

## New England Biolabs Certificate of Analysis

**Product Name:** Bst 2.0 WarmStart® DNA Polymerase  
**Catalog Number:** M0538M  
**Concentration:** 120,000 U/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.  
**Packaging Lot Number:** 10128260  
**Expiration Date:** 11/2023  
**Storage Temperature:** -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 v2.0

| Bst 2.0 WarmStart® DNA Polymerase Component List |   |            |                      |
|--|---|------------|----------------------|
| NEB Part Number                                  | Component Description                           | Lot Number | Individual QC Result |
| M0538MVIAl                                       | Bst 2.0 WarmStart® DNA Polymerase               | 10127488   | Pass                 |
| B1003SVIAl                                       | Magnesium Sulfate (MgSO <sub>4</sub> ) Solution | 10118450   | Pass                 |
| B0537SVIAl                                       | Isothermal Amplification Buffer                 | 10105662   | Pass                 |

| Assay Name/Specification   | Lot # 10128260 |
|--|----------------|
| <p><b>Endonuclease Activity (Nicking)</b><br/>           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 &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>   | Pass           |
| <p><b>Exonuclease Activity (Radioactivity Release)</b><br/>           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 &lt;0.1% of the total radioactivity.</p>   | Pass           |
| <p><b>Inhibition of Primer Extension (Hot Start)</b><br/>           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 &lt;5% extension as determined by capillary electrophoresis.</p> | Pass           |

| Assay Name/Specification  | Lot # 10128260 |
|---|----------------|
| <p><b>RNase Activity (Extended Digestion)</b><br/>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.</p>   | <b>Pass</b>    |
| <p><b>Phosphatase Activity (pNPP)</b><br/>A 200 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 &lt;0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</p>   | <b>Pass</b>    |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>Bst 2.0 DNA Polymerase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>   | <b>Pass</b>    |
| <p><b>qPCR DNA Contamination (E. coli Genomic)</b><br/>A minimum of 120 units of Bst 2.0 WarmStart® DNA Polymerase is screened for the presence of E. coli genomic DNA using SYBR® 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 ≤ 1 E. coli genome.</p> | <b>Pass</b>    |
| <p><b>Non-Specific DNase Activity (16 Hour)</b><br/>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.</p>                                      | <b>Pass</b>    |

This product has been tested and shown to be in compliance with all specifications.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit [www.neb.com/trademarks](http://www.neb.com/trademarks) for additional information.

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15 Nov 2021

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