5x Hot Start Cesium Klentaq C PCR Kit Cat #: HS285

Amount: 125 µl Enzyme
2 x 1.25 ml tubes of 5x KM-PCR-Mix
(sufficient for 500 x 25 µl reactions)
Shipping conditions: Ambient
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 KM-PCR Mix) lacking only the Hot Start Cesium Klentaq C enzyme. The enzyme is provided in a separate vial, which allows an adjustment of its final concentration in PCR.

Cesium Klentaq C is a cold-sensitive mutant of Klentaq1. Due to its suppressed activity at low temperatures this enzyme is designed for hot start PCR performance. It is made with aptamer-based technology further enhancing its hot start performance. This kit can be used for conventional, as well as real-time PCR. For real-time applications you may need to add a fluorescent dye as an alternative to probes. 5x KM-PCR-Mix composition is: 250 mM Tris-Cl pH 9.2, 80 mM ammonium sulfate, 0.125% Brij 58, 17.5 mM magnesium chloride, and 1 mM each dNTP,

TYPICAL PCR PROTOCOL for a 25 µl reaction:

Reagent	Volume	Final Concentration
5x KM-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 Cesium Klentaq C	0.05 – 0.25 µl **	
De-ionized distilled H2O	Adjust final volume to 25 ul	

† 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°-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.