

## New England Biolabs Certificate of Analysis

**Product Name:** *EnGen<sup>®</sup> Spy Cas9 Nickase*  
**Catalog #:** *M0650S*  
**Concentration:** *1 µM*  
**Unit Definition:** *N/A*  
**Lot #:** *0021708*  
**Assay Date:** *08/2017*  
**Expiration Date:** *8/2019*  
**Storage Temp:** *-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*  
**Effective Date:** *09 Aug 2017*

Assay Name/Specification (minimum release criteria)	Lot #0021708
<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 <sup>®</sup> Spy Cas9 Nickase incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	<b>Pass</b>
<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] <i>E. coli</i> DNA and a minimum of 1 pmol of EnGen <sup>®</sup> Spy Cas9 Nickase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Functional Testing (EnGen<sup>®</sup> 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 <sup>®</sup> Spy Cas9 Nickase incubated for 1 hour at 37°C results in ≥90% nicking of the substrate DNA as determined by capillary electrophoresis.	<b>Pass</b>
<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 <sup>®</sup> 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.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - EnGen <sup>®</sup> Spy Cas9 Nickase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>



## New England Biolabs Certificate of Analysis

Assay Name/Specification (minimum release criteria)	Lot #0021708
<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 <sup>®</sup> 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>



Authorized by  
Derek Robinson  
09 Aug 2017



Inspected by  
Bo Wu  
18 Aug 2017

