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## 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: 10160072
Expiration Date: 08/2024
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, S. pyogenes Component List |                              |            |                      |  |
|---|------------------------------|------------|----------------------|--|
| <b>NEB Part Number</b>                      | Component Description        | Lot Number | Individual QC Result |  |
| M0646TVIAL                                  | EnGen® Cas9 NLS, S. pyogenes | 10156777   | Pass                 |  |
| B6003SVIAL                                  | NEBuffer™ r3.1               | 10146824   | Pass                 |  |

| Assay Name/Specification  | Lot # 10160072 |
|---|----------------|
| 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 [ ³H] 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           |
| 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 electrophoresis. | 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              | Pass           |



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| Assay Name/Specification   | Lot # 10160072 |
|--|----------------|
| determined by capillary electrophoresis.   |                |
| 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           |
| Protein Purity Assay (SDS-PAGE) EnGen® Spy Cas9 NLS is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.   | Pass           |

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

One or more products referenced in this document may be covered by a 3rd-party trademark. Please visit www.neb.com/trademarks for additional information.

Bhairavi Jani **Production Scientist** 

02 Aug 2022

Erin Varney

Packaging Quality Control Inspector

02 Aug 2022



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