

## New England Biolabs Certificate of Analysis

*Product Name:* *Bst 3.0 DNA Polymerase*  
*Catalog #:* *M0374M*  
*Concentration:* *120,000 units/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 #:* *0071612*  
*Assay Date:* *12/2016*  
*Expiration Date:* *12/2018*  
*Storage Temp:* *-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-M0374M v1.0*  
*Effective Date:* *02 Sep 2015*

Assay Name/Specification (minimum release criteria)	Lot #0071612
<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> 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.	<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> 3.0 DNA Polymerase incubated for 4 hours at 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> 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.	<b>Pass</b>
<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> -Nitrophenol Phosphate (pNPP) and a minimum of 100 units <i>Bst</i> 3.0 DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - <i>Bst</i> 3.0 DNA Polymerase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>



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<p><b>qPCR DNA Contamination (<i>E. coli</i> Genomic)</b> - A minimum of 120 units of <i>Bst</i> 3.0 DNA Polymerase is screened for the presence of <i>E. coli</i> genomic DNA using SYBR<sup>®</sup> 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 <math>\leq 1 E. coli</math> genome.</p>	<p><b>Pass</b></p>
<p><b>RNase Activity (Extended Digestion)</b> - A 10 <math>\mu</math>l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 <math>\mu</math>l of <i>Bst</i> 3.0 DNA Polymerase 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>	<p><b>Pass</b></p>



Authorized by  
Melanie Fortier  
02 Sep 2015



Inspected by  
David Guo  
01 Dec 2016

