Product Manual

AMPIGENE® HS Taq DNA Polymerase

Catalog #: ENZ-PRT101

ENZ-PRT101-0500 for 500 units

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Product Manual

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions.

Please contact Enzo Life Sciences Technical Support if necessary.

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DESCRIPTION

AmipiGene™ HS Taq DNA Polymerase uses the latest developments in polymerase technology and buffer chemistry to enhance PCR speed, yield and specificity. The enzyme uses advanced hot-start technology for superior sensitivity. The enzyme and buffer system allow for superior PCR performance on complex templates such as mammalian genomic DNA. Due to enhanced efficiency and specificity the enzyme is perfectly suited to difficult PCR.

AMPIGENE® HS Taq is a robust enzyme for all your everyday PCR applications including genotyping, multiplex PCR, screening, library construction, colony PCR and PCR direct from blood and urine. AMPIGENE® HS Taq DNA Polymerase can perform consistently well on a broad range of templates (including both GC and AT rich).

AMPIGENE® HS Taq DNA Polymerase has an error rate of approximately 1 error per 2.0 x 10^5 nucleotides incorporated. PCR products generated with AMPIGENE® HS Taq DNA Polymerase are A-tailed and may be cloned into TA cloning vectors.

Component	500 units
AMPIGENE® HS Taq DNA polymerase (5u/µl)	2 x 50µl
5x AMPIGENE® reaction buffer	4 x 1ml

High-throughput screening has resulted in a buffer system that allows efficient amplification from GC-rich and AT-rich templates, under fast and standard cycling conditions.

AMPIGENE® HS Taq Mix is particularly resistant to PCR inhibitors. The mix is suitable for direct PCR from unprocessed samples including bacterial culture, bacterial colonies, blood and urine.

SHIPPING AND STORAGE

Protect from prolonged exposure to light.

On arrival the kit should be stored at -20°C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4°C for 1 month. The kit can go through 30 freeze/thaw cycles with no loss of activity.

IMPORTANT CONSIDERATIONS

5x AMPIGENE® Reaction buffer: The 5x reaction buffer contains 15mM $MgCl_2$, 5mM dNTPs, enhancers and stabilizers. It is not recommended to add further PCR enhancers or $MgCl_2$ to the reaction. The buffer composition has been optimized to maximize PCR success rates.

Template: For eukaryotic DNA use between 5ng and 500ng per reaction, for cDNA use below 100ng per reaction.

Primers: Primers should have a predicted melting temperature of around 60°C, using default Primer 3 settings. The final primer concentration in the reaction should be between 0.2µM and 0.6µM.

Annealing: We recommend performing a temperature gradient to experimentally determine the optimal annealing temperature. Alternatively, we recommend a 55°C annealing temperature then increase in 2°C increments if non-specific products are present.

Extension: Optimal extension is achieved at 72°C. The optimal extension time is dependent on amplicon length and complexity of template. 15 seconds per kilobase (kb) is recommended for amplification from eukaryotic DNA for amplicons between 1kb and 6kb. For shorter amplicons a 1 second extension is sufficient.

Multiplex PCR: When first performing multiplex PCR it is recommended to run an annealing temperature gradient from 55°C to 65°C. The annealing temperature that results in the best specificity should be used in subsequent experiments. Fast cycling conditions should not be used for multiplex PCR. Initially, we recommend a 90 second extension time. This time may be further extended to increase yield.

Colony PCR: From bacterial colonies use a sterile tip to pick a colony and resuspend into a 50µl reaction as described below. From liquid culture add 5µl of overnight culture to the final mix. Increase initial denaturation time to 10 minutes.

Direct blood/urine PCR: Add 2µl mammalian blood or urine to a 50µl reaction as described below.

REACTION SETUP

1. Prepare a master mix based on the following table:

Reagent	50µl reaction	Final concentration	Notes
5x AMPIGENE® Reaction buffer	10.0µl	1x	
Forward primer (10µM)	2.0µl	400nM	See above for optimal primer design
Reverse primer (10µM)	2.0µl	400nM	
Template DNA	<100ng cDNA, <500ng genomic	variable	See above for template considerations
AMPIGENE® HS Taq DNA polymerase (5u/µl)	0.25µl - 1.0µl		
PCR grade dH_2O	Up to 50µl final volume		

2. Cycle using conditions based on the following table:

Cycles	Temperature	Time	Notes
1	95°C	1-2min	Initial denaturation and enzyme activation. For colony PCR increase denaturation time to 10 minutes
40	95°C 55°C to 65°C 72°C	15 seconds 15 seconds 1-90 seconds	Denaturation Anneal Extension (15 seconds per kb). For multiplex PCR use 90 seconds.

NOTES

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