

## Bst DNA Polymerase, Large Fragment



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



M0275S 051130915091

# M0275S



1,600 units 8,000 U/ml Lot: 0511309  
RECOMBINANT Store at -20°C Exp: 9/15

**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<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>  
10 mM KCl  
2 mM MgSO<sub>4</sub>  
0.1% Triton X-100  
pH 8.8 @ 25°C

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**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<sub>2</sub>, 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 [<sup>3</sup>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 [<sup>3</sup>H] *E. coli* DNA for 4 hours at 65°C releases < 0.1% of the total radioactivity.

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**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)

CERTIFICATE OF ANALYSIS

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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 cannot be used for thermal cycle sequencing or PCR.

**Companion Products Sold Separately:**

Magnesium Sulfate (MgSO<sub>4</sub>) 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

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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.

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

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TRITON® is a registered trademark of Union Carbide Corporation.



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Deoxynucleotide Solution Mix  
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