

## New England Biolabs Certificate of Analysis

**Product Name:** T4 RNA Ligase 2  
**Catalog Number:** M0239L  
**Concentration:** 10,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to ligate 0.4 µg of an equimolar mix of a 23-mer and 17-mer RNAs in a total reaction volume of 20 µl in 30 minutes at 37°C.  
**Packaging Lot Number:** 10200635  
**Expiration Date:** 06/2025  
**Storage Temperature:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl, 50 mM KCl, 0.1 mM DTT, 0.1 mM EDTA, 35 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 50 % Glycerol, (pH 7.5 @ 25°C)  
**Specification Version:** PS-M0239S/L v1.0

T4 RNA Ligase 2 Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0239LVIAL	T4 RNA Ligase 2 (dsRNA Ligase)	10195916	Pass
B0239SVIAL	T4 RNA Ligase 2 Reaction Buffer	10176272	Pass

Assay Name/Specification	Lot # 10200635
<p><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 100 units of T4 RNA Ligase 2 (dsRNA Ligase) 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 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 100 units of T4 RNA Ligase 2 (dsRNA Ligase) incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	Pass
<p><b>Phosphatase Activity (pNPP)</b>            A 200 µl 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 100 units of T4 RNA Ligase 2 (dsRNA Ligase) incubated for 4 hours at 37°C yields &lt;0.00001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</p>	Pass

Assay Name/Specification	Lot # 10200635
<p><b>Protein Purity Assay (SDS-PAGE)</b> T4 RNA Ligase 2 (dsRNA Ligase) is <math>\geq 99\%</math> pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>RNase Activity (Extended Digestion)</b> A 10 <math>\mu</math>l reaction in T4 RNA Ligase Reaction Buffer containing 40 ng of a 300 base single-stranded RNA and a minimum of 10 units of T4 RNA Ligase 2 (dsRNA Ligase) is incubated at 37°C. After incubation for 4 hours, <math>&gt;90\%</math> of the substrate RNA remains intact as determined by gel electrophoresis using agarose gel electrophoresis.</p>	<b>Pass</b>

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit [www.neb.com/trademarks](http://www.neb.com/trademarks) for additional information.



Bo Wu  
Production Scientist  
08 Jun 2023



Josh Hersey  
Packaging Quality Control Inspector  
24 Jul 2023