

## 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:** 10129869  
**Expiration Date:** 11/2023  
**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

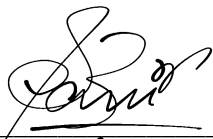
EnGen® Cas9 NLS, <i>S. pyogenes</i> Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0646TVIAL	EnGen® Cas9 NLS, <i>S. pyogenes</i>	10128356	Pass
B6003SVIAL	NEBuffer™ r3.1	10116057	Pass

Assay Name/Specification	Lot # 10129869
<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 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
<b>Protein Purity Assay (SDS-PAGE)</b> EnGen® Spy Cas9 NLS is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<b>Functional Testing (Targeted Digestion)</b> 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
<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 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 # 10129869
electrophoresis.	
<p><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 picomole of EnGen® Spy Cas9 NLS incubated for 4 hours at 37°C results in &lt;10% conversion to the nicked form as determined by agarose gel electrophoresis.</p>	<b>Pass</b>
<p><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 picomole of EnGen® Spy Cas9 NLS incubated for 4 hours at 37°C releases &lt;0.1% of the total radioactivity.</p>	<b>Pass</b>

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

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