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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 3.0 DNA Polymerase
Catalog Number:	M0374S
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:	10035264
Expiration Date:	12/2020
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				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
M0374SVIAL	Bst 3.0 DNA Polymerase	10032982	Pass	
B1003SVIAL	Magnesium Sulfate (MgSO₄) Solution	0021701	Pass	
B0374SVIAL	Isothermal Amplification Buffer II Pack	10029501	Pass	

Assay Name/Specification	Lot # 10035264
<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 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
<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 3.0 DNA Polymerase incubated for 4 hours at 65°C releases <0.1% of the total radioactivity.	Pass
<b>Non-Specific DNase Activity (16 Hour)</b> 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 # 10035264
<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 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
<b>Protein Purity Assay (SDS-PAGE)</b> 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 $\leq$ 1 E. coli genome.	Pass
<b>RNase Activity (Extended Digestion)</b> A 10 $\mu$ I reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 $\mu$ I 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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Tony Spear-Alfonso Production Scientist 07 Jan 2019

Michae 21.

Michael Tonello Packaging Quality Control Inspector 15 Feb 2019

