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New England Biolabs Certificate of Analysis

Product Name: Bst 2.0 WarmStart® DNA Polymerase

Catalog Number: M0538L Concentration: 8,000 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.

Lot Number: 10045187
Expiration Date: 12/2020
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-M0538S/L v1.0

| Bst 2.0 WarmStart® DNA Polymerase Component List | | | | |
|--|---|------------|----------------------|--|
| NEB Part Number | Component Description | Lot Number | Individual QC Result | |
| M0538LVIAL | Bst 2.0 WarmStart® DNA Polymerase | 10032694 | Pass | |
| B1003SVIAL | Magnesium Sulfate (MgSO ₄) Solution | 0021701 | Pass | |
| B0537SVIAL | Isothermal Amplification Buffer | 10035085 | Pass | |

| Assay Name/Specification | Lot # 10045187 |
|--|----------------|
| 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 Bst 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. | Pass |
| qPCR DNA Contamination (E. coli Genomic) 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. | Pass |
| 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. | Pass |



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| Assay Name/Specification | Lot # 10045187 |
|---|----------------|
| 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. | Pass |
| 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. | 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 Bst 2.0 WarmStart® DNA Polymerase incubated for 16 hours at 16°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. | Pass |
| 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. | Pass |
| Protein Purity Assay (SDS-PAGE) Bst 2.0 DNA Polymerase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |

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

Tony Spear-Alfonso Production Scientist

03 Jan 2019

Michael Tonello

Packaging Quality Control Inspector

15 May 2019