

## New England Biolabs Certificate of Analysis

*Product Name:* *Bst DNA Polymerase, Large Fragment*  
*Catalog #:* *M0275M*  
*Concentration:* *120,000 units/ml*  
*Unit Definition:* *One unit is defined at the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 minutes at 65°C.*  
*Lot #:* *0511603*  
*Assay Date:* *03/2016*  
*Expiration Date:* *3/2018*  
*Storage Temp:* *-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-M0275M v1.0*  
*Effective Date:* *16 Oct 2015*

Assay Name/Specification (minimum release criteria)	Lot #0511603
<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 <i>Bst</i> DNA Polymerase, Large Fragment incubated for 4 hours at either 37°C or 65°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<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] <i>E. coli</i> DNA and a minimum of 500 units of <i>Bst</i> DNA Polymerase, Large Fragment incubated for 4 hours at either 37°C or 65°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<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 <i>Bst</i> 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.	<b>Pass</b>
<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 <i>Bst</i> 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.	<b>Pass</b>

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Assay Name/Specification (minimum release criteria)	Lot #0511603
<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 <i>p</i>-Nitrophenyl Phosphate (pNPP) and a minimum of 100 units <i>Bst</i> 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> - <i>Bst</i> 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 (<i>E. coli</i> Genomic)</b> - A minimum of 120 units of <i>Bst</i> DNA Polymerase, Large Fragment is screened for the presence of <i>E. coli</i> genomic DNA using SYBR® Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> 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 <i>Bst</i> 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>



Authorized by  
Melanie Fortier  
16 Oct 2015



Inspected by  
Cathy Rezac  
26 Feb 2016

