EnGen™ Cas9 NLS, *S. pyogenes*

concentrated

NEW ENGLAND BioLabs Inc.
1-800-632-7799
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www.neb.com

M0646T 003160618061

M0646T

RR 37°

400 pmol	**20 µM**	**Lot: 0031606**
RECOMBINANT	**Store at –20°C**	**Exp: 6/18**

Description: EnGen Cas9 NLS, *S. pyogenes*, is an RNA-guided endonuclease that catalyzes site- specific cleavage of double stranded DNA. The location of the break is within the target sequence 3 bases from the NGG PAM (Protospacer Adjacent Motif) (1). The target region must be followed by the NGG PAM sequence. The sgRNA is complementary to the strand of DNA opposite to the target region upstream of the PAM sequence. EnGen Cas9 NLS, *S. pyogenes* contains Simian virus 40 (SV40) T antigen nuclear localization sequence (NLS) on the N- and C- termini of the protein.

Source: An *E. coli* strain that carries the cloned Cas9 gene from *Streptococcus pyogenes* with N- and C-terminal Simian virus 40 (SV40) nuclear localization signals (NLS) and a N- terminal 6XHis tag.

Supplied in: 300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT and 50% glycerol. (pH 7.4 @ 25°C).

Reagents Supplied with Enzyme:
10X Cas9 Nuclease Reaction Buffer

Reaction Conditions: 1X Cas9 Nuclease Reaction Buffer. Incubate at 37°C.

1X Cas9 Nuclease Reaction Buffer:
20 mM HEPES
100 mM NaCl
5 mM $MgCl_2$
0.1 mM EDTA
pH 6.5 @ 25°C

Diluent Compatibility: Diluent Buffer B
300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 µg/ml BSA and 50% glycerol. (pH 7.4 @ 25°C).

Quality Control Assays

Protein Purity (SDS-PAGE): EnGen Cas9 NLS, *S. pyogenes* is > 95% pure as determined by SDS-PAGE analysis using Coomassie Blue detection.

RNase Activity (Extended Digestion): A 10 µl reaction in Cas9 Nuclease Reaction Buffer containing 40 ng of labeled RNA and 1 picomole of EnGen Cas9 NLS, *S. pyogenes* is incubated at 37°C. After incubation for 4 hours, > 90% of the substrate RNA remains intact as determined by agarose gel electrophoresis.

Endonuclease Activity (Nicking): A 50 µl reaction in Cas9 Nuclease Reaction Buffer containing 1 picomole of EnGen Cas9 NLS, *S. pyogenes* with 1 µg ϕX174 RF I DNA for 4 hours at 37°C resulted in < 10% conversion to RF II as determined by agarose gel electrophoresis.

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 1 picomole of EnGen Cas9 NLS, *S. pyogenes* incubated for 4 hours at 37°C releases < 0.1% of the total radioactivity.

Non-Specific DNase Activity (16 hour): A 50 µl reaction in Cas9 Nuclease Reaction Buffer containing 1 µg of λ DNA and 1 picomole of EnGen Cas9 NLS, *S. pyogenes* incubated for 16 hours at 37°C results in a DNA pattern free of detectable nuclease degradation as determined by agarose gel electrophoresis.

Functional Test (EnGen Cas9 NLS, *S. pyogenes* Targeted Digestion): A 30 µl reaction in 1X Cas9 Nuclease Reaction Buffer containing 1 nM PvuII linearized pBR322 DNA (one targeted site CGCTTGTTTCGGCGTGGGTA), 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.

Note: 20 µM is equal to 3.22 mg/ml.

Reference:

1. Jinek M. et al. (2012) *Science* 816–821. doi: 10.1126/*Science*.1225829. Epub 2012 Jun 28. PubMed PMID: 22745249.

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CERTIFICATE OF ANALYSIS

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