AMPIGENE® Bst Polymerase with Dye

REF ENZ-NUC139-1600 1600 Units
REF ENZ-NUC139-8000 8000 Units

INTENDED USE:

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

SUMMARY AND EXPLANATION

AMPIGENE® Bst Polymerase with Dye is a recombinant protein expressed in E. coli and represents the large fragment of Geobacillus stearothermophilus (formerly known as Bacillus stearothermophilus) DNA Polymerase. This portion of the protein maintains the protein 5' to 3' polymerase activity but lacks the 5' to 3' exonuclease activity.[1]

AMPIGENE® Bst Polymerase with Dye displays strong strand displacement activity and is suitable for nucleic acid amplification methods such as whole genome amplification, multiple displacement amplification and isothermal amplification. We recommend a reaction temperature of 65ºC; however, the enzyme works well over a broad temperature range, from 55ºC to 70ºC. It is heat inactivated at 80ºC.

Designed for fast amplification speed, **AMPIGENE® Bst Polymerase with Dye** gives rapid and consistent results across different target sequences and sample types. The enzyme is provided with a 2-part buffer system to ensure high yield and performance even under difficult conditions.

Real-time detection with any qPCR thermocycler can be achieved by adding the supplied 20x AMPIGENE® Fluorescent Dye to the reaction.

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[2].

KNOWN APPLICATION

Amplification of nucleic acid targets with PCR methods.

PRODUCTS SUPPLIED

Component	1600 units	8000 units
AMPIGENE® Bst Polymerase (8 U/µL)	1 x 200 µL	1 x 1 mL
AMPIGENE® Bst Polymerase Buffer A (10X)	1 x 500 µL	2 x 1.25 mL
AMPIGENE® Bst Polymerase Buffer B (5X)	1 x 1mL	3 x 1.7mL
AMPIGENE® Fluorescent Dye (20X)	2 x 125 µL	2 x 625 µL

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® Bst Polymerase Buffer A: The 10x buffer contains 30mM MgSO4, 16mM dNTPs, enhancers and stabilizers. The buffer composition has been optimized to maximize the rate of amplification.

AMPIGENE® Bst Polymerase Buffer B: The 5x buffer contains enhancers designed to further increase the reaction speed.

EXAMPLE USAGE: STRAND DISPLACEMENT

Reaction temperature	Reaction Time	Deactivation temperature	Deactivation time
Recommended: 65˚C Optimal range: 55-70˚C	30-60 minutes	80 ˚C	10 minutes

EXAMPLE USAGE: Loop-Mediated Isothermal Amplification (LAMP)

1. Allow each component to reach room temperature, then briefly vortex.
2. Prepare a master mix based on the following table. Reactions should be setup on ice:

Reagent	25 µL reaction	Final Concentration
AMPIGENE® Bst Polymerase Buffer A (10X)	2.50 µL	1x
AMPIGENE® Bst Polymerase Buffer B (5X)	5.00 µL	1x
AMPIGENE® Fluorescent Dye (20X)	1.25 µL	1x
AMPIGENE® Bst Polymerase (8 U/µL)	1.00 µL	8U
10x Primer Set	2.50 µL	1x *
Template DNA	Variable	
PCR grade dH_2O	Up to 25 µL final volume	

3. Incubate at 65 ˚C for 30 minutes. Time can be extended and temperature can be modified (between 55 ˚C and 70 ˚C) as necessary for low copy targets, challenging templates, or whenever amplification times have been reported to be slow.

If a qPCR instrument is used for signal detection, follow the reaction using the FAM channel, acquiring data every 10-15 seconds. If final products are to be analyzed after the reaction is complete, the enzyme can be inactivated by heating at 80 ˚C for 10 minutes.

* We recommend a predicted melting temperature of around 60˚C using default Primer Explorer v5 settings. A primer set can be prepared with all 4 or 6 (if you include Loop) primers. A 10x primer set should contain: 16µM FIP, 16µM BIP, 2µM F3, 2µM B3, 4-8µM LoopF, 4-8µM LoopB in TE Buffer or water

REFERENCES

1. Mead DA, McClary JA, Luckey JA, Kostichka AJ, Witney FR, Smith LM. Bst DNA polymerase permits rapid sequence analysis from nanogram amounts of template. Biotechniques. 1991 Jul;11(1):76-8, 80, 82-87.
2. 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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