

New England Biolabs Certificate of Analysis

Product Name: EnGen[®] Sau Cas9
Catalog Number: M0654T
Concentration: 20 µM
Lot Number: 10040099
Expiration Date: 03/2021
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-M0654T v1.0

EnGen [®] Sau Cas9 Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0654TVIAL	EnGen [®] Sau Cas9	10040078	Pass
B7203SVIAL	NEBuffer [™] 3.1	10033149	Pass

Assay Name/Specification	Lot # 10040099
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
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 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.	Pass
Protein Purity Assay (SDS-PAGE) EnGen [®] Sau Cas9 is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	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

Assay Name/Specification	Lot # 10040099
<p>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.</p>	Pass
<p>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.</p>	Pass

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



Jessica Cane
Production Scientist
20 Mar 2019



Michael Tonello
Packaging Quality Control Inspector
01 Apr 2019