

## New England Biolabs Product Specification

<b>Product Name:</b>	9°N™ DNA Ligase
<b>Catalog #:</b>	M0238S/L
<b>Concentration:</b>	40,000 units/ml
<b>Unit Definition:</b>	One unit is defined as the amount of enzyme required to give 50% ligation of the 12-base pair cohesive ends of 1 µg of BstEII-digested Lambda DNA in 15 minutes at 45°C.
<b>Shelf Life:</b>	24 months
<b>Storage Temp:</b>	-20°C
<b>Storage Conditions:</b>	10 mM Tris-HCl, 50 mM KCl, 10 mM (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , 1 mM DTT, 0.1 mM EDTA, 200 µg/ml BSA, 50 % Glycerol, (pH 7.5 @ 25°C)
<b>Specification Version:</b>	PS-M0238S/L v1.0
<b>Effective Date:</b>	30 Nov 2016

### Assay Name/Specification (minimum release criteria)

**Endonuclease Activity (Nicking)** - A 50 µl reaction in NEBuffer 4 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 400 units of 9°N™ DNA 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 9°N™ DNA Ligase Reaction Buffer containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA and a minimum of 400 units of 9°N™ DNA Ligase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in NEBuffer 4 containing 1 µg of Lambda-HindIII DNA and a minimum of 80 units of 9°N™ DNA Ligase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**RNase Activity (Extended Digestion)** - A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 µl of 9°N™ DNA 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.

\* The BSA in this product has been granted an EDQM "Certificate of Suitability" from the European Directorate for the Quality of Medicines (# R1-CEP-2003-204-Rev00) and has been granted a USDA Certificate for Export of Bovine Blood Plasma/Serum for Manufacture into Pharmaceutical Products.



Date 30 Nov 2016

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Director of Quality Control

