

## New England Biolabs Certificate of Analysis

**Product Name:** *Thermostable 5' App DNA/RNA Ligase*  
**Catalog Number:** *M0319L*  
**Concentration:** *20 µM*  
**Packaging Lot Number:** *10079372*  
**Expiration Date:** *07/2022*  
**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*

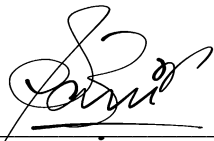
Thermostable 5' App DNA/RNA Ligase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0319LVIAL	Thermostable 5' App DNA/RNA Ligase	10079373	Pass
B7001SVIAL	NEBuffer™ 1	10064408	Pass
B0787AVIAL	MnCl <sub>2</sub>	10061004	Pass

Assay Name/Specification	Lot # 10079372
<b>Phosphatase Activity (pNPP)</b> A 200 µl reaction in 1M Diethanolamine, pH 9.8, 0.5 mM MgCl <sub>2</sub> 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
<b>Functional Testing (Targeted Ligation)</b> 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
<b>Endonuclease Activity (Nicking)</b> 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
<b>RNase Activity (Extended Digestion)</b> A 10 µl reaction in NEBuffer 1 containing 40 ng of a 300 base single-stranded RNA	Pass

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<p>and a minimum of 100 pmol of Thermostable 5' App DNA/RNA 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>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in NEBuffer 1 containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>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 &lt;0.1% of the total radioactivity.</p>	<p><b>Pass</b></p>

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

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