

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>EnGen<sup>®</sup> Spy dCas9 (SNAP-tag<sup>®</sup>)</i>
<i>Catalog #:</i>	<i>M0652S</i>
<i>Concentration:</i>	<i>1 <math>\mu</math>M</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-M0652S v1.0</i>
<i>Effective Date:</i>	<i>09 Aug 2017</i>

### Assay Name/Specification (minimum release criteria)

**Endonuclease Activity (Nicking)** - A 50  $\mu$ l reaction in NEBuffer 3.1 containing 1  $\mu$ g of supercoiled PhiX174 DNA and a minimum of 1 pmol of EnGen<sup>®</sup> Spy dCas9 (SNAP-tag<sup>®</sup>) 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  $\mu$ l reaction in NEBuffer 3.1 containing 1  $\mu$ g of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA and a minimum of 1 pmol of EnGen<sup>®</sup> Spy dCas9 (SNAP-tag<sup>®</sup>) incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Functional Testing (EnGen<sup>®</sup> Spy dCas9 (SNAP-tag<sup>®</sup>), Gel Shift Assay)** - A 20  $\mu$ l reaction in 1X NEBuffer 3.1 containing 20 nM 100 bp FAM labeled double stranded target DNA, 20 nM TAMRA-labeled off target DNA, 100 nM sgRNA and 100 nM EnGen<sup>®</sup> Spy dCas9 (SNAP-tag<sup>®</sup>) incubated for 15 minutes at 37°C results in  $\geq$ 90% binding of the substrate DNA as determined by electrophoretic mobility shift assay.

**Non-Specific DNase Activity (16 Hour)** - A 50  $\mu$ l reaction in NEBuffer 3.1 containing 1  $\mu$ g of Lambda DNA and a minimum of 1 pmol of EnGen<sup>®</sup> Spy dCas9 (SNAP-tag<sup>®</sup>) 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<sup>®</sup> Spy dCas9 (SNAP-tag<sup>®</sup>) is  $\geq$  95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

**RNase Activity (Extended Digestion)** - A 10  $\mu$ l 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 dCas9 (SNAP-tag<sup>®</sup>) 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.



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