

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: 10116049
Expiration Date: 07/2023
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-M0650S v1.0

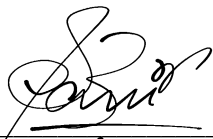
EnGen® Spy Cas9 Nickase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0650SVIAL	EnGen® Spy Cas9 Nickase	10116048	Pass
B6003SVIAL	NEBuffer™ r3.1	10117885	Pass

Assay Name/Specification	Lot # 10116049
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 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.	Pass
Protein Purity Assay (SDS-PAGE) EnGen® Spy Cas9 Nickase 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 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
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 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 electrophoresis.	Pass

Assay Name/Specification	Lot # 10116049
<p>Functional Testing (EnGen® Spy Cas9 Nickase, Targeted Nicking) 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.</p>	Pass
<p>Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer 3.1 containing 1 µg of a mixture of single and double-stranded [³H] 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.</p>	Pass

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

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Production Scientist
22 Sep 2021



Michael Tonello
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22 Sep 2021