NEW ENGLAND BioLabs Inc.
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New England Biolabs
Certificate of Analysis

Product Name:	*EnGen® Sau Cas9*
Catalog Number:	*M0654T*
Concentration:	*20 µM*
Packaging Lot Number:	*10182265*
Expiration Date:	*02/2025*
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	10178567	**Pass**
B6003SVIAL	NEBuffer™ r3.1	10168653	**Pass**

Assay Name/Specification	**Lot # 10182265**
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**
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer 3.1 containing 1 µg of a mixture of single and double-stranded [^{3}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**
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**
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 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**

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Assay Name/Specification	Lot # 10182265
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 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**

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
31 Jan 2023

Michael Tonello
Packaging Quality Control Inspector
28 Mar 2023