TrueLAMP™ Instruction Manual

General Information

Description

TrueLAMP™ is a proprietary colorimetric LAMP kit consisting of 2× TrueLAMP buffer and TrueLAMP polymerase. It is used for detecting DNA or RNA targets by loop-mediated isothermal amplification (LAMP). The unique feature of TrueLAMP is its ability to eliminate primer amplification in the absence of a template—preventing nonspecific or false-positive amplification in no-template control (NTC) reactions.

Specification

- Product Name: TrueLAMP™ Kit
- Catalog #: T0001S, T0001M, T0001L
- Contents:
 - o T0001S: 0.5 mL 2× TrueLAMP buffer and 5 μL TrueLAMP polymerase
 - o T0001M: 1.5 mL 2× TrueLAMP buffer and 15 μL TrueLAMP polymerase
 - o T0001L: 5 × (1.5 mL 2× TrueLAMP buffer and 15 μL TrueLAMP polymerase)
- Reactions:
 - O T0001S: 88 reactions (11 8-strips) at 10 μL each
 - \circ T0001M: 264 reactions (33 8-strips) at 10 μ L each
 - \circ T0001L: 1320 reactions (165 8-strips) at 10 μ L each
- Storage: Store polymerase and buffer at -20 °C for up to 12 months.

Terms & Conditions

- Product Usage: For in vitro laboratory research use only. Not for administration to humans or use in medical diagnosis.
- Warranties and Liabilities: Boltii Diagnostics Inc. accepts no responsibility and shall not be held
 liable for any loss, damage, or expenses—whether consequential or incidental—including
 damage to property, persons, or premises, arising from the use, results of use, or inability to use
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TrueLAMP™ Assay Protocol

A. Before use, warm the 2× buffer at 22 °C for >30 min and vortex to dissolve any microcrystals formed during storage at -20 to 4 °C. Thawed buffer may be capped tightly and stored at 22 °C for a few days. **B.** Because of the small 10 μ L reaction volume, pipettes must be accurately calibrated.

1. Prepare the LAMP Reaction Mix

Combine the following components in a microfuge tube at room temperature to make enough reaction mix for 8 reactions. Use the mix immediately.

Component	1 Reaction (μL)	8 Reactions (μL)	10% Extra (μL)	Total (μL)
Deionized water	3	24	2.4	26.4
10× LAMP primers	1	8	0.8	8.8
2× TrueLAMP buffer	5	40	4	44
TrueLAMP polymerase	0.05	0.4	0.04	0.44

2. Set Up the LAMP Reactions

a. Label each tube in an 8-strip 0.2 mL clear PCR tube set.

b. Add:

- 1 μ L deionized water to the NTC tube.
- 1 μL positive template (~500 copies, pre-determined) to the PTC tube.
- $1 \mu L$ test sample to each of the six remaining tubes.
- **c.** Add 9 μ L of the prepared reaction mix to each tube.

(You should have ~8 μL left; otherwise, pipette accuracy may be off.)

d. Cap the tubes, vortex briefly, and centrifuge the 8-strip to remove bubbles.

3. Start the LAMP Reaction

- **a. For DNA targets**, place the 8-strip upright in a digital oven or submerge it in a water bath or a thermocycler with heated lid at 65 °C for 60–90 min. **For RNA targets**, incubate the 8-strip at 60 °C for 10 min for reverse transcription and then reset the temperature to 65 °C for 50–80 min.
- **b. For semi—real-time colorimetric LAMP**, take snapshots of the 8-strip using a stationary smartphone through the oven window at 15-minute intervals. Keep the position of the phone fixed throughout, as variations in distance or angle may alter magenta readings. **For endpoint colorimetric LAMP**, take a photo at the end of incubation.
- **c.** After incubation, quantify magenta or green intensity in each reaction using a colorimeter app.
 - NTC: Solution should remain red.
 - PTC: Solution should turn orange or yellow.

Frequently Asked Questions

1. Should I store the TrueLAMP Kit at -20 °C?

Yes. While the $2\times$ buffer may be stored at 4–22 °C with the tube tightly capped, CO_2 can dissolve into the buffer over time and lower its pH, causing gradual color fading. Freezing slows this process.

2. How does TrueLAMP eliminate primer amplification in NTCs?

The buffer contains a proprietary inhibitor formulated with the polymerase to prevent nonspecific primer amplification when no template is present.

3. What is the best incubator for TrueLAMP reactions?

The reaction must be heated evenly to prevent evaporation in small volumes. A digital oven or submerged water bath or thermocycler with heated lid works best.

4. Why am I still getting primer amplification in the NTC?

- Warm the buffer before use. Cold storage (–20 to 4 °C) can form microcrystals that reduce inhibition.
- Recalibrate pipettes. Incorrect volumes (< 4.75 μ L buffer per 10 μ L reaction) may also reduce inhibition.
- Verify incubation temperature. Nonspecific amplification can occur at ≤ 63 °C. Temperature
 distribution in digital ovens may be uneven—calibrate and adjust placement of 8-strips as
 needed. For multiple strips, use a water bath.
- Strengthen inhibition. Increase buffer slightly (e.g., $5.25~\mu L~2\times$ buffer per 10 μL reaction) for particularly stubborn primer sets.

5. Why was my positive control not amplified?

• Determine the limit of detection (LOD) for the primer set. Template concentration < LOD will not be amplified consistently.

6. Can I use raw samples (e.g., saliva, plasma)?

Not recommended. TrueLAMP is sensitive to pH and ionic strength. For best results, use purified DNA or RNA. Although water-suspended virions may be used directly with TrueLAMP, its compatibility with other types of cells has not been fully characterized.

7. Can I pre-mix polymerase into the 2× buffer?

No. The polymerase is inactivated within hours, even at -20 °C. Always prepare the reaction mix fresh.

8. How should I quantitate the color change following LAMP amplification?

Color intensity of the reaction may be quantified using a smartphone colorimeter app with its smallest aperture by sampling the region at the center immediately below the liquid meniscus and recording the maximum magenta (CMYK mode) or minimum green (RGB mode).