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 #:	*M0275M*
Concentration:	*120,000 units/ml*
Unit Definition:	*One unit is defined at the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 65°C.*
Lot #:	*0511509*
Assay Date:	*09/2015*
Expiration Date:	*09/2017*
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-M0275M v1.0*
Effective Date:	*16 Oct 2015*

Assay Name/Specification (minimum release criteria)	**Lot #0511509**
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**
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**

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Assay Name/Specification (minimum release criteria)	**Lot #0511509**
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
Denisa Gilaj
26 Oct 2015

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