Bst DNA Polymerase, Large Fragment

1-800-632-7799
info@neb.com
www.neb.com

M0275S 051140916091

M0275S

RR 65° 80°

1,600 units	**8,000 U/ml**	**Lot: 0511409**
RECOMBINANT	**Store at –20°C**	**Exp: 9/16**

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.

Source: *Bst* DNA Polymerase, Large Fragment is prepared from an *E. coli* strain containing a genetic fusion of the *Bacillus stearothermophilus* DNA Polymerase gene, lacking the 5´→ 3´ exonuclease domain, and the gene coding for *E. coli* maltose binding protein (MBP). The fusion protein is purified to near homogeneity and the MBP portion of the fusion is cleaved off *in vitro*. The remaining polymerase is purified free of MBP (1).

Applications:

- Isothermal amplification (LAMP)
- DNA sequencing through high GC regions (2,3)
- Rapid Sequencing from nanogram amounts of DNA template (4)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM dithiothreitol, 0.1% Triton® X-100 and 50% glycerol.

Reagents Supplied with Enzyme:
10X ThermoPol® Reaction Buffer.
100 mM $MgSO_4$

1X ThermoPol Reaction Buffer:
20 mM Tris-HCl
10 mM $(NH_4)_2SO_4$
10 mM KCl
2 mM $MgSO_4$
0.1% Triton X-100
pH 8.8 @ 25°C

Unit 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.

Unit Assay Conditions: 50 mM KCl, 20 mM Tris-HCl (pH 8.8), 10 mM $MgCl_2$, 30 nM M13mp18 SS DNA, 70 nM M13 sequencing primer (–47) 24 mer, 200 µM dATP, 200 µM dCTP, 200 µM dGTP, 100 µM dTTP including [^{3}H]-dTTP and 100 µg/ml BSA.

Heat Inactivation: 80°C for 20 minutes.

Protocol:

Typical LAMP Protocol

Incubate the following reaction at 65°C for 30–60 minutes.

COMPONENT	25 µl REACTION	FINAL CONC.
10X ThermoPol Buffer	2.5 µl	1X (contains 2 mM $MgSO_4$)
$MgSO_4$ (100 mM)	1.5 µl	6 mM (8 mM total)
dNTP Mix (10 mM)	3.5 µl	1.4 mM each
FIP/BIP Primers (25X)	1 µl	1.6 µM
F3/B3 Primers (25X)	1 µl	0.2 µM
LoopF/B Primers (25X)	1 µl	0.4 µM
Bst DNA Polymerase, Large Fragment (8,000 U/ml)	1 µl	320 U/ml
DNA Sample	variable	> 10 copies or more
Nuclease-free Water	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 25X Primer Mix should contain: 40 µM FIP, 40 µM BIP, 5 µM F3, 5 µM B3, 10 µM LoopF, 10 µM LoopB in TE or water.

(see other side)

CERTIFICATE OF ANALYSIS

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2. Reactions should be setup on ice. If room temperature setup is desired, use *Bst* 2.0 WarmStart® DNA Polymerase (NEB #M0538).

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 Mg^{2+} (4–10 mM final) or *Bst* DNA Polymerase, Large Fragment (0.04–0.32 U/µl), or changing reaction temperature (50–68°C).

Quality Control Assays

16-Hour Incubation: Incubation of a 50 µl reaction in ThermoPol Reaction Buffer containing a minimum of 500 units of *Bst* DNA Polymerase, Large Fragment with 1 µg of λ DNA for 16 hours at 65°C results in no detectable change in DNA banding pattern as determined by agarose gel electrophoresis.

Exonuclease Activity: Incubation of a 50 µl reaction in ThermoPol Reaction Buffer containing a minimum of 500 units of *Bst* DNA Polymerase, Large Fragment with 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA for 4 hours at 65°C releases < 0.1% of the total radioactivity.

Endonuclease Activity: Incubation of a 50 µl reaction in ThermoPol Reaction Buffer containing a minimum of 500 units of *Bst* DNA Polymerase, Large Fragment with 1 µg of supercoiled ϕX174 DNA for 4 hours at 65°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

Enzyme Properties

Activity in NEBuffers

ThermoPol Buffer	125%
Unit Assay Conditions	100%
NEBuffer 1	50%
NEBuffer 2	100%
NEBuffer 3	50%
NEBuffer 4	100%

NEBuffers 1, 2, 3 and 4 must be supplemented with 0.1% Triton X-100 or 100 µg/ml BSA.

Approximately 10% activity is observed in these buffers in the absence of BSA or Triton X-100.

Notes On Use: *Bst* DNA Polymerase does not exhibit 3´→ 5´ exonuclease activity.

100 µg/ml BSA or 0.1%Triton X-100 is required for long term storage. Reaction temperatures above 70°C are not recommended.

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

Companion Products Sold Separately:

Magnesium Sulfate ($MgSO_4$) Solution
#B1003S 6.0 ml

ThermoPol Reaction Buffer Pack
#B9004S 6.0 ml

ThermoPol II (Mg-free) Reaction Buffer Pack
#B9005S 6.0 ml

ThermoPol DF (Detergent-free) Reaction Buffer Pack
#B9013S 6.0 ml

Deoxynucleotide Solution Set
#N0446S 25 µmol each

Deoxynucleotide Solution Mix
#N0447S 8 µmol each
#N0447L 40 µmol each

References:

1. Kong, H., Aliotta, J. and Pelletier, J.J., New England Biolabs, unpublished results.
2. Griffin, H. and Griffin, A. (1994). *PCR Technology* (pp.228–229). Florida: CRC Press.
3. McClary, J. et al. (1991) *J. DNA Sequencing and Mapping* 1, 173–180.
4. Mead, D.A. et al. (1991) *BioTechniques* 11, 76–87.
5. Notomi et al. (2000) *Nucleic Acids Res.* 28(12), E63.
6. Hsieh et al. (2014) *Chem. Commun.* 50, 3747–3749.
7. Tanner and Evans (2014) *Curr. Prot. Mol. Biol.* 105, 15.14.

U.S. Patent No. 5,814,506

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The purchase of NEB *Bst* products conveys to the purchaser the limited, nontransferable right to use the purchased products to perform loop-mediated isothermal amplification ("LAMP") for research use only. LAMP is a patented technology belonging to Eiken Chemical Co., Ltd. and any use other than research may require a license from Eiken Chemical Co., Ltd. A patent is pending for NEB's *Bst* DNA Polymerase.