

## OmniTaq 2 LA

Cat #: 312



**Amount:** 125  $\mu$ l (500 x 25  $\mu$ l reactions)

**Shipping conditions:** Ambient

**Storage conditions:** -20°C

**Thermostability:** Retains at least 85% activity after 1 hour at 95°C

**Shelf life:** At least 5 years from date of receipt under proper storage conditions.

### PRODUCT DESCRIPTION:

OmniTaq 2 LA is a DNA polymerase mixture containing OmniTaq 2, a mutant of Taq DNA polymerase that provides strand-displacement and reverse transcriptase activity. It can be used as the sole enzyme in PCR, RT-PCR, and RT-LAMP assays. In addition, this enzyme provides 2-3x faster PCR and provides some inhibition-resistance. The Long-Accurate feature allows for amplification of longer products with higher fidelity and accuracy. LA enzymes are not recommended for use with dUTP. 10x Taq Mutant Reaction Buffer composition is: 500 mM Tris pH 9.1, 160 mM ammonium sulfate, 0.25% Brij 58, and 25 mM magnesium chloride. 10x RT-LAMP Buffer composition is: 500 mM Tris-Cl pH 8.55, 80 mM ammonium sulfate, 0.25% Brij 58, and 29 mM magnesium chloride.

### TYPICAL PCR/RT-PCR PROTOCOL for a 25 $\mu$ l reaction:

Reagent	Volume	Final Concentration
10x Taq Mutant Reaction Buffer	2.5 $\mu$ l	1x
dNTP mix (10 mM each)	0.5 $\mu$ l	200 $\mu$ M each
Left Primer	variable	200 nM
Right Primer	variable	200 nM
DNA or RNA template†	variable	Up to 1 ng/ $\mu$ l
PCR Enhancer Cocktail (optional)*	12.5 $\mu$ l	1x
OmniTaq 2 LA	0.05 – 0.25 $\mu$ l **	
De-ionized distilled H <sub>2</sub> O	Adjust final volume to 25 $\mu$ l	

† DNA (or RNA for RT-PCR) amount depends mostly on genome size and target gene copy number.

\* When inhibition-resistance is needed, we recommend using one of our PCR Enhancer Cocktails (PEC-1, PEC-1GC, PEC-2, or PEC-2-GC) which are specially formulated for use with whole blood, serum or plasma.

\*\* To determine specific optimal enzyme concentration, we strongly recommend an enzyme titration test for each target. Good starting amount of the enzyme per 25  $\mu$ l reaction is 0.05  $\mu$ l for purified DNA templates and 0.25  $\mu$ l for crude samples containing 5-10% whole blood, plasma or serum. Targets larger than 1 kb may require more enzyme.

### CYCLING CONDITIONS:

#### FOR PCR:

1. Initial Denaturing: 94° for 2-8 minutes recommended for crude samples containing 5-10% whole blood, plasma or serum.
2. Denaturing: 94° for 40-60 seconds
3. Annealing: 50°-68° depending on the specific T<sub>m</sub> primers for 40-60 seconds
4. Extension: 68° for 1 min/kb target
5. Repeat steps 2-4 for 25-40 cycles

#### FOR RT-PCR:

- RT: 1. 75° for 2-8 minutes. Some highly folded RNA templates may benefit from an initial 30 seconds at 94°.  
2. 68° for 30 minutes

#### PCR:

3. Denaturing: 94° for 40-60 seconds
4. Annealing: 50°-68° depending on the specific T<sub>m</sub> primers for 40-60 seconds
5. Extension: 68° for 1 min/kb target
6. Repeat steps 3-5 for 25-40 cycles

**RT-LAMP PROTOCOL for 25 ul reaction:**

Reagent	Volume	Final Concentration
10x RT-LAMP Buffer	2.5 µl	1x
5 M Betaine	3.75 ul	0.75 M
dNTP mix	variable	200 µM each
FIP, BIP, FL, and BL Primers	variable	640 nM
F3 and B3 Primers	variable	160 nM
RNA template*	variable	Up to 1 ng/ul
OmniTaq 2 LA	0.05 – 0.25 ul**	
De-ionized distilled H2O	Adjust final volume to 25 ul	

\* RNA amount depends mostly on genome size and target gene copy number.

\*\* To determine specific optimal enzyme concentration, we strongly recommend an enzyme titration test for each target.

Carry-over DNA contamination and false positives may be a concern in labs performing RT-LAMP. To address this, we recommend including the following in your reaction: RNase-Free DNase 10 ug/ml, CaCl<sub>2</sub> 0.1 mM, and DTT 4 mM. A ten minute incubation at room temperature will allow the DNase reaction to proceed. The DNase will be inactivated during the initial denaturation step. Alternately, you may include dUTP in your reactions (at 200 uM) and include Uracil DNA Glycosylase (UDG) to remove previously amplified products. Please refer to References for details.

**ISOTHERMAL REACTION CONDITIONS:**

1. RNA denaturation: 75° for 2-8 minutes. Some highly folded RNA templates may benefit from 30 seconds at 94°.
2. RT-LAMP: 68° for 50-120 minutes

**Please visit us on the web at [www.klentaq.com](http://www.klentaq.com) for troubleshooting and detailed protocols.**

**REFERENCES:**

Barnes, W. M., et al. (2021) A Single Amino Acid Change to Taq DNA Polymerase Enables Faster PCR, Reverse Transcription and Strand-Displacement. *Frontiers in Bioengineering and Biotechnology*. 8:553474. doi: 10.3389/fbioe.2020.553474 <https://doi.org/10.3389/fbioe.2020.553474>

Borst, et. Al (2004) False-Positive Results and Contamination in Nucleic Acid Amplification Assays: Suggestions for a Prevent and Destroy Strategy. *Eur J Clin Microbiol Infect Dis* **23**, 289–299. <https://doi.org/10.1007/s10096-004-1100-1>