NEW ENGLAND BioLabs Inc.
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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:	*10150631*
Expiration Date:	*05/2024*
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	10148881	**Pass**
B6003SVIAL	NEBuffer™ r3.1	10146823	**Pass**

Assay Name/Specification	**Lot # 10150631**
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 37ºC. 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**
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**
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 37ºC releases <0.1% of the total radioactivity.	**Pass**

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Assay Name/Specification	Lot # 10150631
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 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**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 37ºC results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	**Pass**

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Bhairavi Jani
Production Scientist
27 Apr 2022

Erin Varney
Packaging Quality Control Inspector
27 Apr 2022