Version 1.0
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GenCrispr NLS-Cas9-NLS Nuclease
Cat. No. Z03469

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I Description

Cas9 nuclease is an RNA-guided endonuclease that can catalyze cleavage of double stranded 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. The Cas9 RNP complex can localize to the nucleus immediately upon entering the cell with the addition of a nuclear localization signal (NLS). There is no requirement for transcription and translation compared with mRNA or plasmid systems. Additionally, the Cas9 RNP complex is rapidly cleared from the cell minimizing the chance of off-target cleavage when compared to other systems (Kim, et al. 2014). This DNA-free system avoids the risk of inserting foreign DNA into the genome, which can be quite useful for gene editing-based disease therapy. GenScript has developed a NLS-Cas9-NLS nuclease which contains a nuclear localization sequence (NLS) on both ends of the protein to meet all the researchers' requirements (e.g. *in vitro* cleavage assay, RNP complex transfection, and micro injection).

Product Source: GenCrispr NLS-Cas9-NLS is produced by expression in an *E. coli* strain carrying a plasmid encoding the Cas9 gene from *Streptococcus pyogenes* with a nuclear localization signal (NLS) on both ends.

II Kit Contents

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Kit Contents	Quantity	Catalog No.	Components/Concentration
GenCrispr NLS-Cas9-NLS Nuclease	100 μg	Z03469-100	10 mg/mL
	500 μg	Z03469-500	
	1 mg	Z03469-1	
10X Reaction Buffer	1.5 mL		Reagent composition confidentiality,1000μg/mL BSA pH 7.9 at 25°C

III Key Features

- **DNA-free: no external DNA added to system**
- **High cleavage efficiency: Double NLS ensures the efficient entry of Cas9 protein into nuclei**
- **Low off target: 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 and analysis by Image Lab.
- **Low Endotoxin:** Endotoxin level is <0.1EU/μg test by Tachypleus Amebocyte Lysate (TAL) gel-clot method: limit test.
- **Non-specific DNase activity:** A 20 μL reaction in Cas9 reaction buffer containing 100 ng linearized pUC57 plasmid and 0.1 μg GenCrispr Cas9 incubated for 16h at 37°C. No DNA degradation is determined by agarose gel electrophoresis.
- **Non-specific RNase activity:** A 10 μL reaction in Cas9 reaction buffer containing 1800 ng total RNA and 0.1 μg of GenCrispr Cas9 incubated for 2 h at 37°C. No RNA degradation as determined by Agarose gel electrophoresis.
- **High Bioactivity:** 50 ng GenCrispr Cas9 incubated for 2 hours at 37°C result in 90% digestion of the substrate DNA as determined by agarose gel electrophoresis and compared with competing products.

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 a specific gRNA by electroporation or injection.

VI Storage

GenCrispr NLS-Cas9-NLS Nuclease 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) and recommended to be stored at -20°C. Guaranteed stable for 18 months when properly stored.

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VII Diluent Compatibility

In *in vitro* assays, it is recommended to dilute Cas9 nuclease with Diluent Buffer B (validated in our *in vitro* tests).

Diluent Buffer B: 300 mM NaCl, 10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol. (pH 7.4, at 25°C).

VIII Activity test

Cas9 site-specific digestion:

GenScript used *in vitro* digestion of a linearized plasmid to determine the activity of the Cas9 nuclease. It is a sensitive assay for GenCrispr Cas9 quality control. The linearized plasmid containing the target site:

(CATCATTGGAAAACGTTCTT)

can be digested with gRNA:

(CAUCAUUGGAAAACGUUCUUGUUUUAGAGCUAGAAAUAGCAAGUUAAAAUAA GGCUAGUCCGUUAUCAACUUGAAAAAGUGGCACCGAGUCGGUGCUUUUUUU)

and GenCrispr Cas9. Two cleavage DNA fragments (526 bp and 1053 bp) are determined by agarose gel electrophoresis. A 20 μL reaction in 1xCas9 Nuclease Reaction Buffer containing 160 ng linearized pUC57, 100 ng gRNA and 50 ng GenCrispr Cas9 for 2 h at 37°C results in over 90% digestion of linearized pUC57,as determined by agarose gel electrophoresis and compared with competing products .

Reagent	Volume
10×Cas9 Nuclease Reaction Buffer	2 μL
gRNA	100 ng
GenCrispr Cas9 Nuclease	50 ng
DEPC water	Up to 19 μL

Procedure:

1. The above components should be gently mixed, centrifuged, placed in a PCR machine and incubated at 37℃ for 10 minutes.
2. 1 μL (160 ng) of linearized pUC57 was added, mixed and centrifuged, incubated at 37℃ for 2h.
3. 5 μL of cleavage products are resolved on a 1.5% agarose gel.

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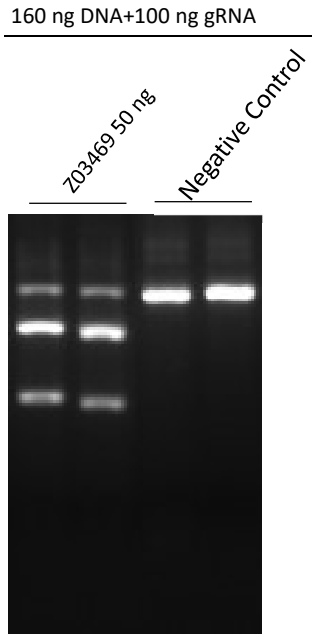

In vitro DNA cleavage assay with GenCrispr NLS-Cas9-NLS nuclease

Reactions were set up according to recommended conditions, and cleavage products were resolved on a 1.5% agarose gel.

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, (2014) Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoprotein. Genome Res. 24(6), 1012-1019.

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/μL.

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