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New England Biolabs Certificate of Analysis

Product Name: EnGen® Sau Cas9

Catalog Number:M0654SConcentration: $1 \mu M$ Packaging Lot Number:10119919Expiration Date:08/2023Storage Temperature: $-20^{\circ}C$

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	10117914	Pass	
B6003SVIAL	NEBuffer™ r3.1	10117885	Pass	

Assay Name/Specification	Lot # 10119919
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%	Pass
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 EnGen® Sau Cas9 incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	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
Protein Purity Assay (SDS-PAGE) EnGen® Sau Cas9 is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
RNase Activity (Extended Digestion) A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA	Pass



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Assay Name/Specification	Lot # 10119919
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.	
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

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.

Bhairavi Jani Production Scientist 14 Sep 2021 Michael Tonello

Packaging Quality Control Inspector

14 Sep 2021



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