

New England Biolabs Certificate of Analysis

Product Name: *Thermostable 5' App DNA/RNA Ligase*
Catalog Number: *M0319L*
Concentration: *20 µM*
Packaging Lot Number: *10221729*
Expiration Date: *10/2025*
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	10209978	Pass
B7001SVIAL	NEBuffer™ 1	10198640	Pass
B0787AVIAL	MnCl ₂	10209979	Pass

Assay Name/Specification	Lot # 10221729
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
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer 1 containing 1 µg of a mixture of single and double-stranded [³ H] 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
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
Phosphatase Activity (pNPP) A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl ₂ containing 2.5 mM	Pass

Assay Name/Specification	Lot # 10221729
<p>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.</p> <p>RNase Activity (Extended Digestion) A 10 µl reaction in NEBuffer 1 containing 40 ng of a 300 base single-stranded RNA 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.</p>	<p>Pass</p>

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

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Production Scientist
25 Oct 2023



Josh Hersey
Packaging Quality Control Inspector
17 Jan 2024