

## New England Biolabs Certificate of Analysis

**Product Name:** *EnGen® Spy Cas9 HF1*  
**Catalog Number:** *M0667T*  
**Concentration:** *20 µM*  
**Packaging Lot Number:** *10249528*  
**Expiration Date:** *06/2026*  
**Storage Temperature:** *-20°C*  
**Storage Conditions:** *10 mM Tris-HCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol (pH 7.4 @ 25°C)*  
**Specification Version:** *PS-M0667T/M v1.0*

EnGen® Spy Cas9 HF1 Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0667TVIAL	EnGen® Spy Cas9 HF1	10245394	Pass
B6003SVIAL	NEBuffer™ r3.1	10237086	Pass

Assay Name/Specification	Lot # 10249528
<b>Endonuclease Activity (Nicking)</b> 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.	<b>Pass</b>
<b>Exonuclease Activity (Radioactivity Release)</b> 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.	<b>Pass</b>
<b>Functional Testing (Targeted Digestion)</b> 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.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 Hour)</b> 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	<b>Pass</b>

Assay Name/Specification	Lot # 10249528
<p>electrophoresis.</p> <p><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® Spy Cas9 HF1 is incubated at 37°C. After incubation for 16 hours, &gt;90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.</p>	<p><b>Pass</b></p>

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

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26 Jun 2024



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