

## New England Biolabs Certificate of Analysis

**Product Name:** SplintR® Ligase  
**Catalog Number:** M0375S  
**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® Ligase Reaction Buffer.  
**Lot Number:** 10040002  
**Expiration Date:** 03/2021  
**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® Ligase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0375SVIAL	SplintR® Ligase	10039999	Pass
B0375SVIAL	10X SplintR® Ligase Reaction Buffer	10020201	Pass

Assay Name/Specification	Lot # 10040002
<b>Endonuclease Activity (Nicking)</b> 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.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> 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.	Pass
<b>Protein Purity Assay (SDS-PAGE)</b> SplintR® Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<b>RNase Activity (Extended Digestion)</b> A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA	Pass

Assay Name/Specification	Lot # 10040002
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.



Bo Wu  
Production Scientist  
12 Sep 2018



Michael Tonello  
Packaging Quality Control Inspector  
18 Apr 2019