

## New England Biolabs Product Specification

<b>Product Name:</b>	<i>Therminator™ DNA Polymerase</i>
<b>Catalog #:</b>	<i>M0261S/L/V</i>
<b>Concentration:</b>	<i>2,000 units/ml</i>
<b>Unit Definition:</b>	<i>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.</i>
<b>Shelf Life:</b>	<i>24 months</i>
<b>Storage Temp:</b>	<i>-20°C</i>
<b>Storage Conditions:</b>	<i>10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)</i>
<b>Specification Version:</b>	<i>PS-M0261S/L v2.0</i>
<b>Effective Date:</b>	<i>12 Feb 2020</i>

### Assay Name/Specification (minimum release criteria)

**Endonuclease Activity (Nicking)** - 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.

**Exonuclease Activity (Radioactivity Release)** - 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 20 units of Therminator™ DNA Polymerase incubated for 4 hours at 37°C and 75°C releases <0.1% of the total radioactivity.

**Non-Specific DNase Activity (16 Hour)** - 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 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.

**Phosphatase Activity (pNPP)** - 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 Therminator™ DNA Polymerase incubated for 4 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

**Protein Purity Assay (SDS-PAGE)** - Therminator™ DNA Polymerase is ≥ 98% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

**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 Therminator™ 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.



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