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New England Biolabs Certificate of Analysis

Product Name: Thermostable 5' App DNA/RNA Ligase

Catalog Number: M0319L
Concentration: 20 µM
Packaging Lot Number: 10160849
Expiration Date: 08/2024
Storage Temperature: -20°C

Storage Conditions: 10 mM Tris-HCl , 50 mM KCl , 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol,

(pH 7.4 @ 25°C)

Specification Version: PS-M0319S/L v1.0

Thermostable 5' App DNA/RNA Ligase Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
M0319LVIAL	Thermostable 5' App DNA/RNA Ligase	10156774	Pass	
B7001SVIAL	NEBuffer™ 1	10150375	Pass	
B0787AVIAL	MnCl ₂	10147746	Pass	

Assay Name/Specification	Lot # 10160849
Phosphatase Activity (pNPP) A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl2 containing 2.5 mM p-Nitrophenyl Phosphate (pNPP) and a minimum of 100 pmol of Thermostable 5' App DNA/RNA Ligase incubated for 16 hours at 37°C yields <0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.	Pass
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer 1 containing 1 µg of a mixture of single and double-stranded [³H] E. coli DNA and a minimum of 100 pmol of Thermostable 5' App DNA/RNA Ligase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
Endonuclease Activity (Nicking) A 50 μl reaction in NEBuffer 1 containing 1 μg of supercoiled PhiX174 DNA and a minimum of 100 pmol of Thermostable 5' App DNA/RNA Ligase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
RNase Activity (Extended Digestion) A 10 µl reaction in NEBuffer 1 containing 40 ng of a 300 base single-stranded RNA	Pass



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Assay Name/Specification	Lot # 10160849
and a minimum of 100 pmol of Thermostable 5' App DNA/RNA 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.	
Functional Testing (Targeted Ligation) A 20 µl reaction in 1X NEBuffer 1 containing 20 pmol of 30 bp FAM-labeled single-stranded RNA, 200 pmol 17 bp 5' pre-adenylated single-stranded DNA linker, and 40 pmol Thermostable 5' App DNA/RNA Ligase incubated for 1 hour at 65°C results in ≥80% ligation of the substrate RNA as determined by capillary electrophoresis.	Pass

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Bhairavi Jani **Production Scientist**

22 Aug 2022

Michael Tonello

Packaging Quality Control Inspector

22 Aug 2022



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