NEW ENGLAND BioLabs Inc.
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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:	*10208063*
Expiration Date:	*10/2024*
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	10168040	**Pass**
B1003SVIAL	Magnesium Sulfate ($MgSO_4$) Solution	10174353	**Pass**
B0537SVIAL	Isothermal Amplification Buffer	10165335	**Pass**

Assay Name/Specification	Lot # 10208063
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 65ºC 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] E. coli DNA and a minimum of 500 units of Bst 2.0 DNA Polymerase incubated for 4 hours at 65ºC 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**

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Assay Name/Specification	Lot # 10208063
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 37ºC 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 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**
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 37ºC. 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**

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

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12 Sep 2023