

## *Bsu* DNA Polymerase, Large Fragment



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



M0330S 001140316031

# M0330S



**200 units**      **5,000 U/ml**      **Lot: 0011403**  
**RECOMBINANT**    **Store at -20°C**    **Exp: 3/16**

**Description:** *Bsu* DNA Polymerase I, Large Fragment retains the 5' → 3' polymerase activity of the *Bacillus subtilis* DNA polymerase I (1), but lacks the 5' → 3' exonuclease domain. This large fragment naturally lacks 3' → 5' exonuclease activity.

**Source:** An *E. coli* strain that contains a genetic fusion of the *Bacillus subtilis* DNA polymerase I gene (starting from codon 297 thus lacking the 5' → 3' exonuclease domain), and the gene coding for maltose binding protein (MBP). The fusion

protein is purified to near homogeneity and the MBP portion is cleaved off *in vitro*. The remaining DNA polymerase is purified free of MBP.

### Applications:

- Random primer labeling
- Second strand cDNA synthesis
- Single dA tailing
- Strand displacement DNA synthesis (2)

Supplied in: 25 mM Tris-HCl (pH 7.4), 50 mM NaCl, 0.1 mM EDTA, 1 mM dithiothreitol and 50% glycerol.

**Reagents Supplied with Enzyme:**  
10X NEBuffer 2

**Reaction Conditions:** 1X NEBuffer 2.  
Supplement with 33 μM dNTPs (not included)  
Incubate at 37°C.

**1X NEBuffer 2:**  
10 mM Tris-HCl  
50 mM NaCl  
10 mM MgCl<sub>2</sub>  
1 mM DTT  
(pH 7.9 @ 25°C)

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**Unit Definition:** One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTPs into acid insoluble material in 30 minutes at 37°C.

**Unit Assay Conditions:** 1X NEBuffer 2, 33 μM dNTP including [<sup>3</sup>H]-dTTP and 70 μg/ml denatured herring sperm DNA. Incubate at 37°C.

### Quality Control Assays

**3' → 5' Exonuclease Activity:** Incubation of a 20 μl reaction in NEBuffer 2 containing a minimum of 50 units of *Bsu* DNA Polymerase, Large Fragment and a 10 nM solution of fluorescent internally labeled oligonucleotide for 30 minutes 37°C yields no detectable 3' → 5' degradation as determined by capillary electrophoresis.

**Exonuclease Activity:** Incubation of a 50 μl reaction in NEBuffer 2 containing minimum of 50 units of *Bsu* 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 37°C releases < 0.1% of the total radioactivity.

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**Endonuclease Activity:** Incubation of a 50 μl reaction in NEBuffer 2 containing a minimum of 50 units of *Bsu* DNA Polymerase, Large Fragment with 1 μg of supercoiled φX174 DNA for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Notes On Use:** *Bsu* DNA Polymerase, Large Fragment is not suitable for generating blunt ends because it lacks the 3' → 5' exonuclease necessary to remove non-templated 3' additions.

*Bsu* DNA Polymerase, Large Fragment retains 50% activity at 25°C and is twice as active as Klenow Fragment (3' → 5' exo-) at this temperature.

**Heat Inactivation:** 75°C for 20 minutes.

### References:

1. Okazaki, T. et al. (1964) *J. Biol. Chem.* 239, 259-268.
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(see other side)

CERTIFICATE OF ANALYSIS

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**Companion Products Sold Separately:**

NEBuffer 2  
#B7002S 6.0 ml

Deoxynucleotide Solution Set  
#N0446S 25 µmol of each

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

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