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OPTIONS AMPLIFIED

CRISPR/Cas9 NLS

In vivo/*in vitro* gene editing

Contents:

CRISPR/Cas9 is provided at a concentration of 10 pmol/µL (10 µM).

Background

CRISPR/Cas9 NLS is a Streptococcus pyogenes Type II Cas9 endonuclease that can be programmed by a small RNA to digest DNA site specifically. The specificity of cleavage is determined by the sequence of a single guide RNA (sgRNA) that contains bases that are complimentary to the target site. Site-specific *in vivo* cleavage of DNA is facilitated by dual nuclear localization signals (NLS). The simplicity of the CRISPR/Cas9 NLS endonuclease system enables precise *in vitro* and *in vivo* DNA digestion.

Application Notes

CRISR/Cas9 NLS can be programmed to cleave DNA precisely by the sequence of the sgRNA that is loaded onto the protein to cleave DNA virtually anywhere. This enables site-specific *in vitro* engineering of genomic, plasmid, or PCR amplified DNA. Furthermore, the nuclear localization signals (NLS) can be used to direct *in vivo* cleavage of genomic DNA.

**These products are intended for research use only, not for diagnostic use. The safety and efficacy of these products in diagnostic or other clinical uses has not been established.*

Protein Details

CRISPR/Cas9 NLS is expressed in *E. coli.* The molecular weight of this protein is 173 kDaltons.

Shipping & Storage

CRISPR/Cas9 NLS is stored at -20 °C in 50% glycerol, 50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 1 mM EDTA, pH 7.5. *Can be supplied in a glycerol-free buffer as a custom order.*

CRISPR/Cas9 NLS is shipped on dry or blue ice. On arrival store at -20 °C for optimum stability. Repeated freeze/thaw cycles should be avoided

Quality Control

- CRISPR/Cas9 NLS activity: >90% cleavage of 1 nM, 4 kb PCR product in 60 minutes at 37 °C using 40 nM targeted gRNA and 1 pmol CRISPR/Cas9 NLS in 50 µL (20 nM).
- Purity: >95% as determined by SDS-PAGE analysis
- <0.2 ng contaminating host DNA per pmol

In vitro digestion of DNA with CRISPR/Cas9 NLS

Product Overview

CRISPR/Cas9 NLS is a recombinant form of *Streptococcus pyogenes* Type II Cas9 nuclease that site specifically digests DNA using the complementarity sequence of a small RNA bound to the protein to guide the nuclease to its target.

A protocol is provided for *in vitro* digestion of double stranded DNA with CRISPR/Cas9 NLS and a single guide RNA.

Useful Information

The DNA, guide RNA and nuclease-free water for this protocol are not supplied with the CRISPR/Cas9 NLS.

Information for the design of a Cas9 nuclease guide RNA containing a target sequence can be viewed at the Addgene website.

10X Reaction Buffer is used in the protocol below. 10X Reaction Buffer is 500 mM NaCl, 100 mM Tris-HCl, 100 mM MgCl2, 10 mM DTT, pH 7.9. This buffer can be substituted with 10X TA Buffer. 10X TA Buffer is 330 mM Tris acetate, 660 mM KCl, 100 mM magnesium acetate, pH 7.5.

General Protocol

1) Assemble the reaction components at room temperature in a microfuge tube.
2) Mix the reaction components and incubate at 37 °C for 1 hour.
3) Stop the reaction by heating at 70 °C for 15 min.

Component	Volume
Nuclease-free water	20 µL
10X Reaction Buffer	3 µL
300 nM guide RNA	3 µL (30 nM final)
1 µM CRISPR/Cas9 NLS	1 µL (~30 nM final)
Pre-incubate the above mixture at RT for 10 min before adding DNA	
30 nM DNA	3 µL (30 nM final)
Total reaction volume	30 µL