

## New England Biolabs Certificate of Analysis

**Product Name:** T7 RNA Polymerase  
**Catalog Number:** M0251L  
**Concentration:** 50,000 U/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.  
**Packaging Lot Number:** 10158543  
**Expiration Date:** 02/2024  
**Storage Temperature:** -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 v3.0

T7 RNA Polymerase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0251LVIAL	T7 RNA Polymerase	10136946	Pass
B9012SVIAL	RNAPol Reaction Buffer	10140898	Pass

Assay Name/Specification	Lot # 10158543
<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] 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
<b>Non-Specific DNase Activity (16 Hour)</b> 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>RNase Activity (Extended Digestion)</b>	Pass

Assay Name/Specification	Lot # 10158543
<p>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, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	
<p><b>Protein Purity Assay (SDS-PAGE)</b> T7 RNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>Promoter Specificity</b> 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 &lt;1.5% of the amount of product incorporated as compared to a control reaction using T7 DNA as a template.</p>	<b>Pass</b>

This product has been tested and shown to be in compliance with all specifications.

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