

## New England Biolabs Certificate of Analysis

*Product Name:* SplintR<sup>®</sup> Ligase  
*Catalog #:* M0375S/L  
*Concentration:* 25,000 units/ml  
*Unit Definition:* 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<sup>®</sup> Ligase Reaction Buffer.  
*Lot #:* 0011803  
*Assay Date:* 03/2018  
*Expiration Date:* 03/2020  
*Storage Temp:* -20°C  
*Storage Conditions:* 10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)  
*Specification Version:* PS-M0375S/L v1.0  
*Effective Date:* 08 Feb 2018

Assay Name/Specification (minimum release criteria)	Lot #0011803
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in SplintR <sup>®</sup> Ligase Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 125 units of SplintR <sup>®</sup> 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 SplintR <sup>®</sup> Ligase 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 125 units of SplintR <sup>®</sup> 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> - SplintR <sup>®</sup> Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<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 25 units of SplintR <sup>®</sup> 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  
08 Feb 2018



Inspected by  
Bo Wu  
16 Mar 2018

