

## New England Biolabs Certificate of Analysis

**Product Name:** T4 RNA Ligase 1 (ssRNA Ligase)  
**Catalog #:** M0204S/L  
**Concentration:** 10,000 units/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.  
**Lot #:** 0671804  
**Assay Date:** 04/2018  
**Expiration Date:** 4/2020  
**Storage Temp:** -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  
**Effective Date:** 28 Mar 2018

Assay Name/Specification (minimum release criteria)	Lot #0671804
<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 <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<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] <i>E. coli</i> DNA and a minimum of 30 units of T4 RNA Ligase 1 (ssRNA Ligase) incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<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.	<b>Pass</b>
<b>qPCR DNA Contamination (<i>E. coli</i> Genomic)</b> - A minimum of 10 units of T4 RNA Ligase 1 (ssRNA Ligase) is screened for the presence of <i>E. coli</i> genomic DNA using SYBR® Green qPCR with primers specific for the <i>E. coli</i> 16S rRNA locus. Results are quantified using a standard curve generated from purified <i>E. coli</i> genomic DNA. The measured level of <i>E. coli</i> genomic DNA contamination is ≤ 1 <i>E. coli</i> genome.	<b>Pass</b>
<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, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	<b>Pass</b>



Authorized by  
Derek Robinson  
28 Mar 2018



Inspected by  
Bo Wu  
24 Apr 2018

