

## New England Biolabs Certificate of Analysis

**Product Name:** EnGen® SpRY Cas9  
**Catalog Number:** M0669M  
**Concentration:** 20 µM  
**Packaging Lot Number:** 10228642  
**Expiration Date:** 01/2026  
**Storage Temperature:** -20°C  
**Storage Conditions:** 10 mM Tris-HCl, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol (pH 7.4 @ 25°C)  
**Specification Version:** PS-M0669T/M v1.0

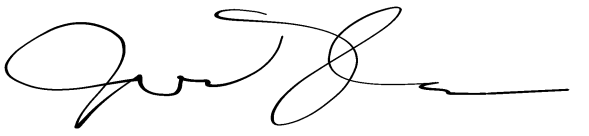
EnGen® SpRY Cas9 Component List			
NEB Part Number	Component Description	Lot Number	Individual QC Result
M0669MVIAl	EnGen® SpRY Cas9	10227910	Pass
B6003SVIAl	NEBuffer™ r3.1	10221488	Pass

Assay Name/Specification	Lot # 10228642
<b>Endonuclease Activity (Nicking)</b> A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 1 picomole of EnGen® SpRY Cas9 incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	Pass
<b>Exonuclease Activity (Radioactivity Release)</b> A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of a mixture of single and double-stranded [ <sup>3</sup> H] E. coli DNA and a minimum of 1 picomole of EnGen® SpRY Cas9 incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	Pass
<b>Functional Testing (Targeted Digestion)</b> A 20 µl reaction in NEBuffer™ r3.1 containing 20 nM of 100 bp FAM and ROX-labeled double-stranded target DNA, 100 nM sgRNA, and 100 nM EnGen® SpRY Cas9 incubated for 30 minutes at 37°C results in ≥90% targeted digestion of the substrate DNA as determined by capillary electrophoresis.	Pass
<b>Non-Specific DNase Activity (16 Hour)</b> A 50 µl reaction in NEBuffer™ r3.1 containing 1 µg of Lambda DNA and a minimum of 1 picomole of EnGen® SpRY Cas9 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 # 10228642
<p><b>Protein Purity Assay (SDS-PAGE)</b> EnGen® SpRY Cas9 is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.</p>	<b>Pass</b>
<p><b>RNase Activity Assay</b> 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® SpRY Cas9 is incubated at 37°C. After incubation for 4 hours, the substrate RNA is assessed by gel electrophoresis using fluorescent detection and compared to the product's RNase QC Standard resulting in no additional non-specific nuclease degradation.</p>	<b>Pass</b>

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

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19 Jan 2024



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Packaging Quality Control Inspector  
29 Jan 2024