

## New England Biolabs Certificate of Analysis

**Product Name:** RtcB Ligase  
**Catalog #:** M0458S  
**Concentration:** 15 µM  
**Lot #:** 0041801  
**Assay Date:** 01/2018  
**Expiration Date:** 01/2020  
**Storage Temp:** -20°C  
**Storage Conditions:** 50 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)  
**Specification Version:** PS-M0458S v1.0  
**Effective Date:** 23 May 2018

Assay Name/Specification (minimum release criteria)	Lot #0041801
<b>Endonuclease Activity (Nicking)</b> - A 50 µl reaction in RtcB Reaction Buffer containing 1 µg of supercoiled PhiX174 DNA and a minimum of 15 pmol of RtcB 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 RtcB 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 15 pmol of RtcB Ligase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Functional Testing (RtcB Ligase)</b> - A 20 µl reaction in 1X RtcB Reaction Buffer supplemented with 0.1 mM GTP and 1 mM MnCl <sub>2</sub> containing 0.5 µM of a 17 mer 5' FAM-labeled 3' phosphorylated RNA, 0.5 µM of a 30 mer 5' OH RNA and 0.75 µM RtcB Ligase incubated for 1 hour at 37°C results in ≥ 80% ligation as determined by capillary electrophoresis.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - RtcB 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 15 pmol of RtcB 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  
23 May 2018



Inspected by  
Bhairavi Jani  
12 Jan 2018

