AMPIGENE® High Fidelity Polymerase

REF ENZ-NUC130-0100 100 Units
REF ENZ-NUC130-0500 500 Units

INTENDED USE:

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

SUMMARY AND EXPLANATION

AMPIGENE® High Fidelity Polymerase is a versatile and robust high fidelity enzyme engineered for PCR applications where greater sequence accuracy is required, together with improved PCR success rates of long and challenging templates.

AMPIGENE® 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.

The high accuracy and enhanced 3'-5' exonuclease activity of **AMPIGENE® High Fidelity Polymerase** result in fidelity that is approximately 100 times higher than Taq DNA polymerase. The enzyme is ideally suited to applications where greater accuracy is required, such as cloning, site-directed mutagenesis and sequencing. PCR products generated with this range of products are blunt ended.

AMPIGENE® High Fidelity Polymerase 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 with minimal or no optimization required.

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 DNA[1].

KNOWN APPLICATION

Amplification of nucleic acid targets with PCR methods.

PRODUCTS SUPPLIED

Component	100 units	500 units
AMPIGENE® High Fidelity Polymerase (2 U/µL)	1 x 50 µL	1 x 250 µL
AMPIGENE® 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 1.7mL

MATERIALS NEEDED (Not Provided)

- Target DNA
- Primers
- dNTPs
- 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

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

GLOBAL HEADQUARTERS

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INSTRUCTIONS FOR USE

AMPIGENE® High Fidelity Buffer: The 5X buffer contains 15 mM $MgCl_2$, 5 mM dNTPs, enhancers and stabilizers. It is not recommended to add further $MgCl_2$ 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® High Fidelity Polymerase 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 however shorter extension times of between 10 and 30 seconds per kb are possible. Two-step cycling protocols may also be used with combined annealing and extension at 68-75 °C.

Fast cycling: If using faster extension times, care must be taken to prevent loading too much template DNA. If non-specific bands are visible after amplification, the amount of template DNA should be decreased.

Reaction Setup

1. Allow 5X AMPIGENE® 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® 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® High Fidelity Polymerase (2 U/µL)	0.25 µL		
PCR grade dH_2O	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	10-30 secs/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.

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