

Product Data Sheet

Product Name: Bst DNA Polymerase, Large Fragment

Cat. No.: GC26131

Protocol

Instructions: 1. Primer Design: Refer to this manual for preliminary selection of primers for loop-mediated isothermal amplification (LAMP). Specific experiments will be needed to identify suitable primers. LAMP primers consist of four or six primers (including a loop). It is recommended to design a six-primer set with a loop. 2. Using LAMP isothermal amplification as an example, set up the reaction system according to the table below.

Reagent	Volume	Final concentration
Nuclease-Free Water	(13.5-x)µl	-
10X Bst Reaction Buffer	2.5µl	1X
MgSO ₄ (100mM)	1.5µl	6mM (8mM total)
dNTP (10mM each)	3.5µl	1.4mM each
FIP/BIP Primers (25X, 40µM)	1µl	1.6µM
F3/B3 Primers (25X, 5µM)	1µl	0.2µM
LoopF/B Primers (25X, 10µM)	1µl	0.4µM
Template	xµl	>10 copies or more
Bst DNA Polymerase, Large Fragment (8U/µl)	1µl	320U/ml
Total volume	25µl	-

Note 1: After preparing the reaction system, add 1µl of high-concentration SYBR Green I to the cap of each 25µl reaction tube. After the isothermal amplification reaction is complete, centrifuge at 8000g for 1 minute. If the reaction system turns fluorescent green, it indicates a positive result; if it remains colorless or brown, it indicates a negative result. Alternatively, the addition of an indicator is not necessary. After the reaction is complete, a noticeable turbidity in the reaction solution indicates a positive result; if the reaction solution remains transparent, it indicates a negative result.

Note 2: To optimize the reaction, adjust the Mg²⁺ concentration (4-10mM), enzyme volume (0.04-

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0.32U/ μ l), or reaction temperature (50-68°C).

Note 3: If analysis is performed by agarose gel electrophoresis or other methods that require opening the LAMP reaction vessel, set up a secondary analysis area and equipment to avoid contamination.

Note 4: Because the reaction is rapid, to ensure experimental reproducibility, it is recommended that template DNA be added last.

Note 5: It is strongly recommended to include a negative control without template to ensure amplification specificity.

Note 6: To prevent contamination when preparing reagents, always perform operations in a clean bench.

Note 7: If possible, preparation of reagents and template DNA should be performed in a separate area from analysis of PCR products, such as electrophoresis, to avoid contamination.

3. Reaction procedure: 65°C for 60 min.

4. Inactivation: 80°C for 20 min.

5. If necessary, electrophoresis analysis can be performed on a 1.5% agarose gel. A gradient band on the electrophoresis graph indicates a positive amplification product, while a negative amplification product is indicated by the absence of a gradient band.

Background

GLPBIO's Bst DNA Polymerase, Large Fragment, also known as *Bacillus stearothermophilus* (Bst) DNA Polymerase I Large Fragment, possesses 5'→3' DNA polymerase activity but lacks 3'→5' and 5'→3' exonuclease activities. Bst DNA Polymerase, Large Fragment exhibits strong strand displacement and can be used in isothermal nucleic acid amplification reactions, such as loop-mediated isothermal amplification (LAMP) and rolling-circle amplification (RCA).

The isothermal amplification temperature mediated by Bst DNA Polymerase, Large Fragment is generally between 50-68°C, typically 65°C. Compared to Bst DNA Polymerase 2.0, this product does not incorporate dUTP into synthesized DNA strands during isothermal amplification.

Bst DNA Polymerase has 5'→3' exonuclease activity, while Bst DNA Polymerase, Large Fragment lacks this 5'→3' exonuclease activity through a deletion mutation.

Activity definition: One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 65°C.

For the enzyme activity of Bst DNA Polymerase, Large Fragment produced by GLPBIO, refer to

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Figure 1.

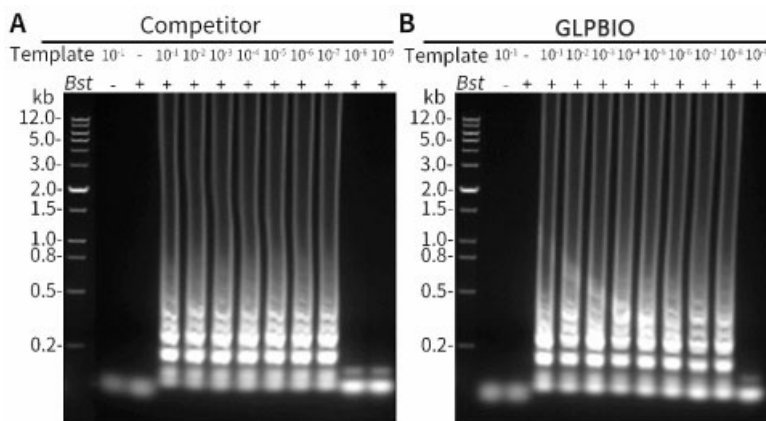


Figure 1. Bst DNA Polymerase, Large Fragment in loop-mediated isothermal amplification. This product or Bst DNA Polymerase, Large Fragment from N Company was used in a 25 μ l system with different amounts (10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶, 10⁻⁷, 10⁻⁸, 10⁻⁹ ng) of the CaMV35 promoter of tobacco mosaic virus as a template, 8U of Bst DNA Polymerase, Large Fragment (abbreviated as Bst in Figure 1), and primers: 1.6 μ M FIP/BIP, 0.2 μ M F3/B3, 0.4 μ M LoopF/B, 1.4mM Each dNTP was added to 1X Bst Reaction Buffer supplemented with MgSO₄ to a final concentration of 8 mM. The reaction was incubated at 65°C for 1 hour, inactivated by heating at 80°C for 20 minutes, and then analyzed by 1.5% agarose gel electrophoresis. As shown in the figure, this product exhibits slightly better or at least equivalent enzyme activity compared to that of Competitor N. M, DNA marker.

Source: The large fragment of *Bacillus stearothermophilus* DNA Polymerase I was obtained by recombinant expression and purification in *Escherichia coli*.

Purity: No DNA endonuclease or exonuclease activity.

Applications: DNA isothermal amplification including loop-mediated isothermal amplification (LAMP), helicase isothermal gene amplification (HDA), multiple displacement amplification (MDA), whole genome amplification (WGA), sequencing of GC-rich DNA, rapid sequencing of nanogram-level DNA templates, and library construction and sequencing.

Enzyme Storage Solution: 10mM Tris-HCl (pH 7.5), 50mM KCl, 0.1mM EDTA, 1mM DTT, 0.1% Triton X-100, 50% (v/v) glycerol.

10X Bst Reaction Buffer: 200mM Tris-HCl, 100mM KCl, 100mM (NH₄)₂SO₄, 20mM MgSO₄, 1%

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Triton X-100, pH 8.8 at 25°C.

Inactivation or Inhibition: Heat at 80°C for 20 minutes.

Storage Conditions:

Store at -20°C.

Precautions:

Bst DNA Polymerase, Large Fragment does not have 3'→5' exonuclease activity.

Isothermal amplification reaction temperatures should not exceed 70°C, as this will inactivate the enzyme.

Bst DNA Polymerase, Large Fragment should not be used for thermal cycle sequencing or PCR.

Always include a negative control without template DNA in each isothermal amplification experiment.

This product is for scientific research use only by professionals. It is not intended for clinical diagnosis or treatment, for food or pharmaceutical use, or for storage in residential settings.

For your safety and well-being, please wear a lab coat and disposable gloves when handling.

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