

## New England Biolabs Certificate of Analysis

**Product Name:** EnGen® Sau Cas9  
**Catalog Number:** M0654S  
**Concentration:** 1 µM  
**Packaging Lot Number:** 10107281  
**Expiration Date:** 12/2022  
**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-M0654S v1.0

EnGen® Sau Cas9 Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0654SVIAL	EnGen® Sau Cas9	10093114	Pass
B6003SVIAL	NEBuffer™ r3.1	10103929	Pass

Assay Name/Specification	Lot # 10107281
<b>Endonuclease Activity (Nicking)</b> 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
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in NEBuffer 3.1 containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] 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
<b>Functional Testing (Targeted Digestion)</b> 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
<b>RNase Activity (Extended Digestion)</b> 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

Assay Name/Specification	Lot # 10107281
<p><b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in NEBuffer 3.1 containing 1 µg of Lambda DNA and a minimum of 1 pmol of EnGen<sup>®</sup> 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.</p>	<b>Pass</b>
<p><b>Protein Purity Assay (SDS-PAGE)</b> EnGen<sup>®</sup> Sau Cas9 is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>

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

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