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 MgCl₂
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 [³²P]-dTTP and 70 µg/ml denatured herring sperm DNA. Incubate at 37°C.

Quality Control Assays
3’→5’ 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 mM 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 a minimum of 50 units of Bsu DNA Polymerase, Large Fragment with 1 µg of a mixture of single and double-stranded [³²P]-E. coli DNA for 4 hours at 37°C releases < 0.1% of the total radioactivity.

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.

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 pX174 DNA for 4 hours at 37°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Heat Inactivation: 75°C for 20 minutes.

References:
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