

New England Biolabs Certificate of Analysis

Product Name: EnGen® Cas9 NLS, *S. pyogenes*
Catalog Number: M0646T
Concentration: 20 µM
Unit Definition: N/A
Packaging Lot Number: 10058698
Expiration Date: 11/2021
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>	10058697	Pass
B7203SVIAL	NEBuffer™ 3.1	10053973	Pass

Assay Name/Specification	Lot # 10058698
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
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 picomole of EnGen® Spy Cas9 NLS 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 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 ≥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 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	Pass

Assay Name/Specification	Lot # 10058698
electrophoresis.	
Protein Purity Assay (SDS-PAGE) EnGen® Spy Cas9 NLS 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 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

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



 Jessica Cane
 Production Scientist
 01 Nov 2019



 Jay Minichiello
 Packaging Quality Control Inspector
 03 Feb 2020