

5x Cesium Klentaq AC LA PCR Kit

Cat #: 270

Amount: 125 µl enzyme and two 1.25 ml tubes of 5x Mastermix
(sufficient for 500 x 25 µl reactions)

Shipping conditions: Ice Pack

Storage conditions: -20°C F

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:

Our 5x ready-to-use PCR kit contains Cesium Klentaq AC LA, 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. LA enzymes are not recommended for use with dUTP.

5X Mastermix 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 Mastermix	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 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.

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.

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