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240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

## New England Biolabs Certificate of Analysis

Product Name:	EnGen® Seq1 Cas9
Catalog Number:	M0668T
Concentration:	20 μM
Packaging Lot Number:	10195510
Expiration Date:	06/2025
Storage Temperature:	-20°C
Storage Conditions:	10 mM Tris-HCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT; 50%, Glycerol (pH 7.4 @ 25°C)
Specification Version:	PS-M0668T v1.0

EnGen® Seq1 Cas9 Component List				
NEB Part Number	Component Description	Lot Number	Individual QC Result	
M0668TVIAL	EnGen® Seq1 Cas9	10195266	Pass	
B6003SVIAL	NEBuffer™ r3.1	10182163	Pass	

Assay Name/Specification	Lot # 10195510
Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 1 pmol of EnGen® Seq1 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™ r3.1 containing 1 µg of a mixture of single and double-stranded [ ³H] E. coli DNA and a minimum of 1 pmol of EnGen® Seq1 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 <sup>™</sup> r3.1 containing 20 nM of 550 bp FAM and ROX-labeled double-stranded target DNA, 200 nM sgRNA, and 200 nM EnGen® Seq1 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™ r3.1 containing 1 µg of Lambda DNA and a minimum of 1 pmol of EnGen® Seq1 Cas9 incubated for 16 hours at 37ºC results in a DNA pattern	Pass





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Assay Name/Specification	Lot # 10195510
free of detectable nuclease degradation as determined by agarose gel electrophoresis.	
<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® Seq1 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 01 Jun 2023

Josh Hersey

Packaging Quality Control Inspector 09 Jun 2023

