

New England Biolabs Certificate of Analysis

Product Name: *Bst 2.0 WarmStart[®] 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 #: *0041612*
Assay Date: *12/2016*
Expiration Date: *12/2018*
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-M0538M v1.0*
Effective Date: *20 May 2016*

Assay Name/Specification (minimum release criteria)	Lot #0041612
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 <i>Bst 2.0</i> DNA Polymerase incubated for 4 hours at 65°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in ThermoPol [®] Reaction Buffer containing 1 µg of a mixture of single and double-stranded [³ H] <i>E. coli</i> DNA and a minimum of 500 units of <i>Bst 2.0</i> DNA Polymerase incubated for 4 hours at 65°C releases <0.1% of the total radioactivity.	Pass
Inhibition of Primer Extension (Hot Start) - A 50 µl reaction in Isothermal Amplification Buffer containing 6 mM MgSO ₄ 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 <i>Bst 2.0</i> WarmStart [®] DNA Polymerase incubated for 2 hours at 25°C yields <5% extension as determined by capillary electrophoresis.	Pass
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 <i>Bst 2.0</i> 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.	Pass
Phosphatase Activity (pNPP) - A 200 reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl ₂ containing 2.5 mM <i>p</i> -Nitrophenyl Phosphate (pNPP) and a minimum of 100 units <i>Bst 2.0</i> DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	Pass

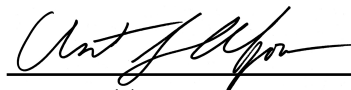


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Assay Name/Specification (minimum release criteria)	Lot #0041612
<p>Protein Purity Assay (SDS-PAGE) - <i>Bst</i> 2.0 DNA Polymerase is $\geq 99\%$ pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p>qPCR DNA Contamination (<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>	Pass
<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>	Pass



Authorized by
Melanie Fortier
20 May 2016



Inspected by
Tony Spear-Alfonso
01 Dec 2016

