

New England Biolabs Certificate of Analysis

Product Name: EnGen® Cas9 NLS, *S. pyogenes*
Catalog Number: M0646T
Concentration: 20 µM
Unit Definition: N/A
Packaging Lot Number: 10192317
Expiration Date: 05/2025
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-M0646T/M v2.0

| EnGen® Cas9 NLS, <i>S. pyogenes</i> Component List | | | |
|--|-------------------------------------|------------|----------------------|
| NEB Part Number | Component Description | Lot Number | Individual QC Result |
| M0646TVIAL | EnGen® Cas9 NLS, <i>S. pyogenes</i> | 10190401 | Pass |
| B6003SVIAL | NEBuffer™ r3.1 | 10182163 | Pass |

| Assay Name/Specification | Lot # 10192317 |
|---|----------------|
| Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer 3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 1 picomole of EnGen® Spy Cas9 NLS 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] E. coli DNA and a minimum of 1 picomole of EnGen® Spy Cas9 NLS 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 3.1 containing 20 nM of 100 bp FAM and ROX-labeled double-stranded target DNA, 100 nM sgRNA, and 100 nM EnGen® Spy Cas9 NLS 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 3.1 containing 1 µg of Lambda DNA and a minimum of 1 picomole of EnGen® Spy Cas9 NLS incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel | Pass |

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| electrophoresis. | |
| Protein Purity Assay (SDS-PAGE) EnGen® Spy Cas9 NLS is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |
| 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 picomole of EnGen® Spy Cas9 NLS 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.

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Jessica Cane
Production Scientist
10 May 2023



Michael Tonello
Packaging Quality Control Inspector
31 May 2023