

5x RapidTaq-LA PCR Kit

Amount: 2 x 1.25 ml (250 reactions)

Shipping conditions: Ice pack

Storage conditions: for best performance, store at -20°C

Shelf life: At least 1 year if stored at -20°C and 10 freeze/thaws or at least 3 months if stored at 4°C.

PRODUCT DESCRIPTION:

Our 5x ready-to-use PCR kit contains RapidTaq-LA, a cold-sensitive double mutant of Taq polymerase with the Long-and-Accurate feature that allows amplification of longer products with higher fidelity and accuracy. 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. This kit can be used for regular, as well as real-time PCR. It contains everything necessary for a PCR reaction to work perfectly, just add your template, primers/probes and water. For real-time reactions you may need to add a fluorescent dye as an alternative to probes. 5X composition is: 5x RapidTaq-LA DNA Polymerase, 1 mM dNTPs, 250 mM Tris-Cl pH 9.2, 80 mM ammonium sulfate, 0.5% Tween 20, and 17.5 mM magnesium chloride.

TYPICAL PCR PROTOCOL for a 50µl reaction:

Reagent	Volume	Final Concentration
5x RapidTaq-LA PCR Kit reagent	10 µl	1x
Left Primer	variable	0.2 µM
Right Primer	variable	0.2 µM
DNA template [†]	Variable	0.5-100ng
Betaine 5M*	13µl (optional)	1.3 M
de-ionized distilled H ₂ O	Adjust final volume to 50µl	-

[†]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.

CYCLING CONDITIONS:

1. Pre-incubation: 94° for 2 minutes for 1 cycle
2. Denaturing: 94° for 40-60 seconds
3. Annealing: 55°-70° depending on the specific primers (5° less than T_m) 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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