

Hot Start Cesium Klentaq AC Cat #: HS250



Amount: 100 μ l (1000 x 50 μ l reactions)

Shipping conditions: Ambient temperature

Storage conditions: -20°C

Thermostability: Retains at least 85% activity after 1 hour at 95°C

Shelf life: At least 1 year from date of receipt under proper storage conditions.

PRODUCT DESCRIPTION:

Hot Start Cesium Klentaq AC is made with aptamer-based technology, enabling room temperature reaction set-up.

Cesium Klentaq AC is a double cold-sensitive mutant of Klentaq1 (5'-exonuclease deficient Taq polymerase with improved fidelity and thermostability). Due to its suppressed activity at low temperatures this enzyme is designed for hot start PCR performance. Addition of the aptamer further enhances its hot start performance.

The aptamer binds to the polymerase at sub-cycling temperatures, inactivating the enzyme and preventing spurious amplification. 10x buffer composition is: 500 mM Tris-Cl pH 9.2, 160 mM ammonium sulfate, 0.25% Brij 58, and 35 mM magnesium chloride.

TYPICAL PCR PROTOCOL for a 25 μ l reaction:

Reagent	Volume	Final Concentration
10x Klentaq Mutant Reaction Buffer	2.5 μ l	1x
dNTP mix (10 mM each)	0.5 μ l	200 μ M each
Left Primer	variable	200 nM
Right Primer	variable	200 nM
DNA template†	variable	0.1-100 ng
PCR Enhancer Cocktail (recommended)*	12.5 μ l	1x
Hot Start Cesium Klentaq AC	0.05 – 0.25 μ l **	
De-ionized distilled H2O	Adjust final volume to 25 μ l	

† 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) or 1.3 M Betaine, which is a general PCR enhancer.

** To determine specific optimal enzyme concentration, we strongly recommend an enzyme titration test for each target. Targets larger than 1 kb may require more enzyme or may benefit from the Long Accurate (LA) version of the 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 T_m primers for 40-60 seconds
4. Extension: 68° for 2 min/kb target
5. Repeat steps 2-4 for 25-40 cycles

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.