NEW ENGLAND BioLabs® Inc.

240 County Road
Ipswich, MA 01938-2723

Tel 978-927-5054
Fax 978-921-1350

www.neb.com
info@neb.com

New England Biolabs
Product Specification

Product Name:	*Bst DNA Polymerase, Large Fragment*
Catalog #:	*M0275S/L*
Concentration:	*8,000 units/ml*
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.*
Shelf Life:	*24 months*
Storage Temp:	*-20°C*
Storage Conditions:	*10 mM Tris-HCl , 50 mM KCl , 1 mM DTT , 0.1 mM EDTA , 0.1 % Triton®X-100 , 50 % Glycerol, (pH 7.1 @ 25° C)*
Specification Version:	*PS-M0275S/L v1.0*
Effective Date:	*04 Aug 2015*

Assay Name/Specification (minimum release criteria)
Endonuclease Activity (Nicking) - A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 500 units of *Bst* DNA Polymerase, Large Fragment incubated for 4 hours at either 37°C or 65°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.
Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *E. coli* DNA and a minimum of 500 units of *Bst* DNA Polymerase, Large Fragment incubated for 4 hours at either 37°C or 65°C releases <0.1% of the total radioactivity.
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer 2 containing 1 µg of T3 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 120 units of *Bst* DNA Polymerase, Large Fragment incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.
Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of Lambda DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 500 units of *Bst* DNA Polymerase, Large Fragment incubated for 16 hours at 65°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.
Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM $MgCl_2$ containing 2.5 mM *p*-Nitrophenol Phosphate (pNPP) and a minimum of 100 units *Bst* DNA Polymerase, Large Fragment incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.
Protein Purity Assay (SDS-PAGE) - *Bst* DNA Polymerase, Large Fragment is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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Assay Name/Specification (minimum release criteria)
qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 120 units of *Bst* DNA Polymerase, Large Fragment is screened for the presence of *E. coli* genomic DNA using SYBR® Green qPCR with primers specific for the *E. coli* 16S rRNA locus. Results are quantified using a standard curve generated from purified *E. coli* genomic DNA. The measured level of *E. coli* genomic DNA contamination is ≤ 1 *E. coli* genome.
RNase Activity (Extended Digestion) - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of *Bst* DNA Polymerase, Large Fragment is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.

Date 04 Aug 2015

Derek Robinson
Director of Quality Control

ISO 9001 Registered Quality Management
ISO 14001 Registered Environmental Management
ISO 13485 Registered Medical Devices