

## New England Biolabs Certificate of Analysis

**Product Name:** *EnGen™ Cas9 NLS, S. pyogenes*  
**Catalog #:** M0646T/M  
**Concentration:** 20 μM  
**Unit Definition:** N/A  
**Lot #:** 0031704  
**Assay Date:** 04/2017  
**Expiration Date:** 04/2019  
**Storage Temp:** -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 v1.0  
**Effective Date:** 30 Jun 2016

Assay Name/Specification (minimum release criteria)	Lot #0031704
<b>Endonuclease Activity (Nicking)</b> - A 50 μl reaction in Cas9 Nuclease Reaction Buffer containing 1 μg of supercoiled PhiX174 DNA and a minimum of 1 picomole of EnGen™ Cas9 NLS, <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.	<b>Pass</b>
<b>Exonuclease Activity (Radioactivity Release)</b> - A 50 μl reaction in Cas9 Nuclease Reaction Buffer containing 1 μg of a mixture of single and double-stranded [ <sup>3</sup> H] <i>E. coli</i> DNA and a minimum of 1 picomole of EnGen™ Cas9 NLS, <i>S. pyogenes</i> incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	<b>Pass</b>
<b>Functional Testing (EnGen™ Cas9 NLS, <i>S. pyogenes</i>, Targeted Digestion)</b> - A 30 μl reaction in 1X Cas9 Nuclease Reaction Buffer containing 1 nM PvuII linearized pBR322 DNA, 40 nM SgRNA and 20 nM EnGen™ Cas9 NLS, <i>S. pyogenes</i> incubated for 1 hour at 37°C results in ≥90% digestion of the substrate DNA as determined by agarose gel electrophoresis.	<b>Pass</b>
<b>Non-Specific DNase Activity (16 Hour)</b> - A 50 μl reaction in Cas9 Nuclease Reaction Buffer containing 1 μg of Lambda DNA and a minimum of 1 picomole of EnGen™ Cas9 NLS, <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.	<b>Pass</b>
<b>Protein Purity Assay (SDS-PAGE)</b> - EnGen™ Cas9 NLS, <i>S. pyogenes</i> is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	<b>Pass</b>



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<b>Assay Name/Specification</b> (minimum release criteria)	<b>Lot #0031704</b>
<b>RNase Activity (Extended Digestion)</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™ Cas9 NLS, <i>S. pyogenes</i> is incubated at 37°C. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	<b>Pass</b>



Authorized by  
Derek Robinson  
30 Jun 2016



Inspected by  
Bo Wu  
10 Apr 2017

