NEW ENGLAND BioLabs Inc.
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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*
Lot Number:	*10009682*
Expiration Date:	*03/2020*
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 v1.0*

EnGen® Cas9 NLS, S. pyogenes Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M0646TVIAL	EnGen® Cas9 NLS, S. pyogenes	0061803	**Pass**
B0386AVIAL	10X Cas9 Nuclease Reaction Buffer	0081801	**Pass**

Assay Name/Specification	**Lot # 10009682**
Protein Purity Assay (SDS-PAGE) EnGen™ Cas9 NLS, S. pyogenes is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
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™ Cas9 NLS, S. pyogenes is incubated at 37ºC. After incubation for 4 hours, >90% of the substrate RNA remains intact as determined by gel electrophoresis using fluorescent detection.	**Pass**
Endonuclease Activity (Nicking) 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, S. pyogenes incubated for 4 hours at 37ºC results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	**Pass**
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in Cas9 Nuclease Reaction Buffer containing 1 µg of a mixture of single and double-stranded [^{3}H] E. coli DNA and a minimum of 1 picomole of EnGen™ Cas9 NLS, S. pyogenes incubated for 4 hours at 37ºC releases <0.1% of the total	**Pass**

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Assay Name/Specification	Lot # 10009682
radioactivity.	
Non-Specific DNase Activity (16 Hour) 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, S. pyogenes incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
Functional Testing (EnGen™ Cas9 NLS, S. pyogenes, Targeted Dige A 30 µl reaction in 1X Cas9 Nuclease Reaction Buffer containing 1 nM PvuII linarized pBR322 DNA, 40 nM SgRNA and 20 nM EnGen™ Cas9 NLS, S. pyogenes incubated for 1 hour at 37°C results in ≥90% digestion of the substrate DNA as determined by agarose gel electrophoresis.	**Pass**

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

Bhairavi Jani
Production Scientist
14 Jun 2018

Michael Tonello
Packaging Quality Control Inspector
14 Jun 2018