Bst II DNA Polymerase (Large Fragment)

REF: EG23101-S/M

Storage Condition

-20℃

Components

Component	EG23101S	EG23101M
Bst II DNA Polymerase (Large Fragment) (8 U/μl)	200 μl	1 ml
10× Bst II Reaction Buffer	1 ml	3×1 ml
$MgSO_4$ (100 mM)	1 ml	3×1 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℃ . Bst II DNA Polymerase displays improved amplification speed, yield, salt tolerance and thermostability compared to wild-type Bst DNA Polymerase, Large Fragment.

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℃ .

Heat Inactivation

Incubation at 85℃ 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 25 μl reaction containing 200 ng of supercoiled plasmid and 40 U of Bst II DNA Polymerase incubated for 4 hours at 37℃ results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 25 μl reaction containing 15 ng of dsDNA fragments and 40 U of Bst II DNA Polymerase incubated for 16 hours at 37 ℃ 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 hours at 37 ℃ 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.

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Protocol

Taking the LAMP reaction as an example

1. Using the online tool http://primerexplorer.jp/lampv5e/index.html to design primers.
2. 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) (8 U/μl) [d]	1 μl	0.32 U/μl
Template DNA	1~5 μl	>10 copies/rxn
ddH_2O	To 25 μl	

a. The Bst Reaction Buffer already contains 2 mM $MgSO_4$, the final concentration of Mg^{2+} can be adjusted between 4~10 mM.
b. The LAMP reaction is very sensitive and can be easily contaminated by aerosols of residual amplification products. To eliminate this contamination, heat-labile HL-Uracil DNA Glycosylase can be used in combination with dUTP (assuming dUTP was used in the previous amplification).
c. A small amount of primer can be added, and it can be pre-mixed into a primer premix solution.
d. The final concentration of Bst II DNA Polymerase (Large Fragment) 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.

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

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

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

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