艾科瑞生物
Accurate Biology

Instruction for Use

Cas9-NLS Nuclease (10mg/mL)

AG51110
Version.V2E1

Research Use Only
Not For Diagnosis Procedures

1. Description

This product is a recombinant protein derived from the wild-type Streptococcus pyogenes Cas9 endonuclease sequence, with nuclear localization signals (NLS) added at both the N-terminal and C-terminal. It is recombinantly expressed and purified from Escherichia coli. This product exhibits excellent in vitro cleavage activity and in vivo gene-editing capabilities. Under the guidance of single-guide RNA (sgRNA), it specifically recognizes the NGG PAM sequence and binds to double-stranded DNA for precise cleavage, generating a double-strand break approximately three bases upstream of the target DNA's PAM site. Additionally, this product retains crRNA and tracrRNA dependency and possesses trans-ssDNA cleavage activity activated by target DNA binding.

2. Kit Information

Kit Name	Cat. No	Specification
Cas9-NLS Nuclease (10mg/mL)	AG 51110	200μg (10 mg/ml)

3. Transportation and Storage

Storage	Store at –20°C
Transportation	Transport at –20°C Dry Ice or Blue Ice Condition

4. Kit Components

Kit Components	Volume
Cas9-NLS Nuclease (10 mg/ml)	20μl
10X Cas9 Reaction Buffer	1 ml

5. Application

1) Gene editing and modification based on CRISPR/Cas9 technology.
2) High-sensitivity, high-specificity nucleic acid detection utilizing trans-cleavage activity.
3) In vitro screening of highly efficient sgRNA sequences.
4) Targeted DNA cleavage guided by sgRNA.
5) Linearization of circular double-stranded DNA containing specific sequences.

6. Precautions

1) Briefly centrifuge Cas9-NLS Nuclease before use to collect all solution to the bottom of the tube, minimizing loss. Gently pipette to mix (avoid foaming). Do not vortex vigorously to prevent enzyme inactivation. Keep on ice during use and store immediately at -20° C after use.
2) The storage buffer of Cas9-NLS Nuclease contains 10% glycerol. For in vivo experiments, dilute the protein appropriately to minimize the impact of glycerol on experimental results.
3) Fully thaw 10X Cas9 Reaction Buffer on ice before use. Briefly centrifuge to collect all solution to the bottom of the tube, gently pipette to mix (avoid foaming), and then proceed with the experiment.
4) This product requires the addition of sgRNA during use. Take precautions to prevent RNase contamination in the experimental environment and during handling.
5) No specific in vivo experimental protocol is provided for this product. Users should refer to relevant literature or transfection kit manuals for guidance.
6) 10 mg/ml Cas9-NLS Nuclease is approximately equivalent to 62,305 nM of Cas9-NLS Nuclease.

7. Protocol

Prepare Reaction Mix as Below.

Components	Volume
Cas9-NLS Nuclease (10 mg/ml) *1	0.5 μg
10X Cas9 Reaction Buffer	3 μL
sgRNA (10 ng/μl)*1	10 μL
RNase free water	Up to 25 μL

*1: Pre-incubate at 37°C for 5 minutes.
*2: Add 5 μl of target DNA substrate (25 ng/μl)[1] and incubate at 37°C for 60 minutes[2].
*3: After the reaction, add 1 μl of Proteinase K (20 mg/ml) and incubate at room temperature for 10 minutes.
*4: Analyze an appropriate amount of the reaction product using agarose gel electrophoresis. If electrophoresis is not performed immediately, store the reaction product at -20°C for future use.

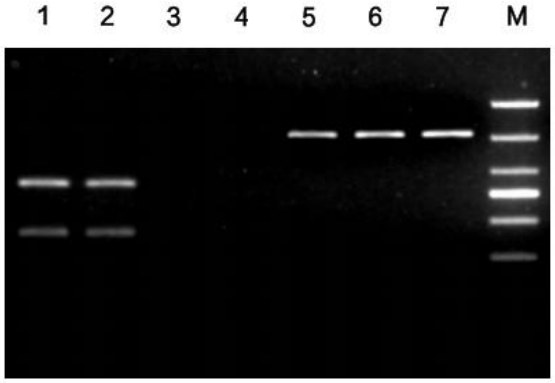
Notes:
*[1]: The recommended molar ratio of Cas9-NLS Nuclease : sgRNA : target DNA is 10 : 10 : 1 or higher.
*[2]: The standard reaction time is 60 minutes but can be extended to 60-120 minutes if necessary.

Experimental Sample

I. In Vitro System

This product exhibits specific double-stranded DNA cleavage activity. The target DNA is approximately 700 bp in length. Under the guidance of a designed sgRNA, this product can cleave the target DNA into two fragments, resulting in two bands at approximately 200 bp and 500 bp on a gel.

[**Note**: For double-stranded cleavage to occur, Cas9, sgRNA, and target DNA are all essential—none can be omitted.]

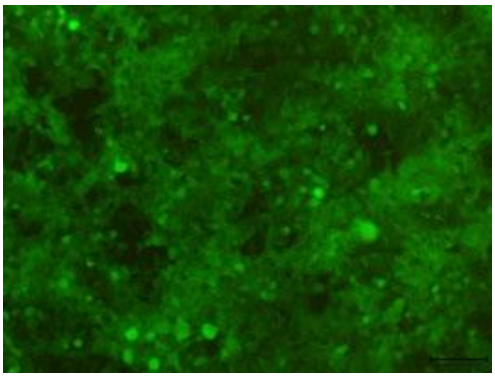


3% TAE Gel 20 μL Sample
1-2：Experiment Group
(Cas9 + sgRNA + Target DNA)
3-4：NTC1 (Cas9 + sgRNA）
5-6：NTC2 (Cas9 + Target DNA)
7：NTC3 (sgRNA + Target DNA)
M：GL1000

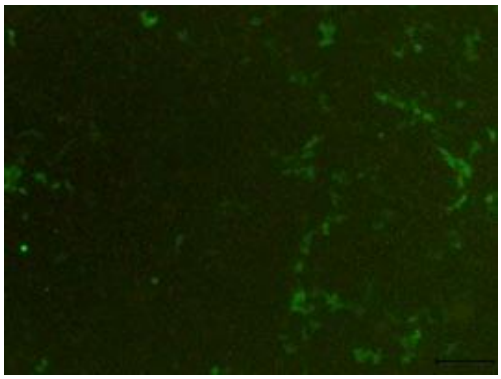
II. In Vivo System

To evaluate the gene-editing efficiency of Cas9-NLS Nuclease, 0.5 μg of the product was co-transfected with sgRNA targeting the EGFP locus into 1.6 × 10^5 EGFP-expressing cells (12-well plate format) using Lipofectamine™ CRISPRMAX™ Cas9 Transfection Reagent (Invitrogen™).

Fluorescence Microscopy (Figure A): The experimental group showed a significant reduction in green fluorescence compared to the blank control group.

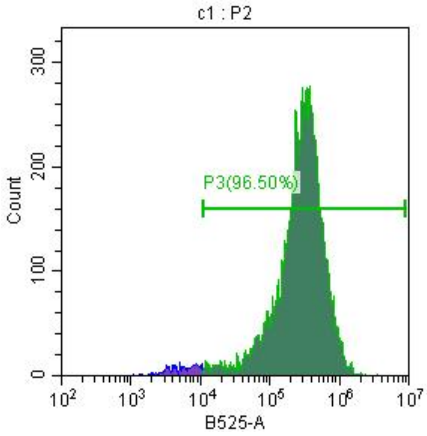


Negative Control Group



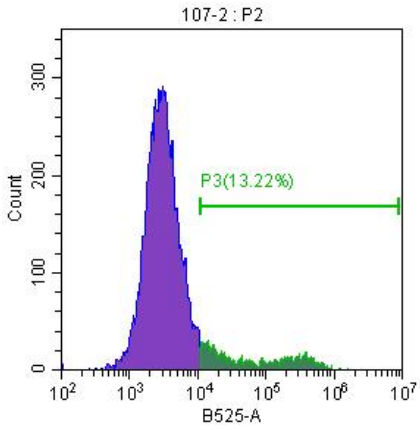
Experimental Group

Flow Cytometry Analysis (Figure B): Among 15,000 analyzed cells, the EGFP-positive cell population decreased by 83.28% in the experimental group compared to the control.



Negative Control Group

Experimental Group



These results demonstrate that Cas9-NLS Nuclease, guided by sgRNA, effectively mediated gene editing at the EGFP target site.

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