Glycerol Free OmniTaq 2 Cat #: GF302

Amount: 1000 x 25 µl reactions
(equivalent to 250 ul standard OmniTaq 2. Volume may be up to 2.5x higher)
Shipping conditions: Ambient
Storage conditions: 4°C
Thermostability: Retains at least 85% activity after 1 hour at 95°C

PRODUCT DESCRIPTION:

A Glycerol-Free preparation of 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. 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 ul reaction:

Reagent	Volume	Final Concentration
10x Taq Mutant Reaction Buffer	2.5 µl	1x
dNTP mix (10 mM each)	0.5 µl	200 µM each
Left Primer	variable	200 nM
Right Primer	variable	200 nM
DNA or RNA template†	variable	Up to 1 ng/ul
PCR Enhancer Cocktail (optional)*	12.5 µl	1x
OmniTaq 2 DNA Polymerase enzyme	0.05 – 0.25 µl **	
De-ionized distilled H2O	Adjust final volume to 25 ul	

† 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 ul reaction is 0.05 ul for purified DNA templates and 0.25 ul for crude samples containing 5-10% whole blood, plasma or serum. Targets larger than 1 kb may require more enzyme or may benefit from the use of an LA (Long Accurate) version of the polymerase.

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 Tm 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 Tm 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	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.

Carrry-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, CaCl2 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 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