

## New England Biolabs Product Specification

<b>Product Name:</b>	<i>T4 RNA Ligase 1 (ssRNA Ligase), High Concentration</i>
<b>Catalog #:</b>	<i>M0437M</i>
<b>Concentration:</b>	<i>30,000 units/ml</i>
<b>Unit Definition:</b>	<i>One unit is defined as the amount of enzyme required to convert 1 nanomole of fluorescently labeled rA16 into a phosphatase-resistant form in 30 minutes at 37°C.</i>
<b>Shelf Life:</b>	<i>24 months</i>
<b>Storage Temp:</b>	<i>-20°C</i>
<b>Storage Conditions:</b>	<i>10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)</i>
<b>Specification Version:</b>	<i>PS-M0437M v2.0</i>
<b>Effective Date:</b>	<i>23 Oct 2024</i>

### Assay Name/Specification (minimum release criteria)

**Endonuclease Activity (Nicking)** - 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), High Concentration incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.

**Exonuclease Activity (Radioactivity Release)** - 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), High Concentration incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Protein Purity Assay (SDS-PAGE)** - T4 RNA Ligase 1 (ssRNA Ligase), High Concentration is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

**qPCR DNA Contamination (*E. coli* Genomic)** - A minimum of 10 units of T4 RNA Ligase 1 (ssRNA Ligase), High Concentration 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.

**RNase Activity (Extended Digestion)** - A 10 µl reaction in NEBuffer 4 containing 40 ng of RNA transcript and a minimum of 30 units of T4 RNA Ligase 1 (ssRNA Ligase), High Concentration is incubated at 37°C. After incubation for 16 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using gel electrophoresis.

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Date 23 Oct 2024

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