NEW ENGLAND BioLabs Inc.
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New England Biolabs
Certificate of Analysis

Product Name:	*EnGen® Spy Cas9 HF1*
Catalog Number:	*M0667T*
Concentration:	*20 µM*
Packaging Lot Number:	*10181559*
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
M0667TVIAL	EnGen® Spy Cas9 HF1	10167636	**Pass**
B6003SVIAL	NEBuffer™ r3.1	10168653	**Pass**

Assay Name/Specification	Lot # 10181559
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 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel	**Pass**

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Assay Name/Specification	Lot # 10181559
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.	**Pass**

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