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240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## 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:	10053592
Expiration Date:	06/2021
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	10046205	Pass	
B1003SVIAL	Magnesium Sulfate (MgSO₄) Solution	10042724	Pass	
B0537SVIAL	Isothermal Amplification Buffer	10035085	Pass	

Assay Name/Specification	Lot # 10053592
Protein Purity Assay (SDS-PAGE) Bst 2.0 DNA Polymerase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<b>Phosphatase Activity (pNPP)</b> 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
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
Inhibition of Primer Extension (Hot Start) A 50 µl reaction in Isothermal Amplification Buffer containing 6 mM MgSO4 and 1.4 mM	Pass





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Assay Name/Specification	Lot # 10053592
dNTPs in the presence of 1.6 $\mu$ M 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.	
<b>Exonuclease Activity (Radioactivity Release)</b> 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 <0.1% of the total radioactivity.	Pass
<b>Endonuclease Activity (Nicking)</b> 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
<b>qPCR DNA Contamination (E. coli Genomic)</b> 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 $\leq$ 1 E. coli genome.	Pass
<b>RNase Activity (Extended Digestion)</b> 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

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

Production Scientist 21 Feb 2019

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Michael Tonello Packaging Quality Control Inspector 15 Aug 2019

