

Bst II DNA Polymerase

(Large Fragment, Glycerol Free)

REF: EG23102S

Storage Condition

-20°C

Components

Component	Amount
Bst II DNA Polymerase (Large Fragment, Glycerol Free) (8 U/μl)	200 μl
10× Bst II Reaction Buffer	1 ml
MgSO ₄ (100 mM)	1 ml

Description

Bst II DNA Polymerase is an *in silico* designed homologue of Bst DNA Polymerase, Large Fragment and expressed in *E. coli*. Bst II DNA Polymerase contains 5'→3' DNA polymerase activity and strong strand displacement activity but lacks 5'→3' exonuclease activity. It is ideal for isothermal applications such as LAMP、RCA、HDA with an optimum of 65°C. Bst II DNA Polymerase displays improved amplification speed, yield, salt tolerance and thermostability compared to wild-type Bst DNA Polymerase, Large Fragment.

This product is glycerol-free and can be used for the preparation of various lyophilized reagents. Avoid repeated freeze-thaw.

Definition of Activity Unit

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.

Heat Inactivation

Incubation at 85°C for 5 minutes.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid and 40 U of Bst II DNA Polymerase incubated for 4 hours at 37°C results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 40 U of Bst II DNA Polymerase incubated for 16 hours at 37 °C results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 40 U of Bst II DNA Polymerase incubated for 1 hour at 37°C results in >90% of the substrate RNA remains intact as determined by agarose.

Residual Host DNA

The product was tested by TaqMan qPCR with primers specific for the *E.coli* 16S rDNA, and the results show that the *E.coli* genome residues less than 10 copies.

Protocol

Taking the LAMP reaction as an example

- Using the online tool <http://primerexplorer.jp/lampv5e/index.html> to design primers.
- Prepare the following reaction mixture on ice. It is recommended to prepare the reagents and template in separate areas, and add the template at last.

Reagent	Amount	Final Concentration
10× Bst II Reaction Buffer	2.5 µl	1×
MgSO ₄ (100 mM)	1.5 µl	6 mM (8 mM total) ^a
dNTP Mix (10 mM each)	3.5 µl	1.4 mM each
dUTP (10 mM) (Optional) ^b	1.5 µl	0.6 mM
HL-Uracil DNA Glycosylase (1 U/µl) (Optional) ^b	1 µl	0.04 U/µl
FIP/BIP Primers (20 µM) ^c	2 µl each	1.6 µM each
F3/B3 Primers (20 µM) ^c	0.25 µl each	0.2 µM each
LoopF/LoopB Primers (20 µM) (Optional) ^c	1 µl each	0.8 µM each
Bst II DNA Polymerase (Large Fragment, Glycerol Free) (8 U/µl) ^d	1 µl	0.32 U/µl
Template DNA	1~5 µl	>10 copies/rxn
ddH ₂ O	To 25 µl	

- The Bst Reaction Buffer already contains 2 mM MgSO₄, the final concentration of Mg²⁺ can be adjusted between 4~10 mM.
- The LAMP reaction is very sensitive and can be easily contaminated by aerosols of residual amplification products. To eliminate this contamination, heatlabile HL-Uracil DNA Glycosylase can be used in combination with dUTP (assuming dUTP was used in the previous amplification).
- A small amount of primer can be added, and it can be pre-mixed into a primer premix solution.
- The final concentration of Bst II DNA Polymerase (Large Fragment, Glycerol Free) can be adjusted between 0.08~0.32 U/µl.

*dNTP mix (REF: EG20907), dUTP (REF: EG20905) and HL-Uracil DNA Glycosylase (REF: EG22906) can be used with this product.

- Gently vortex or briefly mix by shaking, then collect the mixture at the bottom of the tube by brief centrifugation.
- Please react according to the following program:

Step	Temperature	Time
Eliminating residual pollution (Optional)	25°C	5~10 min
LAMP amplification	60~65°C	30~60 min
Thermal inactivation	85°C	5 min

- Use agarose gel electrophoresis or fluorescent dyes to detect the products.