

## New England Biolabs Certificate of Analysis

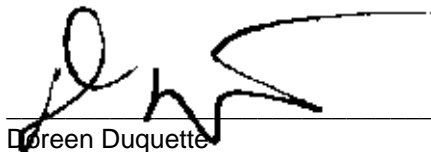
**Product Name:** *Thermostable 5' App DNA/RNA Ligase*  
**Catalog Number:** *M0319L*  
**Concentration:** *20 µM*  
**Packaging Lot Number:** *10059235*  
**Expiration Date:** *11/2021*  
**Storage Temperature:** *-20°C*  
**Storage Conditions:** *10 mM Tris-HCl , 50 mM KCl , 1 mM DTT , 0.1 mM EDTA , 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 | 10059236   | Pass                 |
| B7001SVIAL  | NEBuffer™ 1                        | 10041625   | Pass                 |
| B0787AVIAL  | MnCl <sub>2</sub>                  | 10061004   | Pass                 |

| Assay Name/Specification  | Lot # 10059235 |
|---|----------------|
| <p><b>RNase Activity (Extended Digestion)</b><br/>           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, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>                 | Pass           |
| <p><b>Phosphatase Activity (pNPP)</b><br/>           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 &lt;0.0001 unit of alkaline phosphatase activity as determined by spectrophotometric analysis.</p>                | Pass           |
| <p><b>Functional Testing (Targeted Ligation)</b><br/>           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.</p> | Pass           |
| <p><b>Endonuclease Activity (Nicking)</b><br/>           A 50 µl reaction in NEBuffer 1 containing 1 µg of supercoiled PhiX174 DNA and a</p>  | Pass           |

| Assay Name/Specification   | Lot # 10059235     |
|--|--------------------|
| <p>minimum of 100 pmol of Thermostable 5' App DNA/RNA Ligase incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p> <p><b>Exonuclease Activity (Radioactivity Release)</b><br/>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.



Doreen Duquette  
Production Scientist  
17 Apr 2019



Michael Tonello  
Packaging Quality Control Inspector  
26 Nov 2019