

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 #: *0071709*
Assay Date: *09/2017*
Expiration Date: *09/2019*
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 #0071709
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 <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.	Pass
Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of a mixture of single and double-stranded [³ 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.	Pass
Non-Specific DNase Activity (16 Hour) - 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.	Pass
Phosphatase Activity (pNPP) - A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl ₂ 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.	Pass
Protein Purity Assay (SDS-PAGE) - <i>Bst</i> 3.0 DNA Polymerase is ≥ 99% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass



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<p>qPCR DNA Contamination (<i>E. coli</i> Genomic) - 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® 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> <p>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 <i>Bst</i> 3.0 DNA Polymerase 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.</p>	<p>Pass</p> <p>Pass</p>



Authorized by
Melanie Fortier
02 Sep 2015



Inspected by
Tony Spear-Alfonso
13 Oct 2017

