AMPIGENE® Hot Start *Taq* DNA Polymerase

REF ENZ-NUC123-0200 200 Units
REF ENZ-NUC123-0500 500 Units

INTENDED USE:

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

SUMMARY AND EXPLANATION

AMPIGENE® Hot Start *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® Hot Start *Taq* DNA Polymerase is a robust enzyme for all your everyday PCR applications. **AMPIGENE® Hot Start *Taq* DNA Polymerase** can perform consistently well on a broad range of templates (including both GC and AT rich). It is designed to amplify up to 5 kb DNA fragment from genomic DNA targets.

AMPIGENE® Hot Start *Taq* DNA Polymerase has an error rate of approximately 1 error per 2.0 x 105 nucleotides incorporated. PCR products generated with **AMPIGENE® Hot Start *Taq* DNA Polymerase** are A-tailed and may be cloned into TA cloning vectors.

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.

ASSAY PRINCIPLE

Polymerase chain reaction (PCR) uses *Taq* 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	200 units	500 units
AMPIGENE® Hot Start *Taq* DNA Polymerase (5U/µL)	1 x 40 µL	1 x 100 µL
AMPIGENE® Reaction Buffer (5X)	2 x 1 mL	3 x 1.5 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

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® Hot Start *Taq* DNA Polymerase: AMPIGENE® Hot Start Taq DNA Polymerase is a robust enzyme for all your everyday PCR applications. It is designed to amplify up to 5 kb DNA fragment from genomic DNA targets.

AMPIGENE® Reaction buffer: The 5X reaction buffer contains 8.75 mM $MgCl_2$, 1 mM dNTPs, and enhancers and stabilizers. The buffer composition has been optimized to maximize PCR success rates.

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

Primers: Primers should have a predicted melting temperature of around 60°C. The final primer concentration in the reaction should be between 0.2 µM and 0.5 µM.

Annealing: A 60°C annealing temperature works well for most PCRs. Alternatively, we recommend performing a temperature gradient to experimentally determine the optimal annealing temperature.

Extension: Optimal extension is achieved at 68° - 72°C. The optimal extension time is dependent on amplicon length and complexity of template. Extension time of 1 minute per kilobase (kb) is recommended.

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

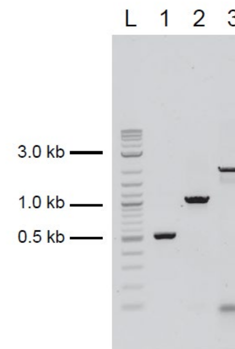
1. Allow 5X AMPIGENE® Reaction Buffer to reach room temperature, then briefly vortex.
2. Prepare a master mix based on the following table:

Reagent	25 µL reaction	Final Concentration
Template DNA	Variable	<100ng for cDNA <500ng for genomic DNA
AMPIGENE® Hot Start *Taq* DNA Polymerase (5U/µL)	0.125-0.5 µL	1.25-5U per rxn
Forward Primer (10 µM)	0.5 µL	200 nM
Reverse Primer (10 µM)	0.5 µL	200 nM
AMPIGENE® Reaction Buffer (5X)	5.0 µL	1X
PCR grade dH_2O	Up to to 25 µL Final volume	

3. Cycle using conditions based on the following table.

Step	Temperature	Time	Cycles
Initial Denaturation	95°C	2-5 mins	1
Denaturation	94°C	15-30 secs	30-40
Annealing	60°C	15-30 secs	
Extension	68-72°C	1-5 mins	
Final Extension	68-72°C	5-10 mins	1

INTERPRETATION OF RESULTS



PCR amplification of human genomic DNA using AMPIGENE® Hot Start *Taq* DNA Polymerase. PCR amplification was performed using three different primers (lane 1-3). Amplification products were analyzed on 1.2% agarose gel. Human genomic DNA (5 ng) was used as template. Annealing temperature was 60°C. Left lane, 100 bp ladder (L).

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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