

## New England Biolabs Certificate of Analysis

**Product Name:** *RtcB Ligase*  
**Catalog Number:** *M0458S*  
**Concentration:** *15 µM*  
**Packaging Lot Number:** *10176763*  
**Expiration Date:** *07/2024*  
**Storage Temperature:** *-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*

RtcB Ligase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
N2080AVIAL	GTP	10156182	Pass
M0458SVIAL	RtcB Ligase	10176036	Pass
B1761SVIAL	10mM MnCl <sub>2</sub>	10167824	Pass
B0790AVIAL	10X RtcB Reaction Buffer	10176038	Pass

Assay Name/Specification	Lot # 10176763
<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] E. coli DNA and a minimum of 15 pmol of RtcB Ligase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<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.	Pass
<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.	Pass
<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	Pass


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<p>16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p> <p><b>Protein Purity Assay (SDS-PAGE)</b> RtcB Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<p><b>Pass</b></p>

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

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