

New England Biolabs Certificate of Analysis

Product Name: EnGen[®] Spy dCas9 (SNAP-tag[®])
Catalog #: M0652T
Concentration: 20 µM
Unit Definition: N/A
Lot #: 0011708
Assay Date: 08/2017
Expiration Date: 8/2019
Storage Temp: -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
Effective Date: 09 Aug 2017

Assay Name/Specification (minimum release criteria)	Lot #0011708
Endonuclease Activity (Nicking) - 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 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 3.1 containing 1 µg of a mixture of single and double-stranded [³ H] <i>E. coli</i> 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
Functional Testing (EnGen[®] Spy dCas9 (SNAP-tag[®]), Gel Shift Assay) - 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
Non-Specific DNase Activity (16 Hour) - 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
Protein Purity Assay (SDS-PAGE) - EnGen [®] Spy dCas9 (SNAP-tag [®]) is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass



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Assay Name/Specification (minimum release criteria)	Lot #0011708
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 dCas9 (SNAP-tag [®]) 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



Authorized by
Derek Robinson
09 Aug 2017



Inspected by
Bo Wu
21 Aug 2017

