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New England Biolabs
Product Specification

Product Name:	*Cas9 Nuclease, S. pyogenes*
Catalog #:	*M0386T/M*
Concentration:	*20 µM*
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-M0386T/M 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 [^{3}H] *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

Derek Robinson
Director of Quality Control

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ISO 14001 Registered Environmental Management
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