

New England Biolabs Product Specification

<i>Product Name:</i>	<i>RtcB Ligase</i>
<i>Catalog #:</i>	<i>M0458S</i>
<i>Concentration:</i>	<i>15 µM</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>50 mM NaCl, 10 mM Tris-HCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-M0458S v1.0</i>
<i>Effective Date:</i>	<i>23 May 2018</i>

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - 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.

Exonuclease Activity (Radioactivity Release) - A 50 µl reaction in RtcB Reaction Buffer containing 1 µg of a mixture of single and double-stranded [³H] *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.

Functional Testing (RtcB Ligase) - A 20 µl reaction in 1X RtcB Reaction Buffer supplemented with 0.1 mM GTP and 1 mM MnCl₂ 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.

Protein Purity Assay (SDS-PAGE) - RtcB Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

RNase Activity (Extended Digestion) - 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.



Date 23 May 2018

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Director of Quality Control

