

New England Biolabs Product Specification

Product Name:	<i>Bst 2.0 DNA Polymerase</i>
Catalog #:	<i>M0537M</i>
Concentration:	<i>120,000 units/ml</i>
Unit Definition:	<i>One unit is defined as the amount of enzyme that will incorporate 25 nmol of dNTP into acid insoluble material in 30 minutes at 65°C.</i>
Shelf Life:	<i>24 months</i>
Storage Temp:	<i>-20°C</i>
Storage Conditions:	<i>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)</i>
Specification Version:	<i>PS-M0537M v2.0</i>
Effective Date:	<i>12 Feb 2020</i>

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* 2.0 DNA Polymerase incubated for 4 hours at 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 [³H] *E. coli* DNA and a minimum of 500 units of *Bst* 2.0 DNA Polymerase incubated for 4 hours at 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 or T7 DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 120 units of *Bst* 2.0 DNA Polymerase incubated for 16 hours at 37°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 MgCl₂ containing 2.5 mM *p*-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units *Bst* 2.0 DNA Polymerase 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* 2.0 DNA Polymerase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.



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qPCR DNA Contamination (*E. coli* Genomic) - A minimum of 120 units of *Bst* 2.0 DNA Polymerase 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 μ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 μ l of *Bst* 2.0 DNA Polymerase 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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