

## New England Biolabs Certificate of Analysis

**Product Name:** EnGen® Spy dCas9 (SNAP-tag)  
**Catalog Number:** M0652T  
**Concentration:** 20 µM  
**Unit Definition:** N/A  
**Packaging Lot Number:** 10129920  
**Expiration Date:** 11/2023  
**Storage Temperature:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl , 300 mM NaCl , 1 mM DTT , 0.1 mM EDTA , 50 % Glycerol, (pH 7.4 @ 25°C)  
**Specification Version:** PS-M0652T v1.0

EnGen® Spy dCas9 (SNAP-tag) Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0652TVIAL	EnGen® Spy dCas9 (SNAP-tag®)	10128360	Pass
B6003SVIAL	NEBuffer™ r3.1	10126635	Pass

Assay Name/Specification	Lot # 10129920
<b>Functional Testing (EnGen® Spy dCas9 (SNAP-tag®), Gel Shift Assay)</b> A 20 µl reaction in 1X NEBuffer 3.1 containing 20 nM 100 bp FAM labeled double stranded target DNA, 20 nM TAMRA-labeled off target DNA, 100 nM sgRNA and 100 nM EnGen® Spy dCas9 (SNAP-tag®) incubated for 15 minutes at 37°C results in ≥90% binding of the substrate DNA as determined by electrophoretic mobility shift assay.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in NEBuffer 3.1 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 dCas9 (SNAP-tag®) incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in NEBuffer 3.1 containing 1 µg of Lambda DNA and a minimum of 1 pmol of EnGen® Spy dCas9 (SNAP-tag®) incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	Pass
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in NEBuffer 3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 1 pmol of EnGen® Spy dCas9 (SNAP-tag®) incubated for 4 hours at 37°C	Pass

Assay Name/Specification	Lot # 10129920
<p>results in &lt;10% conversion to the nicked form as determined by agarose gel 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<sup>®</sup> Spy dCas9 (SNAP-tag<sup>®</sup>) 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>Protein Purity Assay (SDS-PAGE)</b> EnGen<sup>®</sup> Spy dCas9 (SNAP-tag<sup>®</sup>) is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<p style="text-align: center;"><b>Pass</b></p> <p style="text-align: center;"><b>Pass</b></p>

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

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