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## New England Biolabs Certificate of Analysis

Product Name: Cas9 Nuclease, S. pyogenes

Catalog Number: M0386S
Concentration: 1,000 nM
Packaging Lot Number: 10149086
Expiration Date: 05/2024
Storage Temperature: -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

Cas9 Nuclease, S. pyogenes Component List				
<b>NEB Part Number</b>	Component Description	Lot Number	Individual QC Result	
M0386SVIAL	Cas9 Nuclease, S. pyogenes	10149085	Pass	
B6003SVIAL	NEBuffer™ r3.1	10132773	Pass	

Assay Name/Specification	Lot # 10149086
Exonuclease Activity (Radioactivity Release)	Pass
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.	
Endonuclease Activity (Nicking)	Pass
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.	
Non-Specific DNase Activity (16 Hour)	Pass
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.	
Functional Testing (Targeted Digestion)	Pass
A 20 µl reaction in NEBuffer 3.1 containing 20 nM of 100 bp FAM and ROX-labeled	1 433
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	



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Assay Name/Specification	Lot # 10149086
as determined by capillary electrophoresis.	
RNase Activity (Extended Digestion) A 10 $\mu$ I 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.	Pass
Protein Purity Assay (SDS-PAGE) Cas9 Nuclease, S. pyogenes is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass

This product has been tested and shown to be in compliance with all specifications.

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Bhairavi Jani Production Scientist 19 May 2022 Erin Varney

Packaging Quality Control Inspector

19 May 2022



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