

New England Biolabs Product Specification

<i>Product Name:</i>	<i>Bst 2.0 WarmStart® DNA Polymerase</i>
<i>Catalog #:</i>	<i>M0538S/L</i>
<i>Concentration:</i>	<i>8,000 units/ml</i>
<i>Unit Definition:</i>	<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>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<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>
<i>Specification Version:</i>	<i>PS-M0538S/L v1.0</i>
<i>Effective Date:</i>	<i>06 Jan 2016</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.

Inhibition of Primer Extension (Hot Start) - A 50 µl reaction in Isothermal Amplification Buffer containing 6 mM MgSO₄ and 1.4 mM dNTPs in the presence of 1.6 µM of a fluorescent internally labeled oligonucleotide and a minimum of 16 units of *Bst* 2.0 WarmStart® DNA Polymerase incubated for 2 hours at 25°C yields <5% extension as determined by capillary electrophoresis.

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* 2.0 WarmStart® DNA Polymerase incubated for 16 hours at 16°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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<p>qPCR DNA Contamination (<i>E. coli</i> Genomic) - A minimum of 120 units of <i>Bst</i> 2.0 WarmStart[®] DNA Polymerase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR[®] Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.</p>
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<p>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 <i>Bst</i> 2.0 WarmStart[®] 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.</p>



Date 06 Jan 2016

Derek Robinson
Director of Quality Control

