

New England Biolabs Product Specification

Product Name: *Thermostable 5' App DNA/RNA Ligase*
Catalog #: *M0319S/L*
Concentration: *20 µM*
Shelf Life: *24 months*
Storage Temp: *-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*
Effective Date: *01 Nov 2018*

Assay Name/Specification (minimum release criteria)

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.

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.

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.

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.

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.



Date 01 Nov 2018

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Director of Quality Control

