

## New England Biolabs Product Specification

<b>Product Name:</b>	<i>T4 RNA Ligase 2, truncated KQ</i>
<b>Catalog #:</b>	<i>M0373S/L</i>
<b>Concentration:</b>	<i>200,000 units/ml</i>
<b>Unit Definition:</b>	<i>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.</i>
<b>Shelf Life:</b>	<i>24 months</i>
<b>Storage Temp:</b>	<i>-20°C</i>
<b>Storage Conditions:</b>	<i>100 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)</i>
<b>Specification Version:</b>	<i>PS-M0373S/L v2.0</i>
<b>Effective Date:</b>	<i>11 Jul 2018</i>

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

**Endonuclease Activity (Nicking)** - 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 KQ 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 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 KQ incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Phosphatase Activity (pNPP)** - 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 200 units of T4 RNA Ligase 2, truncated KQ incubated for 4 hours at 37°C yields <0.00001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.

**Protein Purity Assay (SDS-PAGE)** - T4 RNA Ligase 2, truncated KQ is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

**RNase Activity (Extended Digestion)** - 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 KQ 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.



Date 11 Jul 2018

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