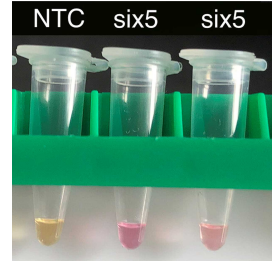


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🌐 Colorimetric LAMP/RT-LAMP Protocol

DOI

<https://dx.doi.org/10.17504/protocols.io.5qpvor3x7v4o/v1>



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Protocol status: Working

We use this protocol and it's working

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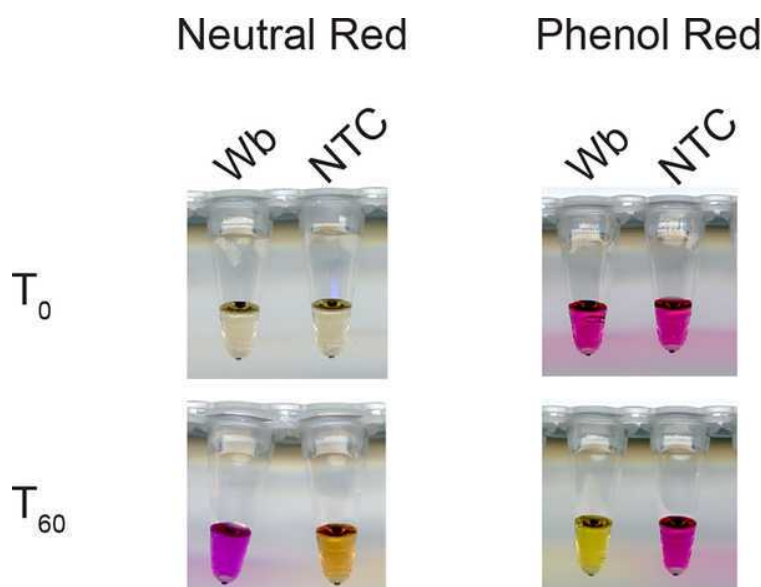
Keywords: lamp reactions with homemade buffer, lamp reaction protocol, colorimetric lamp, lamp reaction, colorimetric method, lamp protocol, reaction ph, lamp protocol this protocol, ph indicator, inherent proton production by dna polymerase, buffered reaction, lamp, drop in the reaction ph, inherent proton production, ph, enzyme, dna polymerase, amplification process, phenol, brewed bstlf, homemade buffer, mmlv enzyme

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 \(2017\)](#)].



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 μL
(NH ₄) ₂ SO ₄	1M	20 mM	10 mM	20 μL
MgSO ₄	1M	16 mM	8 mM	16 μL
KCl	1M	100 mM	50 mM	100 μL
Tween 20	100%	0,2%	0,1%	2 μL
				418 μL

- 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 $^{\circ}\text{C}$ until use.
- 4 Prepare the LAMP or RT-LAMP reaction mix  On ice according to the following tables:

STEP CASE

LAMP

1 step

Component	1X	Final Reaction Concentration
2X Buffer Mix	5 uL	1,4 mM dNTPs 10 mM (NH ₄) ₂ SO ₄ 8 mM Mg SO ₄ 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	
H ₂ O	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 65°C 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:

