

New England Biolabs Certificate of Analysis

Product Name: Cas9 Nuclease, *S. pyogenes*
Catalog Number: M0386T
Concentration: 20 μ M
Packaging Lot Number: 10146848
Expiration Date: 04/2024
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-M0386T/M v1.0

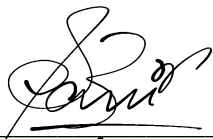
| Cas9 Nuclease, <i>S. pyogenes</i> Component List | | | |
|--|-----------------------------------|------------|----------------------|
| NEB Part Number | Component Description | Lot Number | Individual QC Result |
| M0386TVIAL | Cas9 Nuclease, <i>S. pyogenes</i> | 10146847 | Pass |
| B6003SVIAL | NEBuffer™ r3.1 | 10146823 | Pass |

| Assay Name/Specification | Lot # 10146848 |
|---|----------------|
| Endonuclease Activity (Nicking) A 50 μ l reaction in NEBuffer 3.1 containing 1 μ g of supercoiled PhiX174 DNA and a minimum of 1 pmol of Cas9 Nuclease, <i>S. pyogenes</i> incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis. | Pass |
| Protein Purity Assay (SDS-PAGE) Cas9 Nuclease, <i>S. pyogenes</i> is \geq 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection. | Pass |
| RNase Activity (Extended Digestion) A 10 μ l reaction in NEBuffer 4 containing 40 ng of a 300 base single-stranded RNA and a minimum of 1 pmol of Cas9 Nuclease, <i>S. pyogenes</i> 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 |
| Non-Specific DNase Activity (16 Hour) A 50 μ l reaction in NEBuffer 3.1 containing 1 μ g of Lambda DNA and a minimum of 1 pmol of Cas9 Nuclease, <i>S. pyogenes</i> incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis. | Pass |

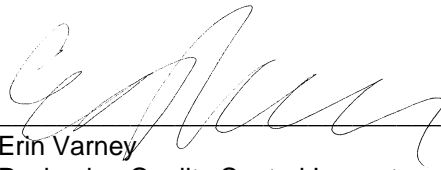
| Assay Name/Specification | Lot # 10146848 |
|---|----------------|
| <p>Functional Testing (Targeted Digestion) A 20 µl reaction in NEBuffer 3.1 containing 20 nM of 100 bp FAM and ROX-labeled double-stranded target DNA, 100 nM sgRNA, and 100 nM Cas9 Nuclease, <i>S. pyogenes</i> incubated for 1 hour at 37°C results in ≥90% targeted digestion of the substrate DNA as determined by capillary electrophoresis.</p> | Pass |
| <p>Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer 3.1 containing 1 µg of a mixture of single and double-stranded [³H] <i>E. coli</i> DNA and a minimum of 1 pmol of Cas9 Nuclease, <i>S. pyogenes</i> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.</p> | Pass |

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

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Bhairavi Jani
Production Scientist
20 Apr 2022



Erin Varney
Packaging Quality Control Inspector
20 Apr 2022