

## New England Biolabs Product Specification

<i>Product Name:</i>	<i>EnGen® Spy Cas9 HF1</i>
<i>Catalog #:</i>	<i>M0667T/M</i>
<i>Concentration:</i>	<i>20 µM</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, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol (pH 7.4 @ 25°C)</i>
<i>Specification Version:</i>	<i>PS-M0667T/M v1.0</i>
<i>Effective Date:</i>	<i>29 Jul 2022</i>

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

**Endonuclease Activity (Nicking)** - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 1 pmol of EnGen® Spy Cas9 HF1 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™ r3.1 containing 1 µg of a mixture of single and double-stranded [<sup>3</sup>H] *E. coli* DNA and a minimum of 1 pmol of EnGen® Spy Cas9 HF1 incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.

**Functional Testing (Targeted Digestion)** - A 20 µl reaction in NEBuffer™ r3.1 containing 20 nM of 100 bp FAM and ROX-labeled double-stranded target DNA, 100 nM sgRNA, and 100 nM EnGen® Spy Cas9 HF1 incubated for 1 hour at 37°C results in ≥90% targeted digestion of the substrate DNA as determined by capillary electrophoresis.

**Non-Specific DNase Activity (16 Hour)** - A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and a minimum of 1 pmol of EnGen® Spy Cas9 HF1 incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

**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 HF1 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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Date 29 Jul 2022

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