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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:	EnGen® Sau Cas9
Catalog Number:	M0654S
Concentration:	1 μΜ
Packaging Lot Number:	10171037
Expiration Date:	07/2024
Storage Temperature:	-20°C
Storage Conditions:	20 mM Tris-HCl, 300 mM NaCl, 0.1 mM TCEP, 50% Glycerol, (pH 7.5 @ 25°)
Specification Version:	PS-M0654S v1.0

EnGen® Sau Cas9 Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
M0654SVIAL	EnGen® Sau Cas9	10156779	Pass	
B6003SVIAL	NEBuffer™ r3.1	10146827	Pass	

Assay Name/Specification	Lot # 10171037
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 EnGen® Sau Cas9 incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel 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 EnGen® Sau Cas9 incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
Functional Testing (Targeted Digestion) A 20 µl reaction in NEBuffer 3.1 containing 20 nM of 515 bp FAM and ROX-labeled double-stranded target DNA, 100 nM sgRNA, and 100 nM EnGen® Sau Cas9 incubated for 15 minutes at 37°C results in ≥90% targeted digestion of the substrate DNA as determined by capillary electrophoresis.	Pass
Protein Purity Assay (SDS-PAGE) EnGen® Sau Cas9 is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
RNase Activity (Extended Digestion)	Pass





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Assay Name/Specification	Lot # 10171037
A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 pmol of EnGen® Sau Cas9 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.	
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 EnGen® Sau Cas9	
incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Jessica Cane Production Scientist 18 Jul 2022

Michae

Michael Tonello Packaging Quality Control Inspector 07 Nov 2022

