

New England Biolabs Product Specification

Product Name:	<i>T7 RNA Polymerase (High Concentration)</i>
Catalog #:	<i>M0460T</i>
Concentration:	<i>1,000,000 units/ml</i>
Unit Definition:	<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>
Shelf Life:	<i>24 months</i>
Storage Temp:	<i>-20°C</i>
Storage Conditions:	<i>100 mM NaCl, 50 mM Tris-HCl (pH 7.9), 1 mM EDTA, 20 mM BME, 0.1 % Triton X-100, 50 % Glycerol</i>
Specification Version:	<i>PS-M0460T v1.0</i>
Effective Date:	<i>04 Oct 2019</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 T7 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 T7 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 T7 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.

Promoter Specificity - A 50 µl reaction in RNAPol Reaction Buffer in the presence of 2 mM NTPs containing 1 µg of Lambda DNA as a template and a minimum of 200 units of T7 RNA Polymerase incubated for 1 hour at 37°C results in <1.5% of the amount of product incorporated as compared to a control reaction using T7 DNA as a template.

Protein Purity Assay (SDS-PAGE) - T7 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 T7 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 04 Oct 2019

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Director of Quality Control

