and thaw cycles are not recommended for DTT solutions, always use a fresh aliquot.

2 To prepare **10 mL** of **10x Storage Buffer** add:

- **6 mL** molecular grade H_2O
- **1 mL** 1M Tris-HCl ph 7.1
- **2 mL** KCl 2.5M
- **20 µl** EDTA 500 mM
- **100 µl** Triton® X-100
- Make up the volume to **10 mL** with H_2O (~ **880 µl**)
- Make aliquots and store at **-20 °C**

In the "**Stocks Solutions**" section you can find the preparation of each solution.

3 To prepare **1.5 mL** of **1x Storage Buffer** add:

- **599 µl** molecular grade H_2O
- **150 µl** 10x Storage Buffer
- **1.5 µl** DTT 1M

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- **500 µl** Glycerol 100%
- Mix properly (by vortex), use immediately or store at **-20 °C**

LAMP and RT-LAMP reaction buffer

4 This (RT-)LAMP assay buffer was made based on NEB 1X Isothermal Amplification Buffer Pack.

10X Isothermal Amplification Buffer is as follow:

Component	10X Buffer concentration	1X Reaction concentration
Tris-HCl (pH 8.8.@25°C)	200 mM	20 mM
$(NH_4)_2SO_4$	100 mM	10 mM
KCl	500 mM	50 mM
$MgSO_4$	20 mM	2 mM
Tween® 20	1%	0.1%

5 Prepare the following **stock solutions:**

Component	Concentration
Tris-HCl (pH 8.8.@25°C)	1M
$(NH_4)_2SO_4$	1M
KCl	2.5M
$MgSO_4$	1M
Tween® 20	100%

In the "**Stocks Solutions**" section you can find the preparation of each solution.

6 To prepare **50 mL** of **10X Isothermal Amplification Buffer** add:

- **20 mL** Molecular H_20
- **10 mL** 1M Tris-HCl (pH 8.8 @ 25°C)
- **5 mL** 1M $(NH_4)_2SO_4$
- **10 mL** 2.5M KCl
- **1 mL** 1M $MgSO_4$
- **0.5 mL** Tween® 20
- Adjust pH **8.8** with NaOH 1 M (~ **560 µl**)
- Make up the volume to **50 mL** with H_2O (~ **3 mL**)
- Filter-sterilize the solution with 0.22 µm filter.

It shouldn't be autoclaved.
The autoclave is not recommended for Tween® 20, $(NH_4)_2SO_4$ and $MgSO_4$ solutions.

- Make aliquots and store at **-20 °C** .

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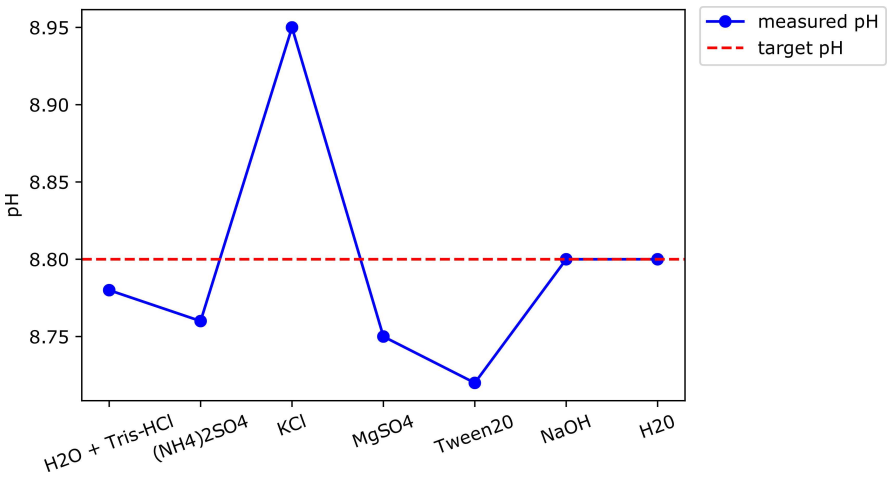

pH variation dynamics (@25 °C) along buffer elaboration.

Stock solutions

7 Potassium chloride (KCl)

To prepare 50 ml of a 1 M solution, weigh 9.3188 g of KCl powder and completely dissolve it in 40 mL of H2O. Make up the volume to 50 mL with H2O (it can be done in a 50 ml conical tube). Filter-sterilize the solution with a 0.22 µm filter and store at room temperature.

8 Ammonium sulfate ($[NH_4]_2SO_4$)

To prepare 50 ml of a 1 M solution, weigh 6.6070 g of (NH4)2SO4 powder and completely dissolve it in 40 mL of H2O. Make up the volume to 50 mL with H2O (it can be done in a 50 ml conical tube). Filter-sterilize the solution with a 0.22 µm filter and store at room temperature.

9 Magnesium sulfate ($MgSO_4$)

To prepare 50 ml of a 1 M solution, weigh 12.3240 g of MgSO4*7H20 powder and completely dissolve it in 40 mL of H2O. Make up the volume to 50 mL with H2O (it can be done in a 50 ml conical tube). Filter-sterilize the solution with a 0.22 µm filter and store at room temperature.

> It is possible to use MgSO4 instead of heptahidated but it is more hygroscopic and would need desiccating before weighing .

10 Tris·HCl

To prepare 250 ml of a 1 M solution:

- Weigh 30.2850 g of Trizma Base powder and completely dissolve it in 200 mL of H2O. It should be done in a magnetic stirrer.
- Adjust to pH 8.8 or 7.1 with concentrated HCl (2.5 M). It should be done in a properly calibrated pH-meter, with continuous stirr and giving enough time to let the pH to equilibrate. It is important to check the temperature with a thermometer and use a heater if necessary to reach a temperature solution of 25°C the whole time.
- Transfer the solution to a graduated cylinder and complete the volume to 250 ml with H2O.
- Filter-sterilize the solution with a 0.22 µm filter

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- Store at room temperature or 4°C up to six months.

Temperature has a significant effect on the pH of Tris buffers. It decreases ~0.028 pH units per each °C increase.

11 Hydrochloric acid (HCl)

To prepare 50 ml of 2.5 M solution:
- Weight 4.55 g HCl and completely dissolve it in 40 mL of H2O, or add 10.25 mL HCl 37% (d = 1.19) solution to 40 ml H20.
- Make up the volume to 50 ml with H2O.

12 EDTA (ethylenediaminetetraacetic acid)

To prepare 500 ml of a 500 mM solution:

- Weigh 93.06 g of EDTA disodium salt dihydrate powder (MW = 372.24 g/mol) and dissolve it in 400 mL of H2O. At this concentration it shouldn't dissolve completely until you up the pH.
- Adjust to pH 8.0 or 7.1 with concentrated NaOH. It would be needed ~25 uL of 10M NaOH. It should be added slowly in a magnetic stirrer letting the EDTA to dissolve and the pH to equilibrate.
- Transfer the solution to a graduated cylinder and complete the volume to 500 ml with H2O.
- Filter-sterilize the solution with a 0.22 µm filter
- Store at room temperature or 4°C.

13 DTT (dithiothreitol)

To prepare 10 ml of 1 M solution, weigh 1.54 g of DTT powder and dissolve it completely in 80 mL of H2O. Make up the volume to 10 mL with H2O (it can be done in a 15 ml conical tube). Filter-sterilize the solution with a 0.22 µm filter, make 100 uL - 1 ml aliquots and store at -20°C.

14 Glycerol

Make an aliquot and sterilize by autoclave.

15 Filters:

To sterilize the solutions and the assembled buffer 0.22 µm syringe filters of MCE or Cellulose acetate membranes could be used.

Specifically we used:

BioFil
MCE membrane
0.22 µm

EDLAB
Cellulose acetate
0.22 µm

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