

## New England Biolabs Certificate of Analysis

**Product Name:** Cas9 Nuclease, *S. pyogenes*  
**Catalog Number:** M0386T  
**Concentration:** 20  $\mu$ M  
**Packaging Lot Number:** 10171881  
**Expiration Date:** 11/2024  
**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>	10169028	Pass
B6003SVIAL	NEBuffer™ r3.1	10168652	Pass

Assay Name/Specification	Lot # 10171881
<b>Protein Purity Assay (SDS-PAGE)</b> Cas9 Nuclease, <i>S. pyogenes</i> is $\geq$ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 $\mu$ l reaction in NEBuffer 3.1 containing 1 $\mu$ g of a mixture of single and double-stranded [ <sup>3</sup> H] <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.	Pass
<b>Endonuclease Activity (Nicking)</b> A 50 $\mu$ l reaction in NEBuffer 3.1 containing 1 $\mu$ 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
<b>RNase Activity (Extended Digestion)</b> A 10 $\mu$ 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

Assay Name/Specification	Lot # 10171881
<p><b>Functional Testing (Targeted Digestion)</b> 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>	<b>Pass</b>
<p><b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in NEBuffer 3.1 containing 1 µg 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.</p>	<b>Pass</b>

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

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14 Nov 2022



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28 Nov 2022