

New England Biolabs Product Specification

<i>Product Name:</i>	<i>EnGen[®] Spy Cas9 Nickase</i>
<i>Catalog #:</i>	<i>M0650S</i>
<i>Concentration:</i>	<i>1 μM</i>
<i>Unit Definition:</i>	<i>N/A</i>
<i>Shelf Life:</i>	<i>24 months</i>
<i>Storage Temp:</i>	<i>-20°C</i>
<i>Storage Conditions:</i>	<i>10 mM Tris-HCl, 300 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50 % Glycerol, (pH 7.4 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-M0650S v1.0</i>
<i>Effective Date:</i>	<i>09 Aug 2017</i>

Assay Name/Specification (minimum release criteria)

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.

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.

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.

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.

Protein Purity Assay (SDS-PAGE) - EnGen[®] Spy Cas9 Nickase is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

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.



Date 09 Aug 2017

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Director of Quality Control

