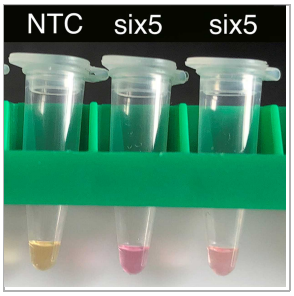

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WORKS FOR ME 1

Colorimetric LAMP/RT-LAMP Protocol

COMMENTS 0

This protocol is published without a DOI.

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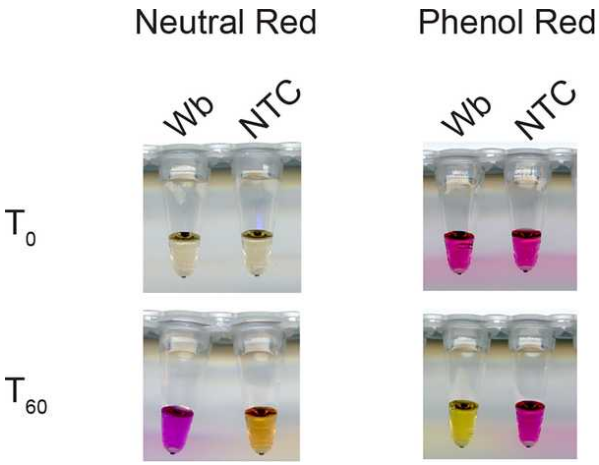
ABSTRACT

This protocol describes how to perform **colorimetric LAMP / RT-LAMP reactions** with homemade buffers, together with home-brewed BstLF and MMLV enzymes.

It is based on previous protocols of our lab (see LAMP/RT-LAMP Reaction protocol), but since two of the most common indicators used in colorimetric LAMP **(phenol red and neutral red) require weakly buffered reactions,** the buffer composition needed to be changed. This was done according to indications by Poole et al (2017).

The colorimetric methods rely on the inherent proton production by DNA polymerases during the amplification process, which leads to a drop in the reaction pH that can be followed using these pH indicators. The results can be seen directly with the "naked eye", without the need for equipment or hands-on processing (Tanner et al., 2015).



Color change of neutral red and phenol red indicator dyes in LAMP reactions.
Before amplification (T_0), reactions containing neutral red are colorless. Samples turn pink if positive or a brownish yellow if negative as shown after a sixty-minute (T_{60}) amplification. Reactions containing phenol red are pink at T_0 and remain pink if negative but turn yellow if positive as shown here after a sixty-minute (T_{60}) amplification. [Figure from Poole et al (2017)].

PROTOCOL CITATION

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2X Buffer Preparation

1 Prepare 1000 µL of 2X Colorimetric Buffer Mix according to the following table:

Reagent	Stock Concentration	2x (Buffer concentration)	1x	Volume for 1 mL
dNTPs	10 mM	2,8 mM	1,4 mM	280 uL
(NH4)2SO4	1M	20 mM	10 mM	20 uL
MgSO4	1M	16 mM	8 mM	16 uL
KCl	1M	100 mM	50 mM	100 uL
Tween 20	100%	0,2%	0,1%	2 uL
				418 uL

- Two main differences compared to non-colorimetric LAMP reaction are the **addition of dNTPs** in the buffer, as they influence the reaction pH, and the **removal of Tris-HCl** as this component is vital to maintain the pH of the buffer at a stable point, which is not desirable for pH-dependent detection
- The optimal concentration of KCl for home-brewed BstLF is 50 mM (see LAMP/RT-LAMP Buffer protocol)

2 Measure the reaction pH with strips and adjust pH using NaOH 250 mM to a final value of **pH 8-9.**

Note

The pH of the original solution should be around 5-6 before adjusting.

3 Add nuclease-free water to a final volume of 1000 µL. Make aliquots and store them at 4 °C until use.

35m

LAMP / RT-LAMP

4 Prepare the LAMP or RT-LAMP reaction mix On ice according to the following tables:

Step 4 includes a Step case.

LAMP

RT-LAMP

step case

LAMP

Component	1X	Final Reaction Concentration
2X Buffer Mix	5 uL	1,4 mM dNTPs 10 mM (NH4)2SO4 8 mM Mg SO4 50 mM KCl 0,1% v/v Tween 20
Neutral Red 1,25 mM	1.2 uL	0,15 mM
10X Primer Mix	1 uL	0,2 uM F3/B3 1,6 uM FIP/BIP 0,4 - 0,8 uM LF/LB
BstLF (0,2 mg/mL)	0.8 uL	
H20	1 uL	
DNA	1 uL	
	10 uL	

- Neutral red can be replaced with another pH indicator like phenol red
- To adjust the concentration of BstLF enzyme use its **1x Storage Buffer**
- 2 uL of DNA could also be added to avoid the use (and possible contamination due to reopening) of nuclease-free water
- Reactions can also be scaled up to higher volumes, especially if you are working with a higher sample volume

5 Incubate the reactions at 65ºc for 35 minutes and observe results with the naked eye.

Expected result

These are expected results with the use of **neutral red** as a colorimetric indicator, with negative reactions (NTC) staying yellow and positive reactions (in this case, six5) turning pink: