

New England Biolabs Certificate of Analysis

Product Name: EnGen® Spy Cas9 Nickase
Catalog Number: M0650T
Concentration: 20 µM
Unit Definition: N/A
Packaging Lot Number: 10182821
Expiration Date: 03/2025
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-M0650T v1.0

EnGen® Spy Cas9 Nickase Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0650TVIAL	EnGen® Spy Cas9 Nickase	10179475	Pass
B6003SVIAL	NEBuffer™ r3.1	10168653	Pass

Assay Name/Specification	Lot # 10182821
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
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 pmol of EnGen® Spy Cas9 Nickase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
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.	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	Pass

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electrophoresis.	
Protein Purity Assay (SDS-PAGE) EnGen® Spy Cas9 Nickase 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 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

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

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Production Scientist
03 Mar 2023



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03 Mar 2023