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240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

New England Biolabs Certificate of Analysis

Product Name:	Cas9 Nuclease, S. pyogenes
Catalog Number:	M0386T
Concentration:	20 μΜ
Packaging Lot Number:	10057222
Expiration Date:	10/2021
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-M0386T/M v1.0

Cas9 Nuclease, S. pyogenes Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
M0386TVIAL	Cas9 Nuclease, S. pyogenes	10057220	Pass	
B7203SVIAL	NEBuffer™ 3.1	10053972	Pass	

Assay Name/Specification	Lot # 10057222
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.	Pass
Functional Testing (Targeted Digestion) A 20 μ I 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.	Pass
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.	Pass
Protein Purity Assay (SDS-PAGE) Cas9 Nuclease, S. pyogenes is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass





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Assay Name/Specification	Lot # 10057222
RNase Activity (Extended Digestion) A 10 μ 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
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.	Pass

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

Doreen Duquette

Production Scientist 16 Sep 2019

Michae m. U.

Michael Tonello Packaging Quality Control Inspector 25 Nov 2019

