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## New England Biolabs Certificate of Analysis

Product Name: Bst 3.0 DNA Polymerase

Catalog Number: M0374L
Concentration: 8,000 U/ml

Unit Definition: One unit is defined at the amount of enzyme that will incorporate 25

nmol of dNTPs into acid insoluble material in 30 minutes at 65°C.

Lot Number: 10012706
Expiration Date: 06/2020
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1 %

Triton®X-100 , 50 % Glycerol, (pH 7.4 @ 25°C)

Specification Version: PS-M0374S/L v1.0

Bst 3.0 DNA Polymerase Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
M0374LVIAL	Bst 3.0 DNA Polymerase	10009987	Pass	
B1003SVIAL	Magnesium Sulfate (MgSO <sub>4</sub> ) Solution	0021701	Pass	
B0374SVIAL	Isothermal Amplification Buffer II Pack	0031711	Pass	

Assay Name/Specification	Lot # 10012706
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 3.0 DNA Polymerase incubated for 4 hours at 65°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
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 3.0 DNA Polymerase incubated for 4 hours at 65°C releases <0.1% of the total radioactivity.	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 3.0 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.	Pass



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Assay Name/Specification	Lot # 10012706
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 3.0 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 3.0 DNA Polymerase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<b>qPCR DNA Contamination (E. coli Genomic)</b> A minimum of 120 units of Bst 3.0 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
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 3.0 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.

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Lynne Apone Production Scientist 15 Jun 2018 Mary Conlon

Packaging Quality Control Inspector

19 Jun 2018