

New England Biolabs Certificate of Analysis

Product Name: EnGen[®] Spy Cas9 HF1
Catalog Number: M0667M
Concentration: 20 µM
Packaging Lot Number: 10181560
Expiration Date: 02/2025
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
M0667MVIAl	EnGen [®] Spy Cas9 HF1	10167637	Pass
B6003SVIAl	NEBuffer [™] r3.1	10168653	Pass

Assay Name/Specification	Lot # 10181560
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 # 10181560
<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.

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.



Jessica Cane
Production Scientist
16 Feb 2023



Josh Hersey
Packaging Quality Control Inspector
02 Mar 2023