NEW ENGLAND BioLabs Inc.
be INSPIRED drive DISCOVERY stay GENUINE
240 County Road
Ipswich, MA 01938-2723
Tel 978-927-5054
Fax 978-921-1350
www.neb.com
info@neb.com

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:	*10030461*
Expiration Date:	*11/2020*
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 v2.0*

EnGen® Cas9 NLS, S. pyogenes Component List			
NEB Part Number	**Component Description**	**Lot Number**	**Individual QC Result**
M0646TVIAL	EnGen® Cas9 NLS, S. pyogenes	10021801	**Pass**
B7203SVIAL	NEBuffer™ 3.1	10021112	**Pass**

Assay Name/Specification	**Lot # 10030461**
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® Spy Cas9 NLS 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**
Protein Purity Assay (SDS-PAGE) EnGen® Spy Cas9 NLS is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	**Pass**
Non-Specific DNase Activity (16 Hour) A 50 µl reaction in NEBuffer 3.1 containing 1 µg of Lambda DNA and a minimum of 1 picomole of EnGen® Spy Cas9 NLS incubated for 16 hours at 37ºC results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.	**Pass**
Endonuclease Activity (Nicking) A 50 µl reaction in NEBuffer 3.1 containing 1 µg of supercoiled PhiX174 DNA and a minimum of 1 picomole of EnGen® Spy Cas9 NLS incubated for 4 hours at 37°C results in <10% conversion to the nicked form as determined by agarose gel electrophoresis.	**Pass**

NEW ENGLAND BioLabs Inc.
be INSPIRED drive DISCOVERY stay GENUINE
240 County Road
Ipswich, MA 01938-2723
Tel 978-927-5054
Fax 978-921-1350
www.neb.com
info@neb.com

Assay Name/Specification	Lot # 10030461
Exonuclease Activity (Radioactivity Release) A 50 µl reaction in NEBuffer 3.1 containing 1 µg of a mixture of single and double-stranded [^{3}H] E. coli DNA and a minimum of 1 picomole of EnGen® Spy Cas9 NLS incubated for 4 hours at 37°C releases <0.1% of the total radioactivity.	**Pass**
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 EnGen® Spy Cas9 NLS incubated for 1 hour at 37°C results in ≥90% targeted digestion of the substrate DNA as determined by capillary electrophoresis.	**Pass**

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

Tony Spear-Alfonso
Production Scientist
14 Nov 2018

Jason Davis
Packaging Quality Control Inspector
30 Nov 2018