

New England Biolabs Certificate of Analysis

Product Name: *EnGen[®] Spy Cas9 Nickase*
Catalog #: *M0650T*
Concentration: *20 µM*
Unit Definition: *N/A*
Lot #: *0021708*
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-M0650T v1.0*
Effective Date: *09 Aug 2017*

Assay Name/Specification (minimum release criteria)	Lot #0021708
Endonuclease Activity (Nicking) - A 50 µl reaction in NEBuffer 3.1 containing 1 µg of supercoiled PhiX174 RF I DNA and a minimum of 1 pmol of EnGen [®] Spy Cas9 Nickase 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 Cas9 Nickase incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
Functional Testing (EnGen[®] Spy Cas9 Nickase, Targeted Nicking) - A 20 µl reaction in 1X NEBuffer 3.1 containing 20 nM 100 bp FAM and ROX labeled double stranded target DNA, 100 nM sgRNA and 100 nM EnGen [®] Spy Cas9 Nickase incubated for 1 hour at 37°C results in ≥90% nicking of the substrate DNA as determined by capillary electrophoresis.	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 Cas9 Nickase 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 Cas9 Nickase 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 #0021708
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 Nickase 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
18 Aug 2017

