



AMPIGENE® Hot Start High Fidelity Polymerase

REF ENZ-NUC133-0100	100 Units
REF ENZ-NUC133-0500	500 Units

INTENDED USE:

For Research Use Only. Not for use in diagnostic procedures.

SUMMARY AND EXPLANATION

AMPIGENE® Hot Start High Fidelity Polymerase is a robust and versatile proofreading enzyme technology for highly precise PCR. The enzyme is designed for all PCR applications where greater sequence accuracy is required, together with improved PCR success rates of long and challenging templates.

AMPIGENE® Hot Start High Fidelity Polymerase is derived from Pfu DNA polymerase for its 3'-5' exonuclease (proofreading) activity. The enzyme is engineered with proprietary mutations that significantly increase processivity, resulting in shorter extension times (30 s/kb), higher yields and the ability to amplify longer and more difficult targets, including eukaryotic genomic templates in excess of 17.5 kb.

AMPIGENE® Hot Start High Fidelity Polymerase uses a proprietary technology aptamer-like molecule that reversibly inhibits both the 3'-5' exonuclease activity and 5'-3' polymerase activity of the enzyme at ambient temperatures. This unique hot start molecule prevents primer dimer formation and non-specific amplification to maximize the sensitivity and specificity of your PCR. This feature makes the enzyme highly suitable for multiplexing and enables reactions to be set up at room temperature.

The enhanced accuracy of **AMPIGENE® Hot Start High Fidelity Polymerase** (2 U/μL) results in fidelity that is approximately 100 times higher than Taq DNA polymerase. The enzyme is ideal for applications where greater accuracy is needed, such as cloning, site-directed mutagenesis and sequencing. PCR products generated with this range of products are blunt ended.

AMPIGENE® Hot Start High Fidelity Polymerase (2 U/μL) is provided with an advanced buffer system including dNTPs, Mg and enhancers, enabling high fidelity PCR of a wide range of targets and fragment sizes regardless of GC content.

ASSAY PRINCIPLE

Polymerase chain reaction (PCR) uses a polymerase enzyme, which directs the synthesis of DNA from deoxynucleotide substrates on a single-stranded DNA template, by adding nucleotides to the 3' end of a custom-designed oligonucleotide annealed to the template DNA¹.

KNOWN APPLICATION

Amplification of nucleic acid targets with PCR methods.

PRODUCTS SUPPLIED

Component	100 units	500 units
AMPIGENE® Hot Start High Fidelity Polymerase (2U/μL)	1 x 50 μL	1 x 250 μL
AMPIGENE® Hot Start High Fidelity Buffer (5X)	1 x 1.7 mL	3 x 1.7 mL
AMPIGENE® High Fidelity Enhancer (10X)	1 x 1.7 mL	2 x 1.7 mL

MATERIALS NEEDED (Not Provided)

- Target DNA
- Primers
- Thermal Cycler

STORAGE AND SHELF-LIFE

- Upon receipt, store kit at -20°C. These products are stable under these conditions up to the expiration date indicated in the vial label.
- Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months from date of receipt. 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.

PERFORMANCE CONSIDERATIONS

1. Do not use reagents past their expiration date.
2. Cross-contamination of samples could cause false results. Use care when working with more than one sample.

LIMITATIONS

- This procedure is for research use only. It is not intended for diagnostic or therapeutic use.

PRECAUTIONS

1. Refer to reagent Safety Data Sheet (SDS) from precautions.
2. Specimens, before and after fixation, and all materials exposed to them should be handled and disposed of with proper precautions.
3. Never pipette reagents by mouth and avoid contact with skin and mucous membranes with reagents and specimens. If reagents and/or specimens come into contact with sensitive areas, rinse thoroughly with water and follow your institution's safety protocols.

TECHNICAL NOTES

GLOBAL HEADQUARTERS

Enzo Life Sciences, Inc.
81 Executive Blvd, Ste 3
Farmingdale, NY 11735 USA
T 1-800-942-0430
F 1-610-941-9252
E info-usa@enzolifesciences.com
www.enzolifesciences.com

EUROPE

Enzo Life Sciences (ELS) AG
Industriestrasse 17, Postfach
CH-4415 Lausen, Switzerland
T +41 61 926 89 89
F +41 61 926 89 79
E info-ch@enzolifesciences.com
www.enzolifesciences.com



For technical support and troubleshooting you can submit a technical enquiry online, call us direct, or alternatively email with the following information:

- Amplicon size
- Reaction setup
- Cycling conditions
- Screen grabs of gel images

INSTRUCTIONS FOR USE

AMPIGENE® Hot Start High Fidelity Buffer: The 5X buffer contains 15 mM MgCl₂, 5 mM dNTPs, enhancers and stabilizers. It is not recommended to add further MgCl₂ to the reaction. The buffer composition has been optimized to maximize PCR success rates.

Reaction Enhancer: In situations where no amplification is observed, we recommend adding the 10X AMPIGENE® High Fidelity Enhancer to the reaction mix. This enhancer can improve the performance of AMPIGENE® Hot Start High Fidelity Polymerase (2 U/μL) on some difficult or long templates, for example GC-rich templates or those with complex secondary structures.

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 0.2 μM and 0.6 μM.

Denaturation: Denaturation should be performed at 95 °C. However, if the presence of high GC regions results in low yields, increasing the melting temperature to 98-100 °C can improve the amount of product.

Annealing: We recommend performing a temperature gradient to experimentally determine the optimal annealing temperature. Alternatively, we recommend a 60 °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. 30 seconds per kilobase (kb) is recommended for most applications. Two-step cycling protocols may also be used with combined annealing and extension at 68-75 °C.

Multiplex PCR: The optimal extension time for multiplex reactions will be dependent on the complexity of template, the length of amplicons, and the number of targets. We recommend starting with the extension time of the longest fragment, and then increasing in increments of between 10 and 30 seconds if necessary.

Reaction Setup

1. Allow 5X AMPIGENE® Hot Start High Fidelity Buffer (and 10X AMPIGENE® High Fidelity Enhancer, if used) to reach room temperature, then briefly vortex.
2. Prepare a master mix based on the following table:

Reagent	25 μL reaction	Final Concentration	Notes
AMPIGENE® Hot Start High Fidelity Buffer (5X)	5.0 μL	1X	
AMPIGENE® High Fidelity Enhancer (10X) (optional)	2.5 μL	1X	See above for use of enhancer
Forward Primer (10 μM)	1.0 μL	400 nM	See above for optimal primer design
Reverse Primer (10 μM)	1.0 μL	400 nM	
Template DNA	< 100 ng genomic DNA < 5 ng less complex DNA	variable	
AMPIGENE® Hot Start High Fidelity Polymerase (2U/ μL)	0.25 μL		
PCR grade dH ₂ O	Up to 25 μL final volume		

3. Cycle using conditions based on the following table.

Step	Temperature	Time	Cycles
Initial Denaturation	95°C	1 min	1
Denaturation	95°C	15 secs	
Annealing	60°C-75°C	15 secs	25-35
Extension	72°C	30 seconds/kb	

REFERENCES

1. Mullis K, Faloona F, Scharf S, Saiki R, Horn G, Erlich H. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harb Symp Quant Biol. 1986;51 Pt 1:263-73.

GLOBAL HEADQUARTERS

Enzo Life Sciences, Inc.
81 Executive Blvd, Ste 3
Farmingdale, NY 11735 USA
T 1-800-942-0430
F 1-610-941-9252
E info-usa@enzolifesciences.com
www.enzolifesciences.com

EUROPE

Enzo Life Sciences (ELS) AG
Industriestrasse 17, Postfach
CH-4415 Lausen, Switzerland
T +41 61 926 89 89
F +41 61 926 89 79
E info-ch@enzolifesciences.com
www.enzolifesciences.com