Биолабмикс

Bst DNA Polymerase, Large Fragment

Description: Bst DNA Polymerase, Large Fragment is the portion of the Bacillus stearothermophilus DNA Polymerase protein that contains the 5′ → 3′ polymerase activity, but lacks 5′ →3′ exonuclease activity. Enzyme provide strong strand displacement activity. The optimum temperature is from 60-65˚C. Bst becomes heat inactivated at 80˚C. *Bst* Polymerase, Large Fragment is prepared from an *E. coli* strain containing a gene of the *Bacillus stearothermophilus* DNA Polymerase gene, lacking the 5′ → 3′ exonuclease domain.

Storage condition: -20º C in 10 mM Tris-HCl (pH 8.0 at 25ºC), 10 mM KCl, 1% BSA, 0,02% Tween 20, 50% glycerol.

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

Quality control: Each lot of enzyme is tested for endonuclease and non specific exonuclease activity.

Applications:

- LAMP
- Reverse transcription
- Whole Genome Amplification

Typical LAMP Protocol:

Component	Final Concentration
10X Buffer for Bst	1X (does not contain MgSO4)
MgSO4 (100 mM)	8 mM total
dNTP Mix (10 mM)	1.4 mM each
FIP/BIP Primers (10X)	1.6 µM
F3/B3 Primers (10X)	0.2 µM
LoopF/B Primers (10X)	0.8 µM
Bst Large fragment (8,000 U/ml)	80-320 U/ml
DNA Sample	> 10 copies or more
Nuclease-free Water	up to 25 µl
Total Reaction Volume	25 µl

General Guidelines:

1. A LAMP Primer Mix can be prepared with all 4 or 6 (with Loop) primers. A 10X Primer Mix should contain: 16 µM FIP, 16 µM BIP, 2 µM F3, 2 µM B3, 8 µM LoopF, 8 µM LoopB in TE or water.
2. Reactions should be setup on ice.
3. If analyzing via agarose gel electrophoresis or other method requiring opening LAMP reaction vessels, setup secondary analysis area and equipment to avoid contamination.
4. Running a no-template control is strongly recommended to ensure amplification specificity.
5. If optimization is desired, try titrating Mg2+ (4–10 mM final) or *Bst* DNA Polymerase, Large Fragment (0.01–0.2 U/µl), or changing reaction temperature (50–68°C).
6. dNTP should be used with dTTP, not dUTP

7. We recommend using LAMP primer design software such as Primer Explorer (http://primerexplorer.jp/e/).

SDS-PAGE: