240 County Road Ipswich, MA 01938-2723 Tel 978-927-5054 Fax 978-921-1350 www.neb.com info@neb.com

New England Biolabs Certificate of Analysis

Product Name: EnGen® Spy dCas9 (SNAP-tag®)

Catalog #:M0652SConcentration: $1 \mu M$ Unit Definition:N/ALot #:0011708Assay Date:08/2017Expiration Date:8/2019Storage Temp: $-20^{\circ}C$

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-M0652S 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