240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

Product Name: T7 RNA Polymerase

Catalog #: M0251S/L
Concentration: 50,000 units/ml

Unit Definition: 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.

 Lot #:
 0091405

 Assay Date:
 05/2014

 Expiration Date:
 5/2016

 Storage Temp:
 -20 °C

Storage Conditions: 100 mM NaCl, 50 mM Tris-HCl (pH 7.9), 1 mM EDTA, 20 mM BME, 0.1 % Triton X-100, 50 % Glycerol

Specification Version: PS-M0251S/L v2.0
Effective Date: 15 May 2014

Assay Name/Specification (minimum release criteria)	Lot #0091405
<b>Endonuclease Activity (Nicking)</b> - 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.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 μl reaction in RNAPol 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 150 units of T7 RNA Polymerase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
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.	Pass
<b>Promoter Specificity</b> - A 50 $\mu$ l reaction in RNAPol Reaction Buffer in the presence of 2 mM NTPs containing 1 $\mu$ g of Lambda DNA as a template and a minimum of 200 units of T7 RNA Polymerase incubated for 4 hours 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.	Pass
<b>Protein Purity Assay (SDS-PAGE)</b> - T7 RNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
RNase Activity (Extended Digestion) - A 10 $\mu$ l 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.	Pass

Authorized by Derek Robinson 15 May 2014







Inspected by
Dongxian Yue
15 May 2014