

## New England Biolabs Certificate of Analysis

**Product Name:** T4 RNA Ligase 1 (ssRNA Ligase)  
**Catalog Number:** M0204L  
**Concentration:** 10,000 U/ml  
**Unit Definition:** One unit is defined as the amount of enzyme required to convert 1 nanomole of 5'-[<sup>32</sup>P] rA16 into a phosphatase-resistant form in 30 minutes at 37°C.  
**Packaging Lot Number:** 10176560  
**Expiration Date:** 09/2024  
**Storage Temperature:** -20°C  
**Storage Conditions:** 50 mM KCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)  
**Specification Version:** PS-M0204S/L v1.0

T4 RNA Ligase 1 (ssRNA Ligase) Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
P0756SVIAL	Adenosine 5'-Triphosphate (ATP)	10176561	Pass
M0204LVIAL	T4 RNA Ligase 1 (ssRNA Ligase)	10164131	Pass
B1004SVIAL	PEG 8000	10153719	Pass
B0216SVIAL	T4 RNA Ligase Reaction Buffer	10153851	Pass

Assay Name/Specification	Lot # 10176560
<p><b>qPCR DNA Contamination (E. coli Genomic)</b>            A minimum of 10 units of T4 RNA Ligase 1 (ssRNA Ligase) is screened for the presence of E. coli genomic DNA using SYBR® Green qPCR with primers specific for the E. coli 16S rRNA locus. Results are quantified using a standard curve generated from purified E. coli genomic DNA. The measured level of E. coli genomic DNA contamination is ≤ 1 E. coli genome.</p>	Pass
<p><b>Protein Purity Assay (SDS-PAGE)</b>            T4 RNA Ligase 1 (ssRNA Ligase) 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 1 µl of T4 RNA Ligase 1 (ssRNA Ligase) 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>	Pass

Assay Name/Specification	Lot # 10176560
<p><b>Endonuclease Activity (Nicking)</b> A 50 µL reaction in T4 RNA Ligase 1 Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 30 units of T4 RNA Ligase 1 (ssRNA Ligase) incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in T4 RNA Ligase 1 Reaction Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] E. coli DNA and a minimum of 30 units of T4 RNA Ligase 1 (ssRNA Ligase) incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	<b>Pass</b>

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

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17 Jan 2023