5x Cesium Klentaq AC LA PCR Kit Cat #: 270



Amount: 125 μl Cesium Klentaq AC LA 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.

Expiration: On tube label

PRODUCT DESCRIPTION:

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

Cesium Klentaq AC is a cold-sensitive double mutant of Klentaq1 DNA polymerase. Due to its suppressed activity at low temperatures this enzyme is designed for hot-start PCR performance. It also features the Long-Accurate feature that allows amplification of longer products with higher fidelity and accuracy. 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
Cesium Klentaq AC LA	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 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.

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.