AMPIGENE® High Fidelity Red Mix

REF ENZ-NUC132-0050 50 Reactions

INTENDED USE:

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

SUMMARY AND EXPLANATION

AMPIGENE® High Fidelity Red Mix is a convenient high fidelity 2X mix designed for PCR applications where greater sequence accuracy is required, together with improved PCR success rates of long and challenging templates. The inclusion of a red dye enables direct loading and tracking during agarose gel electrophoresis.

AMPIGENE® High Fidelity Red Mix contains the engineered and highly processive AMPIGENE® High Fidelity Polymerase, developed for fast and versatile high-fidelity PCR. The enzyme is derived from Pfu DNA polymerase for its 3'- 5' exonuclease (proofreading) activity. Several proprietary mutations significantly improve DNA binding and processivity, resulting in shorter extension times (10-30 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 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 Red Mix uses 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	50 x 50 uL reactions
AMPIGENE® High Fidelity Red Mix (2X)	1 x 1.25 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® High Fidelity Red Mix: The 2X mix contains AMPIGENE® High Fidelity Polymerase, 6 mM $MgCl_2$, 2 mM dNTPs, enhancers, stabilizers and a red dye for tracking during agarose electrophoresis. It is not recommended to add further PCR enhancers or $MgCl_2$ to the reaction. The mix composition has been optimized to maximize PCR success rates.

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 0.6 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.

Agarose gel electrophoresis dye migration: The 2X mix contains a red dye for tracking during agarose gel electrophoresis. In a 2% agarose TAE gel the dye migrates at a rate equivalent to 350 bp of DNA. In a 1% agarose TAE gel the dye migration rate is equivalent to 600 bp of DNA.

Reaction Setup

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

Reagent	50 µL reaction	Final Concentration
AMPIGENE® High Fidelity Red Mix (2X)	25 µL	1x
Forward Primer (10 µM)	2.0 µL	400 nM
Reverse Primer (10 µM)	2.0 µL	400 nM
Template DNA	<100 ng genomic DNA <5 ng less complex DNA	variable
PCR grade dH_2O	Up to 50 µL final volume	

2. 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 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.

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