

## New England Biolabs Certificate of Analysis

**Product Name:** SplintR<sup>®</sup> Ligase  
**Catalog Number:** M0375L  
**Concentration:** 25,000 U/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.  
**Packaging Lot Number:** 10159592  
**Expiration Date:** 07/2024  
**Storage Temperature:** -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

SplintR <sup>®</sup> Ligase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0375LVIAL	SplintR <sup>®</sup> Ligase	10156776	Pass
B0375SVIAL	10X SplintR <sup>®</sup> Ligase Reaction Buffer	10156815	Pass

Assay Name/Specification	Lot # 10159592
<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] E. coli 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	<b>Pass</b>

Assay Name/Specification	Lot # 10159592
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.	

This product has been tested and shown to be in compliance with all specifications.

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