

## 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:** *10072178*  
**Expiration Date:** *02/2022*  
**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			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0275LVIAL	Bst DNA Polymerase, Large Fragment	10069446	Pass
B9004SVIAL	ThermoPol® Reaction Buffer Pack	10067018	Pass
B1003SVIAL	Magnesium Sulfate (MgSO <sub>4</sub> ) Solution	10071233	Pass

Assay Name/Specification	Lot # 10072178
<p><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 DNA Polymerase, Large Fragment incubated for 4 hours at 37°C and 65°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	Pass
<p><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 DNA Polymerase, Large Fragment incubated for 4 hours at 37°C and 65°C releases &lt;0.1% of the total radioactivity.</p>	Pass
<p><b>Non-Specific DNase Activity (16 Hour)</b>            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.</p>	Pass

Assay Name/Specification	Lot # 10072178
<p><b>Non-Specific DNase Activity (16 Hour)</b> 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.</p>	<b>Pass</b>
<p><b>Phosphatase Activity (pNPP)</b> A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl<sub>2</sub> containing 2.5 mM p-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units Bst DNA Polymerase, Large Fragment incubated for 4 hours at 37°C yields &lt;0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> Bst DNA Polymerase, Large Fragment is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>qPCR DNA Contamination (E. coli Genomic)</b> 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.</p>	<b>Pass</b>
<p><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 DNA Polymerase, Large Fragment is incubated at 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	<b>Pass</b>

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

*Christie Vazquez*

Christie Vazquez  
Production Scientist  
29 Apr 2020

*Jay Minichiello*

Jay Minichiello  
Packaging Quality Control Inspector  
29 Apr 2020