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## New England Biolabs Certificate of Analysis

Product Name: Bst DNA Polymerase, Large Fragment

Catalog Number: M0275L
Concentration: 8,000 U/ml

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 10

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

Packaging Lot Number: 10167676
Expiration Date: 06/2024
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-M0275S/L v2.0

Bst DNA Polymerase, Large Fragment Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
M0275LVIAL	Bst DNA Polymerase, Large Fragment	10154459	Pass	
B9004SVIAL	ThermoPol® Reaction Buffer Pack	10165338	Pass	
B1003SVIAL	Magnesium Sulfate (MgSO <sub>4</sub> ) Solution	10159437	Pass	

Assay Name/Specification	Lot # 10167676
Protein Purity Assay (SDS-PAGE) Bst DNA Polymerase, Large Fragment is ≥ 99% pure as determined by SDS-PAGE analysis	Pass
using Coomassie Blue detection.	
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 DNA Polymerase, Large	Pass
Fragment incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	
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 DNA Polymerase, Large Fragment included for 4 hours at 37°C and 65°C results in x10°C conversion to the picked form	Pass
incubated for 4 hours at 37°C and 65°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of a mixture of	Pass



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Assay Name/Specification	Lot # 10167676
single and double-stranded [ ³H] E. coli DNA and a minimum of 500 units of Bst DNA Polymerase, Large Fragment incubated for 4 hours at 37°C and 65°C releases <0.1% of the total radioactivity.	
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 DNA Polymerase, Large Fragment incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of Lambda DNA in addition to a reaction containing Lambda-HindIII DNA and a minimum of 500 units of Bst DNA Polymerase, Large Fragment incubated for 16 hours at 65°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
qPCR DNA Contamination (E. coli Genomic) A minimum of 120 units of Bst DNA Polymerase, Large Fragment 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 DNA Polymerase, Large Fragment 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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02 Aug 2022

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13 Oct 2022