SpCas9-GFP Nuclease User Guide

I. Basic Information

Cat. No.	EDE0014
Product Name	SpCas9-GFP Nuclease
Molecular Weight	195.15 kDa
Form	Liquid

- **Product Description**

Cas9 Nuclease (SpCas9), derived from *Streptococcus pyogenes*, is an RNA-guided endonuclease that cleaves double-stranded DNA. In the presence of a PAM (protospacer adjacent motif) sequence in the target DNA, Cas9 Nuclease can specifically cut the target DNA under the guidance of sgRNA, generating double-strand breaks with blunt ends. The PAM sequence is essential for Cas9 to recognize and cleave the target DNA. The cleavage site is located three nucleotides upstream of the PAM within the target sequence.

When SpCas9-GFP is transfected into cells, its nuclear localization can be observed under a microscope, allowing visualization of Cas9-mediated gene editing. The nuclease can also be applied for in vitro cleavage of target DNA, cloning of desired fragments, and other molecular biology experiments.

- **Product Components**

Components	500 pmol	1000 pmol
SpCas9-GFP Nuclease (100 μM)	100 μM* 5 μL* 1 vial	100 μM* 10 μL* 1 vial

- **Storage Conditions and Shelf Life**

  Shelf life: 1 year. Store at -20°C; for long-term storage, -80°C is recommended.

  It is advisable to aliquot the product according to usage to avoid repeated freeze-thaw cycles.

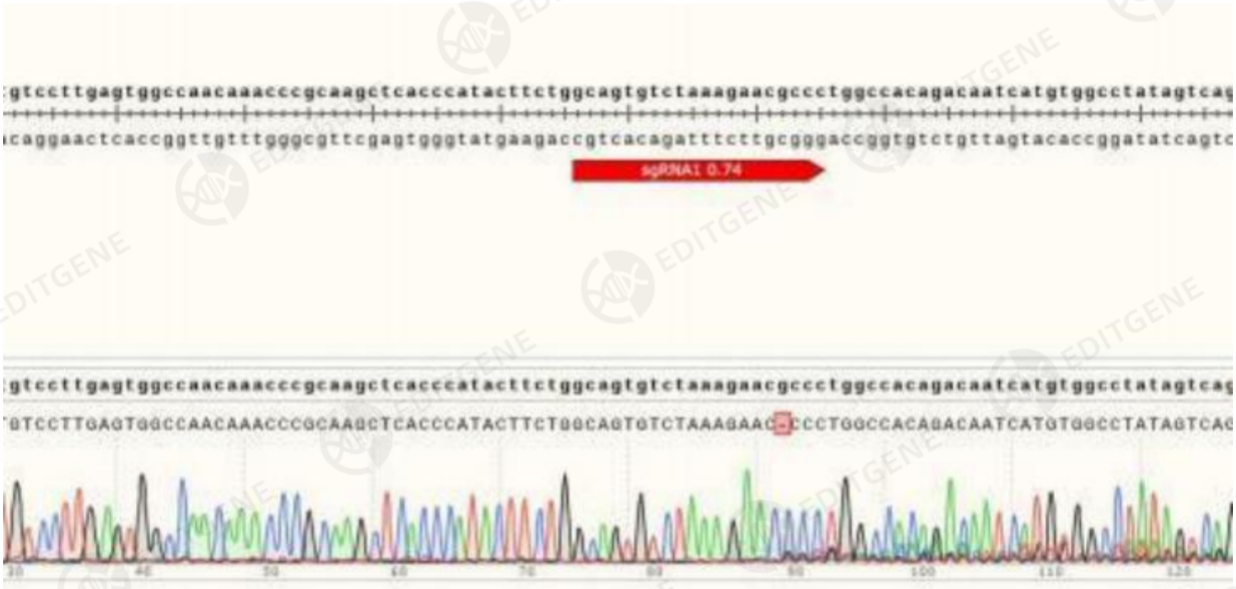
- **Product Features**

  Purified using a one-step method to maximize enzymatic activity. Activity tests show that the nuclease is significantly more active than comparable products.

- **Quality Control**

  Sample Purity: ~95% (verified by SDS-PAGE)

  Functional Validation: When delivered as an RNP complex via electroporation into THP-1 cells, the nuclease achieves a knockout efficiency of 80%.



II. Instructions

- **RNP Electroporation Procedure**

1. Cell Culture and Seeding (Using a 24-well plate as an example)

Culture cells to a healthy, actively growing state. 24 hours before transfection, seed the cells into a

24-well plate so that the confluency reaches approximately 60% at the time of transfection. Use cells in good condition and ensure they are free of bacterial, fungal, or mycoplasma contamination. If using cells recently thawed from liquid nitrogen, passage them at least twice before transfection.

2. Preparation of Ingenio® Electroporation Buffer / RNP / Cell Mixture (Perform Immediately Before Electroporation)

According to the recommended volumes in the table below, mix Cas9-EGFP nuclease and sgRNA in an Eppendorf tube using pipette tip. Mix gently to avoid bubbles, label as Tube 1, and incubate at room temperature for 15 minutes.

Components	Volume (μL)	Final concentration
sgRNA (100 μM)	1.2	1.2 μM
Cas9-EGFP (100 μM)	1	1 μM

***Note**: It is recommended to use a 1:1 molar ratio of Cas9-EGFP to sgRNA. Depending on editing efficiency, cytotoxicity, and off-target effects, the ratio can be adjusted appropriately within the range of 1:1.2 to 1:2.*

3. Preparation of Electroporation Buffer / Cell Mixture

Centrifuge the cells and wash them with ion-free PBS to remove any residual PBS. Transfer the cells into the electroporation buffer (Ingenio® Electroporation Buffer) and label as Tube 2. The total volume of the electroporation buffer should correspond to the size of the electroporation cuvette.

4. Preparation of Electroporation Buffer / Cell / RNP Mixture

Add 2.2 μL of the RNP mixture to the cell suspension and gently mix by pipetting. Transfer the mixture to the electroporation cuvette, taking care to avoid introducing bubbles.

Note:

0.2 cm electroporation cuvette: 100 μL per cuvette

0.4 cm electroporation cuvette: 250 μL per cuvette

5. Transfection of Target Cells

Perform electroporation according to the instructions of the electroporator. After electroporation, allow the cells to recover for 10 minutes. Then, add 100 µL of pre-warmed complete medium and transfer the electroporated cells to a 12-well plate containing 0.8 mL of pre-warmed complete medium per well for further culture.

6. Analysis of Transfected Cells

48 hours after transfection, extract the genomic DNA from the transfected cells and amplify the target region using specific primers. The amplicon should include the sgRNA target cleavage site.

Gene editing efficiency can then be analyzed using tools such as **TIDE** (https://tide.nki.nl/) or **ICE** (https://ice.synthego.com/#/). For instructions on ICE analysis, refer to: https://www.synthego.com/guide/how-to-use-crispr/ice-analysis-guide.

- **RNP Lipid-Based Transfection Procedure**

1. Cell Culture and Seeding (Using a 24-well plate as an example)

Seed cells in advance and allow them to reach approximately 60% confluency before transfection. The number of cells to be seeded should be based on their growth rate: rapidly growing cells should be seeded at a lower density so that the target confluency is reached at the time of transfection.

2. Preparation of Transfection Complexes

Use Lipofectamine 3000 (ThermoFisher) for transfection, . Prepare the complexes in two separate parts, Solution A and Solution B, according to the protocol. Mix the two systems together immediately before transfection.

Solution A

Reagent	Volume (µL)	Final concentration
sgRNA (100 µM)	1.2	1.2 µM

Cas9-EGFP (100 μM)	1	1 μM
Opti-MEM	25	-

Mix thoroughly and incubate at room temperature for **15 minutes**.

Solution B

Reagent	Volume (μL)	Final concentration
Opti-MEM	25	-
Lipofectamine 3000	1.5	-

Mix Solution B gently; no incubation is required.

Gently mix the solutions from tubes A and B, and incubate the mixture at room temperature for 10 minutes.

3. Cell Transfection

Add the prepared mixture dropwise to the cells and swirl gently to ensure even distribution.

4. Analysis of Transfected Cells

After 48 hours, extract the genomic DNA from the transfected cells. Amplify the target region using specific primers; the resulting amplicon should include the sgRNA target site.

Analyze the gene-editing efficiency using tools such as **TIDE** (https://tide.nki.nl/) or **ICE** (https://ice.synthego.com/#/; user guide available at https://www.synthego.com/guide/how-to-use-crispr/ice-analysis-guide).

- **Precautions**

  To prevent RNase contamination, maintain a clean and organized workspace. Always wear clean gloves and a mask during the experiment. Use only RNase-free consumables, including pipette tips, centrifuge tubes, and other labware.

III. Publishing Requirements

When using this product in publications, please acknowledge our company: Guangzhou Editgene Co. Ltd, China, SpCas9-GFP Nuclease (CAS：EDE0014). Or EDITGENE CO.LTD, SpCas9-GFP Nuclease (CAS：EDE0014), if used within U.S. or Europe territory.