5x Klentaq-S PCR Kit Cat #: 125

Amount: 100 µl enyzme
2 x 1.25 ml tubes of 5X KT-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 KTS-PCR Mix) lacking only the Klentaq-S enzyme. The enzyme is provided in a separate vial, which allows an adjustment of its final concentration in PCR.

Our 5x ready-to-use PCR kit contains Klentaq-S, a mutant of Klentaq1 that has the feature of incorporating both dNTPs and ddNTPS, for use in Pyrophosphorolysis-Activated Polymerization (PAP) for excellent specificity of primer binding. 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 KTS-PCR Mix composition is: 450 uM Na4PPi, 250 mM Tris-Cl pH 7.8, 80 mM ammonium sulfate, 10% DMSO, 0.125% Brij 58, 17.5 mM Magnesium Chloride, and 1 mM each dNTP.

TYPICAL PCR PROTOCOL for Pyrophosphorolysis-Activated Polymerization (PAP) for a 25 µl reaction:

Reagent	Volume	Final Concentration
5X KTS-PCR Mix	5 µl	1x
Left Primer	variable	200 nM
Right Primer	variable	200 nM
DNA template†	variable	100-200 ng
Klentaq-S*	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.
* 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 LA (Long-Accurate) version of the enzyme.

CYCLING CONDITIONS*:

Initial denaturing: 95° for 2 minutes

25 "Touchdown" cycles: 94° for 15 seconds
60° for 30 seconds
64° for 30 seconds
68° for 1 minute
72° for 1 minute

*Suggested conditions for PAP for 25 µl reactions. Optimal temperatures may vary depending on primer sequence. Extension times may be increased for longer targets. We typically recommend 1 minute + 1 minute per kb target.

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

REFERENCES:
Barnes, W.M. (1994) PCR amplification of up to 35 kb DNA with high fidelity and high yield from bacteriophage templates, PNAS 91, 2216-2220.

US Patent No 5.436.149