Product information

Product Name: Hot-start KlenTaq DNA Polymerase

Catalog No: VN100HS and VN101HS

Packing Size: 125 µl and 50 µl

Shipping Condition: Ambient temperature

Storage Condition: -20^{0}C

Hot-start KlenTaq DNA Polymerase is a mixture of KlenTaq DNA Polymerase and an aptamer-based inhibitor. The inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at low temperatures. This prevents primer-dimer formation and spurious amplification, increasing specificity. During normal cycling conditions, the inhibitor releases the enzyme, allowing efficient amplification.

KlenTaq DNA Polymerase is a Taq polymerase variant that lacks 5'-exonuclease activity due to an N-terminal deletion of Taq. This modification results in improved fidelity and thermostability compared to the wild-type Taq. Hot-start KlenTaq DNA Polymerase can be used in real-time PCR with DNA binding dyes, such as SYBR Green and Eva Green. However, it cannot be used in real-time PCR TaqMan assays, which require 5'-exonuclease activity.

It is supplied with 10X KlenTaq Mutant Buffer**:**

500 mM Tris-HCl 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.

Protocol:

PCR setting for a 25 µl reaction

Reagent	Volume	Final Concentration
10x KlenTaq Mutant Buffer	2.5 µl	1x
dNTP Mix (10 mM)	0.5-1.0 µl	200-400 µM each
Left Primer	Variable	0.2-0.4 µM
Right Primer	Variable	0.2-0.4 µM
DNA template† / Blood / Serum / Plasma	Variable	0.1-100 ng < 5 µl
PCR Enhancer Cocktail (recommended for crude samples)*	Titration	Variable
Hot-start KlenTaq Polymerase**	0.05-0.25 µl	
De-ionized Distilled H_2O	Adjust final volume to 25 µl	-

† The amount of DNA template required for PCR depends primarily on the genome size and target gene copy number.
* For optimal PCR performance, we recommend using one of our PCR Enhancer Cocktails (see PCR enhancers for details) if you encounter difficulty amplifying your target. The PEC is not included with the enzyme and must be ordered separately if needed. A titration of PEC is also recommended to determine the optimal concentration for your targets and to mitigate inhibition in the samples.
**To determine the specific optimal enzyme concentration, we strongly recommend performing an enzyme titration test for each target, starting from 0.05 µl per 25 µl reaction volume, to determine the optimal amount for the application. Targets larger than 1 kb may require a higher enzyme concentration.

Typical Cycling Parameters

Initial denaturation	95^{0}C	2-5 min (for purified DNA) 5-15 min (for crude samples)
25-40 cycles		
Denaturation	94^{0}C	20 to 30 sec
Annealing	50^{0}C to 68^{0}C	20 to 60 sec
Extension	70^{0}C	1-2 min / 1kb target
Final Extension	70^{0}C	5 min
Hold	4^{0}C	

Troubleshooting guide

No PCR products	Please check your PCR settings to ensure that all necessary components are included in the PCR mix. It's also recommended to perform a gradient annealing temperature experiment to determine the optimal temperature for your specific target. This can help to improve PCR efficiency and specificity, resulting in more reliable and consistent results.
The bands in agarose gel are smear	Enzyme titration for a particular target. It involves testing a range of enzyme concentrations to find the concentration that produces the most efficient and accurate results. It's important to note that using too much enzyme can actually inhibit the PCR reaction or overamplify the target, especially when working with short and simple target genes. Additionally, it's important to check for DNA and primer degradation as degraded DNA or primers can also negatively impact the PCR results.
Low yield of products	If you are using crude samples, it's important to initially denature the samples for 5-15 minutes to ensure proper release of DNA. Additionally, you can try the following strategies to improve PCR performance: • Conduct an enzyme titration experiment to optimize the enzyme concentration for your specific target. • Increase the extension time to ensure complete amplification of the target DNA. • Try a gradient annealing temperature experiment to identify the optimal annealing temperature for your primers. • Consider redesigning your primers if the amplification is not specific or efficient enough.
Non-specific products are observed	If you are observing non-specific products in your PCR reaction, there are several strategies you can try: • Perform a gradient annealing temperature experiment to identify the optimal temperature for your primers, which can help to reduce non-specific products. • Check the GC content of your target sequence. If it's above 65%, it may be necessary to use our PEC-GC. • Evaluate your primers and redesign them if necessary to improve specificity and reduce non-specific products.

Note: This product is for R&D use only

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