

New England Biolabs Certificate of Analysis

Product Name: *EnGen™ Cas9 NLS, S. pyogenes*
Catalog #: M0646T/M
Concentration: 20 μM
Unit Definition: N/A
Lot #: 0031610
Assay Date: 10/2016
Expiration Date: 10/2018
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 #0031610
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, <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
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] <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.	Pass
Functional Testing (EnGen™ Cas9 NLS, <i>S. pyogenes</i>, Targeted Digestion) - 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.	Pass
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, <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.	Pass
Protein Purity Assay (SDS-PAGE) - EnGen™ Cas9 NLS, <i>S. pyogenes</i> is ≥ 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.	Pass



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Assay Name/Specification (minimum release criteria)	Lot #0031610
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, <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.	Pass



Authorized by
Derek Robinson
30 Jun 2016



Inspected by
Bo Wu
31 Oct 2016

