New England Biolabs
Product Specification

Product Name: Bst 2.0 WarmStart® DNA Polymerase
Catalog #: M0538S/L
Concentration: 8,000 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.
Shelf Life: 24 months
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-M0538S/L v3.0
Effective Date: 14 Apr 2021

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 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 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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<thead>
<tr>
<th>Assay Name/Specification (minimum release criteria)</th>
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<tr>
<td><strong>qPCR DNA Contamination (<strong>E. coli</strong> Genomic)</strong> - A minimum of 120 units of Bst 2.0 WarmStart® DNA Polymerase is screened for the presence of <strong>E. coli</strong> genomic DNA using SYBR® Green qPCR with primers specific for the <strong>E. coli</strong> 16S rRNA locus. Results are quantified using a standard curve generated from purified <strong>E. coli</strong> genomic DNA. The measured level of <strong>E. coli</strong> genomic DNA contamination is $\leq 1$ <strong>E. coli</strong> genome.</td>
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<td><strong>RNase Activity (Extended Digestion)</strong> - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of Bst 2.0 WarmStart® DNA Polymerase is incubated at 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</td>
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Derek Robinson  
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Date 14 Apr 2021