

## New England Biolabs Certificate of Analysis

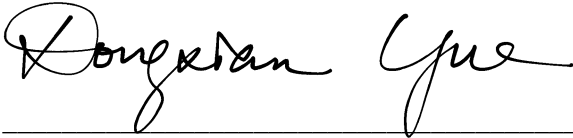
**Product Name:** *Hi-T7 RNA Polymerase*  
**Catalog Number:** *M0658S*  
**Concentration:** *50,000 U/ml*  
**Unit Definition:** *One unit is defined as the amount of enzyme required to incorporate 1 nmol ATP into acid-insoluble material in 1 hour at 50°C.*  
**Packaging Lot Number:** *10183471*  
**Expiration Date:** *03/2025*  
**Storage Temperature:** *-20°C*  
**Storage Conditions:** *50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 0.1% Triton®X-100, 50% Glycerol, (pH 7.9 @ 25°C)*  
**Specification Version:** *PS-M0658S v1.0*

Hi-T7 RNA Polymerase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0658SVIAL	Hi-T7® RNA Polymerase	10182659	Pass
B0658AVIAL	10X Hi-T7™ RNA Polymerase Reaction Buffer	10164455	Pass

Assay Name/Specification	Lot # 10183471
<p><b>Endonuclease Activity (Nicking)</b>            A 50 µl reaction in NEBuffer 4 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 150 units of Hi-T7™ RNA Polymerase incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	Pass
<p><b>Exonuclease Activity (Radioactivity Release)</b>            A 50 µl reaction in NEBuffer 4 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 Hi-T7™ RNA Polymerase incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	Pass
<p><b>Protein Purity Assay (SDS-PAGE)</b>            Hi-T7™ RNA Polymerase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	Pass
<p><b>RNase Activity (Extended Digestion)</b>            A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 50 units of Hi-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>	Pass

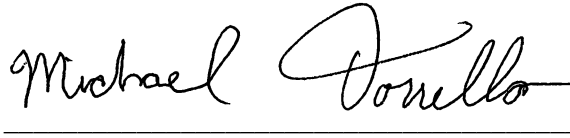
This product has been tested and shown to be in compliance with all specifications.

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09 Mar 2023



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13 Mar 2023