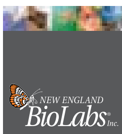
Bsu DNA Polymerase, Large Fragment



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

M0330S 001120914091

M0330S

200 units **5,000 U/ml** **Lot: 0011209**
RECOMBINANT **Store at –20°C** **Exp: 9/14**

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 $MgCl_2$
1 mM DTT
(pH 7.9 @ 25°C)

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 $[^3H]$-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 $[^3H]$ *E. coli* DNA for 4 hours at 37°C releases < 0.1% of the total radioactivity.

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.
2. Piepenburg, O. et al. (2006) *PLOS Biology*, 4, 1115–1121.

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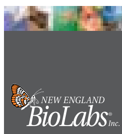
CERTIFICATE OF ANALYSIS

Bsu DNA Polymerase, Large Fragment



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CERTIFICATE OF ANALYSIS

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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NEBuffer 2
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Deoxynucleotide Solution Set
#N0446S 25 µmol of each

Deoxynucleotide Solution Mix
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