

New England Biolabs Certificate of Analysis

Product Name: *EnGen® Spy Cas9 HF1*
Catalog Number: *M0667T*
Concentration: *20 µM*
Packaging Lot Number: *10230394*
Expiration Date: *05/2026*
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	10230392	Pass
B6003SVIAL	NEBuffer™ r3.1	10227734	Pass

Assay Name/Specification	Lot # 10230394
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.	Pass
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of a mixture of single and double-stranded [³ 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.	Pass
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.	Pass
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	Pass

Assay Name/Specification	Lot # 10230394
<p>electrophoresis.</p> <p>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.</p>	<p>Pass</p>

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

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 Jessica Cane
 Production Scientist
 15 May 2024



 Michael Tonello
 Packaging Quality Control Inspector
 22 May 2024