

New England Biolabs Certificate of Analysis

Product Name: EnGen[®] Sau Cas9
Catalog Number: M0654T
Concentration: 20 µM
Packaging Lot Number: 10065477
Expiration Date: 02/2022
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	10065476	Pass
B7203SVIAL	NEBuffer [™] 3.1	10072160	Pass

Assay Name/Specification	Lot # 10065477
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
Protein Purity Assay (SDS-PAGE) EnGen [®] Sau Cas9 is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	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
RNase Activity (Extended Digestion)	Pass

Assay Name/Specification	Lot # 10065477
<p>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.</p> <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>	<p>Pass</p>

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



Bhairavi Jani
Production Scientist
20 May 2020



Michael Tonello
Packaging Quality Control Inspector
20 May 2020