Product information

Product Name: ZipTaq LA DNA Polymerase

Catalog No: VN3010E, VN3011E

Packing Size: 125 µl and 50 µl

Shipping Condition: Ambient temperature

Storage Condition: -20^{0}C

Product Description:

ZipTaq LA DNA Polymerase is a new generation mutant of Taq polymerase with multiple mutations that make it the fastest enzyme in our collection. With the addition of one of our PECs, it can tolerate up to 40% blood in the reaction. The Long-and-Accurate feature allows for amplification of longer products with higher fidelity and accuracy. This enzyme can be used in real-time PCR with DNA binding dyes, such as SYBR Green and Eva Green, however, it is better to choose non-LA version in real-time PCR TaqMan assay.

Protocol:

PCR setting for a 25 µl reaction

Reagent	Volume	Final Concentration
10x ZipTaq Reaction Buffer	2.5 µl	1x
dNTP Mix (10 mM)	0.5 µl	200 µM each
Left Primer	Variable	0.4 µM
Right Primer	Variable	0.4 µM
DNA template†	Variable	0.1-100 ng
PCR Enhancer Cocktail (recommended for crude samples)*	Titration	Variable
ZipTaq LA Polymerase**	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.
** The typical enzyme amount is 0.25 µl. Targets larger than 1 kb may require more enzyme. Enzyme titration is highly recommended.

Typical Cycling Parameters
Three-step PCR

Initial denaturation	95^{0}C	1-2 min (for purified DNA) 5 min (for crude samples)
25-40 cycles		
Denaturation	94^{0}C	1 to 5 sec
Annealing	50^{0}C to 68^{0}C (depending on Tm of primers)	1 to 5 sec*
Extension	68-70^{0}C	1-5 sec / 1kb target*
Final Extension	68-70^{0}C	1-5 min
Hold	4^{0}C	

Two-step PCR

Initial denaturation	95^{0}C	1-2 min (for purified DNA) 5 min (for crude samples)
25-40 cycles		
Denaturation	94^{0}C	1 to 5 sec*
Annealing/Extension	60^{0}C to 68^{0}C (depending on Tm of primers)	1 to 5 sec / 1kb target*
Final Extension	65-70^{0}C	1-5 min
Hold	4^{0}C	

*Exact number of seconds will depend on the thermocycler and target. If you use crude sample, the extension time will increase to 20 sec / 1kb. We recommend experimentation to determine precise cycling parameters.

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 at least 5 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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