

Rapid Klentaq Cat #: 440

Amount: 25 µl (25 units/µl or 250 reactions) per tube

Shipping conditions: Ambient temperature

Storage conditions: -20°C for enzyme, 4°C for 10x Rapid Klentaq buffer

Thermo stability: 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:

Rapid Klentaq is a double cold-sensitive mutant of Klentaq1 (5'-exonuclease deficient Taq polymerase with improved fidelity and thermostability). This enzyme is designed to provide robust amplification with a very short extension time. Due to its suppressed activity at low temperatures, it can perform hot-start PCR as well. 10x buffer composition is: 500 mM Tris-Cl pH 9.2, 160 mM ammonium sulfate, 0.25% Brij 58, and 35 mM magnesium chloride. We also offer (upon request) 10x buffer at pH 7.9 for better fidelity.

TYPICAL PCR PROTOCOL for a 25µl reaction:

Reagent	Volume	Final Concentration
10x Rapid Klentaq PCR buffer [†]	2.5µl	1x
dNTP mix (10 mM)	0.5µl	200µM each
Left Primer	variable	200 nM
Right Primer	variable	200 nM
DNA template [†]	variable	0.1-100 ng
Betaine 5M*	6.5 µl (optional)	1.3 M
Rapid Klentaq**	0.05 µl	2.5 unit
de-ionized distilled H ₂ O	Adjust final volume to 25 µl	-

[†] DNA amount depends mostly on genome size and target gene copy number.

*Betaine is a general PCR enhancer. It usually improves the yield and specificity of amplification especially for longer targets.

**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 use of an LA (Long Accurate) version of the polymerase.

CYCLING CONDITIONS

1. Denaturing: 94° for 2 minutes for 1 cycle
2. Denaturing: 94° for 30-45 seconds
3. Annealing: 50°-68° (depending on the specific T_m of primers) for 40-60 seconds
4. Extension: 68° for as little as 10 seconds for a 600 bp target (longer targets may require longer extension for optimal results. Try 2 min/kb to start.)
5. Repeat steps 2-4 for 25-40 cycles

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.

Please visit us on the web at www.klentaq.com for troubleshooting and detailed protocols.

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