

New England Biolabs Certificate of Analysis

Product Name: EnGen[®] Cas9 NLS, *S. pyogenes*
Catalog Number: M0646T
Concentration: 20 μ M
Unit Definition: N/A
Packaging Lot Number: 10102330
Expiration Date: 08/2023
Storage Temperature: -20°C
Storage Conditions: 10 mM Tris-HCl , 300 mM NaCl , 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol, (pH 7.4 @ 25°C)
Specification Version: PS-M0646T/M v2.0

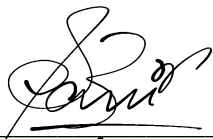
EnGen [®] Cas9 NLS, <i>S. pyogenes</i> Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0646TVIAL	EnGen [®] Cas9 NLS, <i>S. pyogenes</i>	10102329	Pass
B6003SVIAL	NEBuffer [™] r3.1	10110766	Pass

Assay Name/Specification	Lot # 10102330
RNase Activity (Extended Digestion) A 10 μ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 picomole of EnGen [®] Spy Cas9 NLS 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
Functional Testing (Targeted Digestion) A 20 μ l reaction in NEBuffer 3.1 containing 20 nM of 100 bp FAM and ROX-labeled double-stranded target DNA, 100 nM sgRNA, and 100 nM EnGen [®] Spy Cas9 NLS incubated for 1 hour at 37°C results in \geq 90% targeted digestion of the substrate DNA as determined by capillary electrophoresis.	Pass
Non-Specific DNase Activity (16 Hour) A 50 μ l reaction in NEBuffer 3.1 containing 1 μ g of Lambda DNA and a minimum of 1 picomole of EnGen [®] Spy Cas9 NLS incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
Exonuclease Activity (Radioactivity Release) A 50 μ l reaction in NEBuffer 3.1 containing 1 μ g of a mixture of single and	Pass

Assay Name/Specification	Lot # 10102330
double-stranded [³ H] E. coli DNA and a minimum of 1 picomole of EnGen [®] Spy Cas9 NLS incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	
Protein Purity Assay (SDS-PAGE) EnGen [®] Spy Cas9 NLS is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer 3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 1 picomole of EnGen [®] Spy Cas9 NLS incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass

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

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Bhairavi Jani
Production Scientist
17 Aug 2021



Michael Tonello
Packaging Quality Control Inspector
17 Aug 2021