5x Hot Start OmniTaq PCR Kit Cat #: HS320



Amount: 125 μl EnzymePOLYMERA2 x 1.25 ml tubes of 5x TM-PCR-Mix
(sufficient for 500 x 25 μl reactions)POLYMERAShipping conditions: Ice PackStorage conditions: Ice PackStorage conditions: For best performance, store at -20°CThermostability: Retains at least 85% activity after 1 hour at 95°C

Shelf life: At least 1 year if stored at -20°C and 10 freeze/thaws or at least 1 month if stored at 4°C. **Expiration:** On tube label

PRODUCT DESCRIPTION:

Our PCR kit contains 5X concentrated master mix (TM-PCR Mix) lacking only the enzyme. The enzyme is provided in a separate vial, which allows an adjustment of its final concentration in PCR.

OmniTaq is a Triple mutant of Taq polymerase that makes the enzyme resistant to the inhibitory effects of blood, soil and more. It is made with aptamer-based technology, enabling room temperature reaction set-up. This kit can be used for conventional as well as real-time PCR. For real-time reactions you may need to add a fluorescent dye as an alternative to probes. LA enzymes are not recommended for use with dUTP. 5x TM-PCR-Mix composition is: 250 mM Tris-Cl, 80 mM ammonium sulfate, 0.13% Brij 58, 12.5 mM Magnesium Chloride, and 1 mM each dNTP. Final pH is 9.1.

TYPICAL PCR PROTOCOL for a 25 µl reaction:

| Reagent | Volume | Final Concentration |
|--------------------------------------|------------------------------|----------------------------|
| 5x TM-PCR-Mix | 5 µl | 1x |
| Left Primer | variable | 200 nM |
| Right Primer | variable | 200 nM |
| DNA template† | variable | 0.1-100 ng |
| PCR Enhancer Cocktail (recommended)* | 12.5 μl | 1x |
| Hot Start OmniTaq | 0.05 – 0.25 µl ** | |
| De-ionized distilled H2O | Adjust final volume to 25 µl | |

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

* For optimal performance, 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 or 1.3M Betaine, a generic PCR enhancer.

** To determine specific optimal enzyme concentration, we strongly recommend an enzyme titration test for each target. A good starting amount of the enzyme per 25 µl reaction is 0.05 µl for purified DNA templates and 0.25 µl 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 LA (Long-Accurate) version of the kit.

CYCLING CONDITIONS:

- 1. Denaturing: 94° for 2-8 minutes for 1 cycle *
- 2. Denaturing: 94° for 40-60 seconds
- 3. Annealing: $50^{\circ}-68^{\circ}$ depending on the specific Tm primers for 40-60 seconds
- 4. Extension: 68° for 2 min/kb target
- 5. Repeat steps 2-4 for 25-40 cycles

Initial 2-8 min heating step is recommended for crude samples containing 5-10% whole blood, plasma or serum.

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

REFERENCES:

Kermekchiev, M.B., et al. (2003) Cold-sensitive mutants of Taq DNA polymerase provide a hot start for PCR. Nucl Acids Res. 31, 6139-6147.

Kermekchiev, M.B. et al. (2009) Mutants of Taq DNA polymerase resistant to PCR inhibitors allow DNA amplification from whole blood and crude soil samples. Nucl. Acids Res., 37 (5):e40 E pub.