Bst DNA Polymerase, Large Fragment

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

M0275S 051140316031

M0275S

1,600 units	**8,000 U/ml**	**Lot: 0511403**
RECOMBINANT	**Store at –20°C**	**Exp: 3/16**

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:

- 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.

Reaction Conditions:
1X ThermoPol Reaction Buffer.
Incubate at 65°C.

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.

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.

(see other side)

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(see other side)

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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.

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#B9013S 6.0 ml

Deoxynucleotide Solution Set
#N0446S 25 µmol each

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

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Page 2 (M0275)