

## New England Biolabs Certificate of Analysis

**Product Name:** *RtcB Ligase*  
**Catalog Number:** *M0458S*  
**Concentration:** *15 µM*  
**Packaging Lot Number:** *10115450*  
**Expiration Date:** *12/2022*  
**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                      | 10114990   | Pass                 |
| M0458SVIAL                 | RtcB Ligase              | 10092638   | Pass                 |
| B1761SVIAL                 | 10mM MnCl <sub>2</sub>   | 10091462   | Pass                 |
| B0790AVIAL                 | 10X RtcB Reaction Buffer | 10092639   | Pass                 |

| Assay Name/Specification   | Lot # 10115450 |
|--|----------------|
| <b>Exonuclease Activity (Radioactivity Release)</b><br>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><br>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><br>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>Protein Purity Assay (SDS-PAGE)</b><br>RtcB Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.   | Pass           |


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|--|--------------------|
| <p><b>RNase Activity (Extended Digestion)</b><br/>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, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent 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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