

## New England Biolabs Certificate of Analysis

*Product Name:* Therminator™ DNA Polymerase  
*Catalog #:* M0261S/L  
*Concentration:* 2,000 units/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 75°C.  
*Lot #:* 0201703  
*Assay Date:* 03/2017  
*Expiration Date:* 3/2019  
*Storage Temp:* -20°C  
*Storage Conditions:* 10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)  
*Specification Version:* PS-M0261S/L v1.0  
*Effective Date:* 11 Sep 2015

Assay Name/Specification (minimum release criteria)	Lot #0201703
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in ThermoPol® Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 20 units of Therminator™ DNA Polymerase incubated for 4 hours at 37°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 20 units of Therminator™ DNA Polymerase incubated for 4 hours at either 37°C or 75°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 2 units of Therminator™ 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 Therminator™ 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> - Therminator™ DNA Polymerase is ≥ 98% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>



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<b>Assay Name/Specification</b> (minimum release criteria)	<b>Lot #0201703</b>
<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 Thermo <sup>™</sup> 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.	<b>Pass</b>



Authorized by  
Melanie Fortier  
11 Sep 2015



Inspected by  
Tony Spear-Alfonso  
28 Feb 2017

