

## New England Biolabs Certificate of Analysis

**Product Name:** T4 RNA Ligase 2, truncated K227Q  
**Catalog Number:** M0351S  
**Concentration:** 200,000 U/ml  
**Unit Definition:** 200 units is defined as the amount of enzyme required to give 80% ligation of a 31-mer RNA to the pre-adenylated end of a 17-mer DNA in a total reaction volume of 20 µl in 1 hour at 25°C.  
**Packaging Lot Number:** 10163626  
**Expiration Date:** 09/2024  
**Storage Temperature:** -20°C  
**Storage Conditions:** 100 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)  
**Specification Version:** PS-M0351S/L v2.0

T4 RNA Ligase 2, truncated K227Q Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0351SVIAL	T4 RNA Ligase 2, truncated K227Q	10163625	Pass
B1004SVIAL	PEG 8000	10115452	Pass
B0216SVIAL	T4 RNA Ligase Reaction Buffer	10122381	Pass

Assay Name/Specification	Lot # 10163626
<b>Protein Purity Assay (SDS-PAGE)</b> T4 RNA Ligase 2, truncated K227Q is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in T4 RNA Ligase Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 200 units of T4 RNA Ligase 2, truncated K227Q 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 T4 RNA Ligase Reaction Buffer containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 200 units of T4 RNA Ligase 2, truncated K227Q incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>RNase Activity (Extended Digestion)</b>	Pass

Assay Name/Specification	Lot # 10163626
<p>A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 200 units of T4 RNA Ligase 2, truncated K227Q is incubated at 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p> <p><b>Phosphatase Activity (pNPP)</b> A 200 ul reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl<sub>2</sub> containing 2.5 mM p-Nitrophenyl Phosphate (pNPP) and a minimum of 200 units of T4 RNA Ligase 2, truncated K227Q incubated for 4 hours at 37°C yields &lt;0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</p>	<p><b>Pass</b></p>

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

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Bo Wu  
Production Scientist  
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Josh Hersey  
Packaging Quality Control Inspector  
02 Nov 2022