NEW ENGLAND BioLabs Inc.

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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 #:	*0131507*
Assay Date:	*07/2015*
Expiration Date:	*07/2017*
Storage Temp:	*-20°C*
Storage Buffer:	*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 v3.0*
Effective Date:	*06 Jan 2016*

Assay Name/Specification (minimum release criteria)	**Lot #0131507**
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.	**Pass**
Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in RNAPol Reaction Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] *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.	**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**
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.	**Pass**
Protein Purity Assay (SDS-PAGE) - T7 RNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
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.	**Pass**

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Authorized by
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06 Jan 2016

Inspected by
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11 Jan 2016