

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: 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.

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 ul 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.

** 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 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.