# TrueLAMP™ Instruction Manual

**General Information**

**Description**

TrueLAMP™ is a proprietary colorimetric LAMP reagent system consisting of 2× TrueLAMP buffer and TrueLAMP polymerase for rapid, high-specificity nucleic acid amplification. TrueLAMP chemistry suppresses primer-driven amplification in the absence of template, preventing nonspecific or false-positive reactions in no-template controls (NTCs). The liquid format provides flexibility for custom reaction design and scalable workflows while maintaining robust inhibition control.

**Specification**

- Product Name: TrueLAMP™
- Catalog #: TL0001S, TL0001M, TL0001L
- Contents:
    - TL0001S: 0.5 mL 2× TrueLAMP buffer and 5 µL TrueLAMP polymerase
    - TL0001M: 1.5 mL 2× TrueLAMP buffer and 15 µL TrueLAMP polymerase
    - TL0001L: 5 × (1.5 mL 2× TrueLAMP buffer and 15 µL TrueLAMP polymerase)
- Reactions:
    - TL0001S: 88 reactions (11 8-strips) at 10 µL each
    - TL0001M: 264 reactions (33 8-strips) at 10 µL each
    - TL0001L: 1320 reactions (165 8-strips) at 10 µL each

Storage: Store polymerase at –20 °C and buffer at 22 °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 this product. Boltii Diagnostics Inc. makes no warranties, expressed or implied, including but not limited to warranties of merchantability or fitness for a particular purpose.

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# TrueLAMP™ Assay Protocol

### 1. Prepare Reaction Mix

Prepare enough for 8 reactions (+10% excess). Use immediately.

| Component | 1 Reaction (µL) | 8 Reactions (µL) | +10% (µL) | Total (µL) |
|---|---|---|---|---|
| Nuclease-free 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 |

* It's critical to use calibrated pipettes for the 10 µL reactions.

### 2. Reaction Setup

1) Label each tube in an 8-strip 0.2 mL clear PCR tube set.
2) Add:
   - 1 µL nuclease-free water to NTC tube
   - 1 µL positive template (~500 copies, pre-validated) to PTC tube
   - 1 µL test sample to each test tube
3) Add 9 µL reaction mix to each tube.
4) Cap the tubes, vortex briefly, and centrifuge the 8-strip to remove bubbles.

### 3. Amplification

Incubate in submerged water bath or smart coffee mug or digital oven or thermocycler with heated lid:

• 58 °C for 10 min, then
• 65 °C for 50–80 min

### 4. Detection

**Colorimetric Readout:**
• Endpoint: photograph after incubation
• Semi-real-time: photograph every 15 min without moving camera
**Quantify using smartphone colorimeter app**:
• RGB mode → minimum green value
• CMYK mode → maximum magenta value

**Expected Results:**

NTC: red
PTC: yellow

# Frequently Asked Questions

**1. Should the kit be stored at -20 °C?**

Store polymerase at -20 °C and 2x buffer at 22 °C.

**2. How does TrueLAMP suppress nonspecific amplification?**

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

**3. What incubator works best?**

Uniform heating is critical to avoid evaporation and condensation in 10 µL reactions. Recommended: Fully submerged water bath or temperature-controlled smart coffee mug (POC) or thermocycler with heated lid. Evenly air-circulated digital oven may also work. Do not use heat bloc.

**4. Why is my NTC positive?**

1) Recalibrate pipettes. Incorrect volumes (< 4.75 µL buffer per 10 µL reaction) may reduce inhibition. 2) Verify incubation temperature. Nonspecific amplification can occur at ≤ 63 °C. 3) Increase buffer slightly (e.g., 5.5 µL 2× buffer per 10 µL reaction) for stubborn primer sets.

**5. Why did my positive control fail?**

Template concentration may be below LOD. Determine LOD for each primer set.

**6. Can raw samples (e.g., saliva, plasma) be used?**

Purified DNA/RNA is recommended. TrueLAMP is sensitive to pH and ionic strength. Water-suspended cells or virions may be compatible after validation.

**7. Can polymerase be premixed with 2× buffer for storage?**

No. Polymerase activity declines rapidly when premixed. Prepare reaction mix fresh.

**8. Why is a 58 °C pre-incubation needed and how long should reactions run?**

An initial 58 °C 10 min pre-incubation followed by 65 °C for 50-80 min improves amplification consistency and robustness across DNA and RNA targets, LAMP primer sets, and incubation systems (oven, bath, thermocycler). Amplification is generally visible by 30 min and reaches maximum by 90 min. However, strict endpoint timing is not required. Extended incubation does not typically alter endpoint color.

**9. How should color be quantified?**

Use a smartphone colorimeter app. Sample the region just below the liquid meniscus with the smallest aperture and record: minimum green (RGB mode) or maximum magenta (CMYK mode).