Hot Start Klentaq-S Cat #: HS105

Amount: 100 µl enzyme (sufficient for 2000 x 25 µl reactions up to 1 kb)
Shipping conditions: Ambient
Storage conditions: -20°C
Expiration: On tube label

PRODUCT DESCRIPTION:
Hot Start Klentaq-S is made with aptamer-based technology, enabling room temperature reaction set-up. Klentaq-S is a mutant of Klentaq that has the feature of incorporating both dNTPs and ddNTPS. It can be used in Pyrophosphorolysis-Activated Polymerization (PAP) for excellent specificity of primer binding. The hot-start 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 7.8, 160 mM ammonium sulfate, 0.25% Brij 58, and 35 mM magnesium chloride. The enzyme may not perform as well at a higher pH.

TYPICAL PCR PROTOCOL for a 25 µl reaction:

Reagent	Volume	Final Concentration
10x Klentaq-S reaction buffer	2.5 µl	1x
dNTP mix (10 mM)	0.0625 - 0.5 ul	25 - 200 uM each
Left Primer	variable	25 - 200 nM
Right Primer	variable	25 - 200 nM
Na4PPi	variable	90 uM
DMSO	variable	2%
BSA (optional)	variable	0.15 mg/ml
DNA template†	variable	100 - 200 ng
Hot Start Klentaq-S**	0.05 – 0.25 µl **	
De-ionized distilled H_2O	Adjust final volume to 25 ul	-

† 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 polymerase.

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 ul 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:
Liu Q and Sommer SS. (2002) Pyrophosphorolysis-activatable oligonucleotides may facilitate detection of rare alleles, mutation scanning and analysis of chromatin structures. Nucleic Acids Res. 30(2):598-604.

Liu Q, et al. (2006) Multiplex dosage pyrophosphorolysis-activated polymerization: application to the detection of heterozygous deletions. Biotechniques 40(5):661-8.