GenCrispr NLS-Cas9-D10A Nickase
Cat. No. Z03390 **Version 03072017**

I DESCRIPTION

NLS-Cas9-D10A Nickase is a mutant form of Cas9 nuclease. Cas9 nuclease is an RNA-guided endonuclease that can catalyze cleavage of double strand DNA. This kind of targeted nuclease is a powerful tool for genome editing with high precision. Cas9 protein forms a very stable ribonucleoprotein (RNP) complex with the guide RNA (gRNA) component of the CRISPR/Cas9 system. Cas9 nuclease cleaves the double stranded DNA generating two break sites based on its two active domains. NLS-Cas9-D10A Nickase is a mutant form of Cas9 nuclease which deactivates one active domain, thus it can only cut a single strand of DNA that is complementary to the guide-RNA. Combined with two different gRNA, NLS-Cas9-D10A Nickase produces two cut sites respectively and causes a double strand break. Compared with the wild type Cas9 nuclease, the two-gRNA guided cleavage significantly reduces off target effects. GenCrispr NLS-Cas9-D10A Nickase is highly purified mutant protein expressed in an *E. coli* strain carrying a plasmid encoding the mutated Cas9 gene from *Streptococcus pyogenes*, which meets all the researchers' requirements with lower off-target effects (e.g. *in vitro* cleavage assay, RNP complex transfection, and micro injection).

II KIT CONTENTS

Kit Contents	Quantity	Catalog No.	Components/Concentration
GenCrispr NLS-Cas9-D10A Nickase	10 ug	Z03390-10	1 mg/ml
	50 ug	Z03390-50	1 mg/ml
	100 ug	Z03390-100	4 mg/ml
10X Reaction Buffer	1.5 ml		200 mM HEPES, 1 M NaCl, 50 mM $MgCl_2$, 1 mM EDTA, pH 6.5 at 25°C

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III KEY FEATURES

- **DNA-free: no external DNA added to system**
- **High cleavage efficiency: NLS ensures the efficient entry of Cas9 protein into nuclei**
- **Lower off target: Double gRNA-guided cleavage and transient expression of Cas9 nuclease**
- **Time-saving: no need for transcription and translation**

IV Quality Control Analysis

- **High Protein purity:** GenCrispr Cas9 is > 95% pure as determined by SDS-PAGE using Coomassie Blue detection.
- **Low Endotoxin:** Endotoxin level is <0.1EU/ug test by gel-clot method: limit test.
- **Non-specific DNase activity:** A 20 ul reaction in Cas9 reaction buffer containing 100 ng linearized pUC57 plasmid and 0.1 ug GenCrispr Cas9 incubated for 16 h at 37°C. No DNA degradation is determined by agarose gel electrophoresis.
- **Non-specific RNase activity:** A 10 ul reaction in Cas9 reaction buffer containing 1600 ng total RNA and 0.1 ug of GenCrispr Cas9 incubated for 2 h at 37°C. No RNA degradation is determined by agarose gel electrophoresis.
- **High Bioactivity:** 20 nM GenCrispr Cas9 incubated for 2 h at 37°C result in over 90% digestion of the substrate DNA as determined by agarose gel electrophoresis.

V Utilities of Product

1. Screening for highly efficient and specific targeting gRNAs by in vitro DNA cleavage.
2. In vivo gene editing when combined with TWO specific gRNA by electroporation or injection.

VI STORAGE

GenCrispr NLS-Cas9-D10A Nickase is supplied with 1X storage buffer (10 mM Tris, 300 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol pH7.4 at 25°C). The recommended storage temperature is -20°C.

VII 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 at 25°C).

VIII Activity test

Cas9 site-specific digestion:

To test the activity of NLS-Cas9-D10A Nickase, GenScript used an *in vitro* digestion approach to determine the single strand cleavage activity. The purified plasmid pUC57 was used as a substrate with a target site:

(CATCATTGGAAAACGTTCTT)

It can be digested by gRNA:

(CAUCAUUGGAAAACGUUCUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAAGGCUAGUCC GUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUUU)

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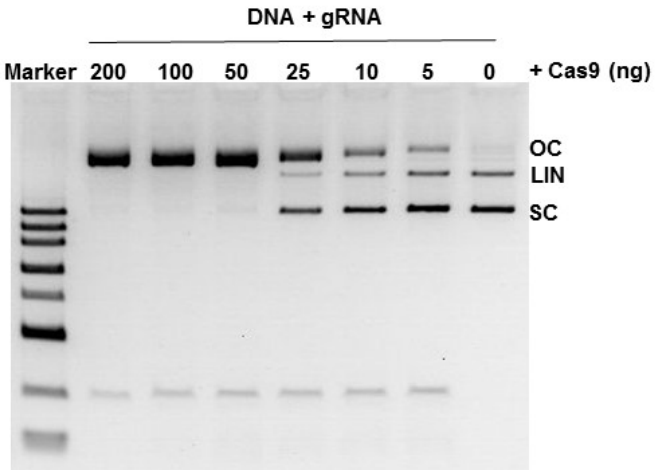
and GenCrispr NLS-Cas9-D10A Nickase. Once cleaved at single strand DNA, the plasmid substrate behaves as three different pattern (open circular, linearized, supercoiled), which can be distinguished by agarose gel electrophoresis because of different running speeds. A 20 µl reaction in 1xCas9 Nuclease Reaction Buffer containing 160 ng plasmid, 40 nM gRNA and 20 nM GenCrispr NLS-Cas9-D10A Nickase for 2 h at 37°C results in over 90% digestion as determined by agarose gel electrophoresis.



In vitro DNA cleavage assay with GenCrispr NLS-Cas9-D10A Nickase

Reactions were set up according to recommended conditions, and cleavage products were resolved on a 1% agarose gel. Input DNA is pUC57 plasmid DNA (OC, open circular; LIN, linearized; SC, supercoiled)

IX References

1. Jinek et al. A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity. (2012) Science 337 (6096) 816-821 (2012).
2. Larson, M. H., et al. CRISPR interference (CRISPRi) for sequence-specific control of gene expression.Nature Protocols. 8, (11), 2180-2196 (2013).
3. Ran, F. A., et al. Genome engineering using the CRISPR-Cas9 system. Nature Protocols. 8, (11), 2281-2308 (2013).
4. Kim, S., Kim, D., Cho, S.W., Kim, J., Kim, J.S. Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoprotein. Genome Res. 24(6), 1012-1019 (2014).
5. Mali P, Yang L, Esvelt KM, Aach J, Guell M, Dicarlo JE, Norville JE, Church GM. RNA-Guided Human Genome Engineering via Cas9. Science. 2013 Jan 3.

Note:

1. This is a basic protocol. The reagent concentrations, conditions, and parameters may need to be optimized.
2. 1000 nM is equal to 160 ng/ul.

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