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## New England Biolabs Product Specification

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.

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-M0275M v2.0
Effective Date: 12 Feb 2020

## Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50  $\mu$ l reaction in ThermoPol® Reaction Buffer containing 1  $\mu$ g of supercoiled PhiX174 DNA and a minimum of 500 units of Bst DNA Polymerase, Large Fragment incubated for 4 hours at 37°C and 65°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

Exonuclease Activity (Radioactivity Release) - A 50  $\mu$ l reaction in ThermoPol® Reaction Buffer containing 1  $\mu$ g 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 37°C and 65°C releases <0.1% of the total radioactivity.

Non-Specific DNase Activity (16 Hour) - A 50  $\mu$ l reaction in NEBuffer 2 containing 1  $\mu$ g of T3 or T7 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.

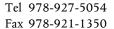
Phosphatase Activity (pNPP) - A 200 μl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl<sub>2</sub> containing 2.5 mM *p*-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units *Bst* DNA Polymerase, Large Fragment incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

Protein Purity Assay (SDS-PAGE) - Bst DNA Polymerase, Large Fragment is  $\geq$  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  $\leq 1$  *E. coli* genome.

RNase Activity (Extended Digestion) - A 10  $\mu$ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1  $\mu$ l 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.

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Derek Robinson Director, Quality Control







Date 12 Feb 2020