

## New England Biolabs Certificate of Analysis

**Product Name:** EnGen® Spy Cas9 Nickase  
**Catalog Number:** M0650S  
**Concentration:** 1 µM  
**Unit Definition:** N/A  
**Packaging Lot Number:** 10239965  
**Expiration Date:** 03/2026  
**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-M0650S v1.0

EnGen® Spy Cas9 Nickase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0650SVIAL	EnGen® Spy Cas9 Nickase	10235969	Pass
B6003SVIAL	NEBuffer™ r3.1	10227734	Pass

Assay Name/Specification	Lot # 10239965
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in NEBuffer 3.1 containing 1 µg of supercoiled PhiX174 RF I DNA and a minimum of 1 pmol of EnGen® Spy Cas9 Nickase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<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 pmol of EnGen® Spy Cas9 Nickase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Functional Testing (EnGen® Spy Cas9 Nickase, Targeted Nicking)</b> A 20 µl reaction in 1X NEBuffer 3.1 containing 20 nM 100 bp FAM and ROX labeled double stranded target DNA, 100 nM sgRNA and 100 nM EnGen® Spy Cas9 Nickase incubated for 1 hour at 37°C results in ≥90% nicking 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 pmol of EnGen® Spy Cas9 Nickase incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel	Pass

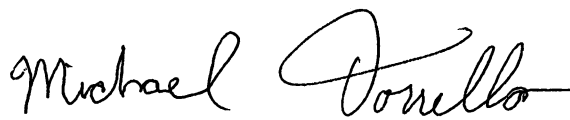
Assay Name/Specification	Lot # 10239965
electrophoresis.	
<b>Protein Purity Assay (SDS-PAGE)</b> EnGen® Spy Cas9 Nickase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>
<b>RNase Activity (Extended Digestion)</b> A 10 µl reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 pmol of EnGen® Spy Cas9 Nickase 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.	<b>Pass</b>

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

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