

## New England Biolabs Certificate of Analysis

**Product Name:** Bst 3.0 DNA Polymerase  
**Catalog Number:** M0374M  
**Concentration:** 120,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme that will incorporate 25 nmol of dNTPs into acid insoluble material in 30 minutes at 65°C.  
**Packaging Lot Number:** 10072234  
**Expiration Date:** 03/2022  
**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-M0374M v2.0

Bst 3.0 DNA Polymerase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0374M VIAL	Bst 3.0 DNA Polymerase	10071629	Pass
B1003S VIAL	Magnesium Sulfate (MgSO <sub>4</sub> ) Solution	10068556	Pass
B0374S VIAL	Isothermal Amplification Buffer II Pack	10061978	Pass

Assay Name/Specification	Lot # 10072234
<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 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 ≤ 1 E. coli genome.	Pass
<b>RNase Activity (Extended Digestion)</b>	Pass

Assay Name/Specification	Lot # 10072234
<p>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 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>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 &lt;0.1% of the total radioactivity.</p>	<b>Pass</b>
<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 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.</p>	<b>Pass</b>
<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 3.0 DNA Polymerase incubated for 4 hours at 65°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>

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



Christie Vazquez  
Production Scientist  
08 Apr 2020



Jay Minichiello  
Packaging Quality Control Inspector  
08 Apr 2020