

## 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:** 10204912  
**Expiration Date:** 06/2025  
**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

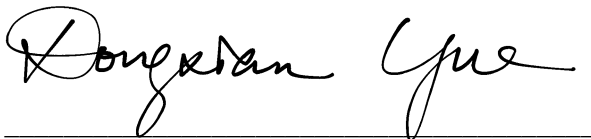
| T7 RNA Polymerase Component List |                        |            |                      |
|----------------------------------|------------------------|------------|----------------------|
| NEB Part Number                  | Component Description  | Lot Number | Individual QC Result |
| M0251LVIAL                       | T7 RNA Polymerase      | 10191106   | Pass                 |
| B9012SVIAL                       | RNAPol Reaction Buffer | 10201967   | Pass                 |

| Assay Name/Specification   | Lot # 10204912 |
|--|----------------|
| <b>Endonuclease Activity (Nicking)</b><br>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><br>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><br>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>  | Pass           |

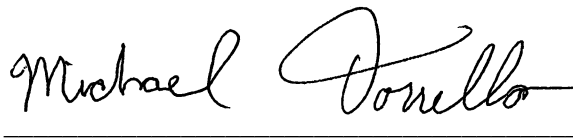
| Assay Name/Specification   | Lot # 10204912 |
|--|----------------|
| <p>A 50 µl reaction in RNAPol Reaction Buffer in the presence of 2 mM NTPs containing 1 µg of Lambda DNA as a template and a minimum of 200 units of T7 RNA Polymerase incubated for 1 hour at 37°C results in &lt;1.5% of the amount of product incorporated as compared to a control reaction using T7 DNA as a template.</p>                                      |                |
| <p><b>Protein Purity Assay (SDS-PAGE)</b><br/>T7 RNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>   | <b>Pass</b>    |
| <p><b>RNase Activity (Extended Digestion)</b><br/>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> | <b>Pass</b>    |

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

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