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## New England Biolabs Certificate of Analysis

Product Name:RtcB LigaseCatalog Number:M0458SConcentration:15 μMPackaging Lot Number:10102982Expiration Date:01/2023Storage 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				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
N2080AVIAL	GTP	10094852	Pass	
M0458SVIAL	RtcB Ligase	10102983	Pass	
B1761SVIAL	10mM MnCl2	10091462	Pass	
B0790AVIAL	10X RtcB Reaction Buffer	10092639	Pass	

Assay Name/Specification	Lot # 10102982
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	Pass
<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.	Pass
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.	Pass
Protein Purity Assay (SDS-PAGE) RtcB Ligase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass



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Assay Name/Specification	Lot # 10102982
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.	Pass

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

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Bhairavi Jani Production Scientist

25 Mar 2021

Michael Tonello

Packaging Quality Control Inspector

25 Mar 2021