# **TrueLAMP™ Instruction Manual**

#### **General Information**

#### Description

TrueLAMP<sup>™</sup> is a proprietary colorimetric LAMP kit consisting of 2× TrueLAMP buffer and TrueLAMP polymerase. It is used to detect 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 reactions (NTCs).

### **Specification**

- Product Name: TrueLAMP™ Kit
- Catalog #: T0001S, T0001M, T0001L
- Contents:
  - 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 + 15 μL TrueLAMP polymerase)
- Reactions:
  - $\circ$  T0001S: 88 reactions (11 8-strips) at 10  $\mu$ L each
  - $\circ$  T0001M: 264 reactions (33 8-strips) at 10  $\mu$ L each
  - T0001L: 1320 reactions (165 8-strips) at 10 μL 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
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## TrueLAMP™ Assay Protocol

**A.** Before use, warm the TrueLAMP buffer to 22 °C (or in hand) for 30 min and vortex to dissolve any microcrystals (which may be barely visible against a light source) formed during storage at -20 to 4 °C. **B.** Because of the small 10  $\mu$ 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	3.00	24.00	2.40	26.40
10× LAMP primers	1.00	8.00	0.80	8.80
2× TrueLAMP buffer	5.00	40.00	4.00	44.00
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:
  - ο **1 μL deionized water** to the NTC tube.
  - $\circ$  1 µL positive template (500 copies) to the PTC tube.
  - 1 μL test sample to each of the six remaining tubes.
- c. Add  $9 \mu L$  of the LAMP reaction mix to each tube.

(You should have  $\sim$ 8  $\mu$ L left; otherwise, your pipettes may be inaccurate.)

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

#### 3. Start the LAMP Reaction

(Optional: Photograph the 8-strip before starting the reaction for before-after comparison.)

- a. Submerge the 8-strip (weighted) in a water bath at 65 °C.
- b. Incubate for **60–90 minutes**, or even overnight.
- c. After incubation, visually inspect and photograph the results.

(Optional: Quantify magenta color intensity with a colorimeter app, e.g., ColorMeter RGB Colorimeter.)

- 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× buffer may be kept at 4–22 °C, CO₂ 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 LAMP reactions?

The reaction must be heated evenly to prevent evaporation from small volumes. A submerged water bath works best. If using a heat block or thermocycler, overlay each tube with 10 µL mineral oil.

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

- Warm the buffer before use. Cold storage (-20 to 4 °C) can cause microcrystals that reduce inhibition.
- Recalibrate pipettes. Incorrect volumes ( $<4.75 \mu L 2x$  buffer or  $>0.07 \mu L$  polymerase per 10  $\mu L$  reaction) may cause failure of inhibition.
- Verify incubation temperature. Nonspecific amplification can occur at  $\leq$ 63 °C. Be aware that temperature distribution can be uneven in digital ovens, which may require calibration and adjust the placement of the 8-strips in the oven. For multiple 8-strip reactions, a water bath is advised.
- $\bullet$  Strengthen inhibition. Increase buffer slightly (e.g., 5.25  $\mu L$  2x buffer per 10  $\mu L$  reaction) for stubborn primer sets.

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

Not recommended. TrueLAMP is sensitive to pH and ionic strength. Using viral, bacterial, fungal, plant or mammalian cells has not been fully characterized. For best results, use purified DNA or RNA.

#### 6. 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.

## 7. How can I monitor TrueLAMP reactions kinetically at 65 °C?

If available, use a fluorescent dye and real-time PCR thermocycler with TrueLAMP. Alternatively, use a digital oven. Photograph the 8-strip at intervals (e.g., 0, 15, 30, 45, 60 min) with a stationed smartphone through the glass door. Quantify magenta intensity with a CMYK colorimeter app and plot intensity vs. time.