5x Hot Start OmniTaq 3 PCR Kit Cat #: HS323

Amount: 125 µl Enzyme
2 x 1.25 ml tubes of 5x TM-PCR-Mix
(sufficient for 500 x 25 µl reactions)
Shipping conditions: Ice Pack
Storage conditions: For best performance, store at -20°C
Thermostability: 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 (5x TM-PCR Mix) lacking only the Hot Start OmniTaq 3 enzyme. The enzyme is provided in a separate vial, which allows an adjustment of its final concentration in PCR.

OmniTaq 3 is a mutant of Taq Polymerase showing enhanced performance. It is made with aptamer-based technology, enabling room temperature reaction set-up. It 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 TM-PCR-Mix 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 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 3	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. 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.

CYCLING CONDITIONS:
1. Denaturing: 94° for 2-8 minutes for 1 cycle *
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 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.