

New England Biolabs Product Specification

Product Name: Cas9 Nuclease, *S. pyogenes*
Catalog #: M0386S/L
Concentration: 1,000 nM
Shelf Life: 24 months
Storage Temp: -20°C
Storage Conditions: 10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)
Specification Version: PS-M0386S/L v1.0
Effective Date: 14 Jun 2018

Assay Name/Specification (minimum release criteria)

Endonuclease Activity (Nicking) - A 50 µl reaction in NEBuffer 3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 1 pmol of Cas9 Nuclease, *S. pyogenes* 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 3.1 containing 1 µg of a mixture of single and double-stranded [³H] *E. coli* DNA and a minimum of 1 pmol of Cas9 Nuclease, *S. pyogenes* incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

Functional Testing (Targeted Digestion) - A 20 µl reaction in NEBuffer 3.1 containing 20 nM of 100 bp FAM and ROX-labeled double-stranded target DNA, 100 nM sgRNA, and 100 nM Cas9 Nuclease, *S. pyogenes* incubated for 1 hour at 37°C results in ≥90% targeted digestion of the substrate DNA as determined by capillary electrophoresis.

Non-Specific DNase Activity (16 Hour) - A 50 µl reaction in NEBuffer 3.1 containing 1 µg of Lambda DNA and a minimum of 1 pmol of Cas9 Nuclease, *S. pyogenes* incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Protein Purity Assay (SDS-PAGE) - Cas9 Nuclease, *S. pyogenes* is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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 pmol of Cas9 Nuclease, *S. pyogenes* 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 14 Jun 2018

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Director of Quality Control

