Product information

Product Name: Hot-start OmniTaq 3 DNA Polymerase

Catalog No: VN1800HS and VN1801HS

Packing Size: 125 µl and 50 µl

Shipping Condition: Ambient temperature

Storage Condition: -20^{0}C

Hot-start OmniTaq 3 DNA Polymerase is a novel enzyme mixture composed of OmniTaq 3 DNA Polymerase and an aptamer-based inhibitor. This inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at low temperatures to prevent non-specific amplification such as primer dimer formation, but releases the enzyme during normal cycling conditions to enable efficient amplification. The hot-start feature of this enzyme allows reactions to be set up at room temperature, increasing specificity and sensitivity of PCR.

This enzyme is a new generation mutant of Taq DNA polymerase that is able to tolerate high levels of PCR inhibitors, making it suitable for challenging samples such as blood and soil. It is compatible with real-time PCR using both DNA binding dyes, such as SYBR Green and Eva Green, and TaqMan assays that require 5'-exonuclease activity.

It is supplied with 10X Taq Mutant Buffer**:**
500 mM Tris-HCl pH 9.1, 160 mM ammonium sulfate, 0.25% Brij-58, and 25 mM magnesium chloride.

Protocol:

PCR setting for a 25 µl reaction

Reagent	Volume	Final Concentration
10x Taq 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 OmniTaq 3 Polymerase**	0.1-0.25 µl	
De-ionized Distilled H_2O	Adjust final volume to 25 µl	-

†The amount of DNA used in PCR reactions should be optimized based on the size of the target gene, the number of copies of the target gene in the sample, and the overall genome size of the organism.
* For optimal performance, we recommend using one of our PCR Enhancer Cocktails (see PCR enhancers for details) that are specially formulated for use with whole blood, serum, plasma, or other crude samples. A titration of PEC is recommended to determine the optimal amount for your target.
** To determine the specific optimal enzyme concentration, we strongly recommend conducting an enzyme titration test for each target. A good starting amount of enzyme per 25 µl reaction is 0.05 µl for purified DNA templates and 0.125 µl for crude samples containing 5% or more whole blood, plasma, or serum. Targets larger than 1 kb may require more enzyme or the use of the Long Accurate version.

Typical Cycling Parameters

Initial denaturation	95^{0}C	2-5 min (for purified DNA) 5-15 min (for crude sample)
25-40 cycles		
Denaturation	94^{0}C	20 to 40 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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