

New England Biolabs Product Specification

<i>Product Name:</i>	<i>T3 RNA Polymerase</i>
<i>Catalog #:</i>	<i>M0378S</i>
<i>Concentration:</i>	<i>50,000 units/ml</i>
<i>Unit Definition:</i>	<i>One unit is defined as the amount of enzyme that will incorporate 1 nmol ATP into acid-insoluble material in a total reaction volume of 50 µl in 1 hour at 37°C in 1X RNA Polymerase Reaction Buffer.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 20 mM βME, 0.1% Triton®X-100, 50% Glycerol, (pH 7.9 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-M0378S v1.0</i>
<i>Effective Date:</i>	<i>13 Jun 2018</i>

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 µl reaction in RNAPol Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 150 units of T3 RNA 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 RNAPol Reaction Buffer containing 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 150 units of T3 RNA Polymerase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in RNAPol Reaction Buffer containing 1 µg of Lambda DNA and a minimum of 250 units of T3 RNA Polymerase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Protein Purity Assay (SDS-PAGE) - T3 RNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

RNase Activity (Extended Digestion) - A 10 µl reaction in RNAPol Reaction Buffer containing 40 ng of a 300 base single-stranded RNA and a minimum of 50 units of T3 RNA Polymerase is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.



Date 13 Jun 2018

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Director of Quality Control

