NEW ENGLAND BioLabs Inc.

240 County Road
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New England Biolabs
Certificate of Analysis

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.*
Lot #:	*0511609*
Assay Date:	*09/2016*
Expiration Date:	*9/2018*
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:	*16 Oct 2015*

Assay Name/Specification (minimum release criteria)	**Lot #0511609**
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.	**Pass**
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.	**Pass**
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.	**Pass**
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.	**Pass**

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Assay Name/Specification (minimum release criteria)	**Lot #0511609**
Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM $MgCl_2$ containing 2.5 mM *p*-Nitrophenyl 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.	**Pass**
Protein Purity Assay (SDS-PAGE) - *Bst* DNA Polymerase, Large Fragment is ≥ 99% pure as determined by SDS -PAGE analysis using Coomassie Blue detection.	**Pass**
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.	**Pass**
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.	**Pass**

Authorized by
Melanie Fortier
16 Oct 2015

Inspected by
Cathy Rezac
17 Aug 2016

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