

5x OmniTaq 2 PCR/RT-PCR Kit

Cat #: 322

rev 9/6/21



Amount: 125 μ l enzyme and two 1.25 ml tubes of 5x Mastermix
(sufficient for 500 x 25 μ l reactions)

Shipping conditions: Ice Pack

Storage conditions: -20°C F

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

Shelf life: At least 1 year from date of receipt under proper storage conditions.

PRODUCT DESCRIPTION:

OmniTaq 2 is a mutant of Taq DNA polymerase that provides strand-displacement and reverse transcriptase activity. It can be used as the sole enzyme in RT-PCR and RT-LAMP assays. In addition, this enzyme provides 2-3x faster PCR and inhibition-resistance. This kit can be used for conventional as well as realtime PCR. For real-time reactions you may need to add a fluorescent dye as an alternative to probes. 5x Mastermix composition is: 250 mM Tris-Cl, 80 mM ammonium sulfate, 0.13% Brij 58, 1 mM each dNTP, and 12.5 mM magnesium chloride. Final pH is 9.1

TYPICAL PCR PROTOCOL for a 25 μ l reaction:

Reagent	Volume	Final Concentration
5x Mastermix	5 μ l	1x
Left Primer	variable	200 nM
Right Primer	variable	200 nM
DNA template†	variable	Up to 1 ng/ul
PCR Enhancer Cocktail (optional)*	12.5 μ l	1x
OmniTaq 2 DNA Polymerase enzyme	0.05 – 0.25 μ l **	
De-ionized distilled H2O	Adjust final volume to 25 μ l	

† DNA amount depends mostly on genome size and target gene copy number.

* If 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 μ l reaction is 0.05 μ l for purified DNA templates and 0.25 μ l 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 reverse transcriptase PCR (RT PCR) the only difference (besides the template being RNA) is to incubate for about 30 minutes with the cDNA primer (or both primers) before cycling. If this RT step is skipped, or changed to less than one minute, this could be the “no RT” control. Some highly folded RNA templates benefit from an initial 30 sec. at 94°.

We have not tried hexamer primers, and we have one bad report for trying this. More feedback is welcome.

Primers must be well mixed into the reaction before the RT step, whereas for plain PCR, convection can provide the mixing.

For PCR: For genomic DNA, Initial Denaturing: 94° for 2-3 minutes once, to break DNA and allow denaturation.

Each cycle:

Denaturing: 94° for 10-55 seconds (the 55 sec improves reliability if rxn volume is near maximum).

Annealing/Extension 50°-68° depending on the specific Tm of the primers for 40-300 seconds

Repeat for 25-40 cycles

Initial up to 8 min heating step is recommended for crude samples containing 5-10% whole blood, plasma or serum.

REFERENCE:

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>

Please visit us on the web at www.klentaq.com for troubleshooting and detailed protocols.

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