

## New England Biolabs Product Specification

<b>Product Name:</b>	<i>Bst 2.0 WarmStart® DNA Polymerase</i>
<b>Catalog #:</b>	<i>M0538S/L</i>
<b>Concentration:</b>	<i>8,000 units/ml</i>
<b>Unit Definition:</b>	<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>
<b>Shelf Life:</b>	<i>24 months</i>
<b>Storage Temp:</b>	<i>-20°C</i>
<b>Storage Conditions:</b>	<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>
<b>Specification Version:</b>	<i>PS-M0538S/L v3.0</i>
<b>Effective Date:</b>	<i>14 Apr 2021</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 [<sup>3</sup>H] *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 MgSO<sub>4</sub> 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 or T7 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 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 MgCl<sub>2</sub> 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 WarmStart<sup>®</sup> DNA Polymerase is screened for the presence of *E. coli* genomic DNA using SYBR<sup>®</sup> 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* 2.0 WarmStart<sup>®</sup> 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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