

Bst DNA Polymerase, Large Fragment

Product description

Bst DNA Polymerase, Large Fragment, the large fragment of DNA Polymerase I from *Bacillus stearothermophilus* (Bst), is obtained by recombinant expression in *Escherichia coli*. Bst DNA Polymerase, Large Fragment possesses 5'→3' DNA polymerase activity and strong strand displacement activity, while it lacks 5'→3' exonuclease activity due to deletion mutation.

The product is widely applied in nucleic acid isothermal amplification reactions, including loop-mediated isothermal amplification (LAMP) and rolling-circle amplification (RCA). It is also suitable for sequencing of high-GC content DNA, rapid sequencing of nanogram-level DNA templates, library construction and sequencing, and other related applications.

Composition and storage conditions

Components	Size	800 U	4000 U	Storage
	Bst DNA Polymerase, Large Fragment (8 U/μL)		100 μL	500 μL
10 × Bst Reaction Buffer		300 μL	1.5 mL	-20 °C
100 mM MgSO ₄		200 μL	1 mL	-20 °C
Shipping: Dry Ice		Shelf life: 12 months		

Experimental operation

Taking LAMP isothermal amplification as an example

1. Primer design: For loop-mediated isothermal amplification (LAMP) primer design, please refer to the website: <http://primerexplorer.jp/e/>. Version V5 is recommended. The detailed operation manual can be downloaded at: http://primerexplorer.jp/e/v5_manual/index.html. Preliminary screening of LAMP primers can be performed according to the manual, and the optimal primers need to be further verified by experiments.

2. Prepare the reaction system on ice according to the table below:

Total Reaction Volume	25 μL	
10× Bst Reaction Buffer	2.5 μL	1×

MgSO ₄ (100 mM)	1.5 µL	6 mM (8 mM total)
dNTP (10 mM each)	3.5 µL	1.4 mM each
FIP/BIP Primers (25×, 40 µM)	1 µL	1.6 µM
F3/B3 Primers (25×, 5 µM)	1 µL	0.2 µM
LoopF/B Primers (25×, 10 µM)	1 µL	0.4 µM
Bst DNA Polymerase, Large Fragment (8 U/µL)	1 µL	320 U/mL
Template DNA	X µL	>10 copies or more
Nuclease-free Water	To 25 µL	

*Note: LAMP amplification proceeds rapidly. To ensure reproducible results, add the template DNA last. Meanwhile, set up a no-template negative control to guarantee amplification specificity.

- Gently mix the reaction mixture by slow pipetting or flicking the tube wall. Do not vortex. Briefly centrifuge to collect residual liquid at the bottom of the tube.
- Incubate at 65°C for 60 min, then heat at 80°C for 20 min to terminate the reaction.
- Analyze the products by 1.5% agarose gel electrophoresis. A ladder-like band pattern indicates a positive amplification result; the absence of ladder bands indicates a negative result.

Product properties

- Unit Definition (U): One unit is defined as the amount of enzyme required to incorporate 10 nmol of dNTP into acid-insoluble material within 30 min at 65 °C.
- Storage Buffer: 10 mM Tris-HCl (pH 7.1), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Triton X-100, 50% (v/v) glycerol.
- 10× Bst Reaction Buffer: 200 mM Tris-HCl (pH 8.8), 100 mM KCl, 100 mM (NH₄)₂SO₄, 20 mM MgSO₄, 1% Triton X-100.
- Quality assurance:
 - Free of DNA endonuclease and exonuclease activities.
- Inactivation conditions: Complete inactivation can be achieved by incubation at 80°C for 20 min.

Notes

- Bst DNA Polymerase, Large Fragment is not suitable for thermal cycle sequencing or conventional PCR assays. In addition, the reaction temperature for isothermal amplification should not exceed 70°C; otherwise, enzyme inactivation will occur.
- For reaction optimization, adjust the Mg²⁺ concentration (4–10 mM), enzyme dosage (0.04–0.32 U/µL), or reaction temperature (50–68 °C).
- This product is for research use only.



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