

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>SplintR® Ligase</i>
<i>Catalog #:</i>	<i>M0375S/L</i>
<i>Concentration:</i>	<i>25,000 units/ml</i>
<i>Unit Definition:</i>	<i>One unit is defined as the amount of enzyme needed to ligate (to 50% completion) 2 picomoles of a tripartite FAM-labeled DNA:RNA hybrid substrate in a 20 µl reaction at 25°C in 15 minutes in 1X SplintR® Ligase Reaction Buffer.</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-M0375S/L v1.0</i>
<i>Effective Date:</i>	<i>08 Feb 2018</i>

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

**Endonuclease Activity (Nicking)** - A 50 µl reaction in SplintR® Ligase Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 125 units of SplintR® Ligase 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 SplintR® 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 125 units of SplintR® Ligase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Protein Purity Assay (SDS-PAGE)** - SplintR® Ligase 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 25 units of SplintR® 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.



Date 08 Feb 2018

Derek Robinson  
Director of Quality Control

