NEW ENGLAND BioLabs Inc.
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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:	*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	$MnCl_2$	10061004	**Pass**

Assay Name/Specification	**Lot # 10059235**
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.	**Pass**
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**
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**
Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer 1 containing 1 µg of supercoiled PhiX174 DNA and a	**Pass**

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Assay Name/Specification	Lot # 10059235
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.	
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**

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