

## New England Biolabs Certificate of Analysis

*Product Name:* Bst 2.0 WarmStart<sup>®</sup> DNA Polymerase  
*Catalog #:* M0538M  
*Concentration:* 120,000 units/ml  
*Unit Definition:* 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.  
*Lot #:* 0051706  
*Assay Date:* 06/2017  
*Expiration Date:* 06/2019  
*Storage Temp:* -20°C  
*Storage Conditions:* 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1 % Triton<sup>®</sup>X-100, 50 % Glycerol, (pH 7.1 @ 25°C)  
*Specification Version:* PS-M0538M v1.0  
*Effective Date:* 18 Nov 2016

Assay Name/Specification (minimum release criteria)	Lot #0051706
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in ThermoPol <sup>®</sup> 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.	<b>Pass</b>
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 µl reaction in ThermoPol <sup>®</sup> Reaction Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> 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.	<b>Pass</b>
<b>Inhibition of Primer Extension (Hot Start)</b> - 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 <sup>®</sup> DNA Polymerase incubated for 2 hours at 25°C yields <5% extension as determined by capillary electrophoresis.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 Hour)</b> - 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 <sup>®</sup> 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.	<b>Pass</b>
<b>Phosphatase Activity (pNPP)</b> - A 200 reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl <sub>2</sub> containing 2.5 mM <i>p</i> -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.	<b>Pass</b>



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<p><b>Protein Purity Assay (SDS-PAGE)</b> - <i>Bst</i> 2.0 DNA Polymerase is <math>\geq 99\%</math> pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (<i>E. coli</i> Genomic)</b> - A minimum of 120 units of <i>Bst</i> 2.0 WarmStart<sup>®</sup> DNA Polymerase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR<sup>®</sup> 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 <math>\leq 1</math> <i>E. coli</i> genome.</p>	<b>Pass</b>
<p><b>RNase Activity (Extended Digestion)</b> - A 10 <math>\mu</math>l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 <math>\mu</math>l of <i>Bst</i> 2.0 WarmStart<sup>®</sup> DNA Polymerase is incubated at 37°C. After incubation for 16 hours, <math>&gt;90\%</math> of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	<b>Pass</b>



Authorized by  
Karen Moreira  
18 Nov 2016



Inspected by  
Tony Spear-Alfonso  
20 Jun 2017

