

Recombinant Cas9 Nuclease, GMP Grade

Cat No.: GMP-1701

01/ Product Description

CRISPR-Cas9 is an adaptive immune system developed by bacteria and archaea during the long-term evolution. The CRISPR-Cas9 system integrates fragments of invaded phage and plasmid DNA into CRISPR sequences and uses the corresponding CRISPR RNAs (crRNAs) to guide the cutting of homologous sequences by Cas9 protein and the system has immune memory ability. The artificially modified Cas9/sgRNA system can guide Cas9 protein to recognize and cut double-stranded DNA with sgRNA targets through sgRNA (single guide RNA), which can be used for gene knockout and precise DNA editing.

The recombinant *S. pyogenes* Cas9 protein expressed in *E. coli* which is cultured in animal-free medium and the protein is purified by multi-step column chromatography. It is produced with pharmaceutical raw materials and the host-cell protein residues and nucleic acid residues are strictly controlled. Product production and quality control conform to GMP standards, ensure the production process and all raw materials traceable.

02/ Product Features

- 1) Genetically engineered production without animal ingredients.
- 2) Preparation of raw materials and accessories for pharmaceutical specifications.

03/ Application

- 1) Non-viral vector CRISPR gene knockout based on protein and sgRNA transfection.
- 2) Non-viral vector CRISPR gene knock-in based on protein and sgRNA transfection.

04/ Quality Elements

Element	Standard
Appearance	clear, transparent solution
Visible Particles	meet the specification
pH	7.5-8.5
Purity	≥ 95% as analyzed by SDS-PAGE ≥ 95% as analyzed by SEC-HPLC
Molecular Weight	163±16.3kDa (Reduced type)
Protein Content	9.5~10.5mg/ml (Bradford assay)
Activity	In vitro cutting efficiency ≥ 90% (300ng)
Bacterial Endotoxins	<10EU/mg
Host-bacterial Protein Residues	≤ 0.005% of total protein
Exogenous DNA Residues	≤ 10ng per 1mg of protein
Endonuclease Residues	The degradation of substrate was ≤ 10%
Exonuclease Residues	The degradation of substrate was ≤ 10%
RNase Residues	The degradation of substrate was ≤ 10%
Mycoplasma	Negative (Mycoplasma kit)
Heavy Metals	≤ 10 ppm
Sterility	Meet the specification

05/ Complying to Following Regulations

- 1) ISO 9001:2015, certified facility.
- 2) Good Manufacturing Practice (GMP) for Pharmaceutical Products (current version) and its appendices.
- 3) Chinese Pharmacopoeia – General Chapter 0232: Quality Control of Raw Materials and Excipients Used in the Production of Biological Products.
- 4) USP Chapter <1043>, Ancillary Materials for Cell, Gene, and Tissue-Engineered Products.
- 5) Ph. Eur. General Chapter 5.2.12, Raw Materials of Biological Origin for the Production of Cell-based and Gene Therapy Medicinal Products.

06/ Storage and Transport Conditions

Store at -20±5°C, avoid repeated freezing and thawing; Valid for 2 years; Cold transport on dry ice.

07/ Storage Buffer

30mM Tris, 300mM NaCl, 50% Glycerol, 0.1mM EDTA, pH 7.4

08/ Product Packaging

Components	GMP-1701-U100 Size: 100µg	GMP-1701-M001 Size: 1mg
Recombinant Cas9 Nuclease, GMP Grade (10mg/ml)	100µg	1mg

Note: Recombinant Cas9 Nuclease (160ng=1pmol).

09/ In Vitro Cutting System

Components	Quantity
dsDNA	200ng
sgRNA	200ng
Recombinant Cas9 Nuclease, GMP Grade	0.5-1µg
10×Reaction buffer	2µl
RNase free water	Up to 20µl

The reaction was incubated at 37°C for 1h and terminated by holding at 70°C for 10 mins.

In vitro cutting results:

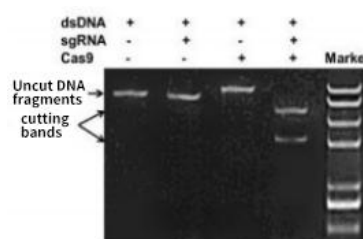


Figure 1: Detection of DNA fragment cutting activity of Cas9 endonuclease. The DNA fragment with the target site is 6100bp, and the digested fragments are 3900bp and 2200bp

10/ Intracellular Gene Editing System (20µl)

1. Preparation of RNP complexes

Components	By Quantity	By Volume
sgRNA	80pmol	1.5µl
Recombinant Cas9 Nuclease, GMP Grade	80pmol	1.5µl
Electroporation buffer	7µl	7µl

mix and incubate for 10 mins at room temperature

2. Prepare 10µl of cells (2.0×10^5) in the logarithmic growth phase (resuspended cells must to use the corresponding electroporation buffer) and gently mixed with the prepared RNP complex. The mix is incubated for 10 mins.
3. Choose the right conditions for electroporation.
4. After the electroporation, the cells are transferred to 6-well plates and 2ml of Penicillin-Streptomycin free medium is added.
5. The efficiency of gene editing can be verified after 48 hours.

The results of gene editing efficiency are shown in graphs:

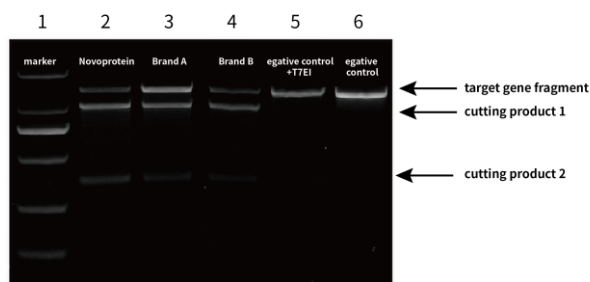


Figure 2: Different Cas9/sgRNA RNP complexes were delivered to 293T cells by electroporation. Cells were collected after 48 hours, and DNA was extracted.

The results were the size of products after DNA cutting by different Cas9 proteins from different companies, under the same experimental conditions.

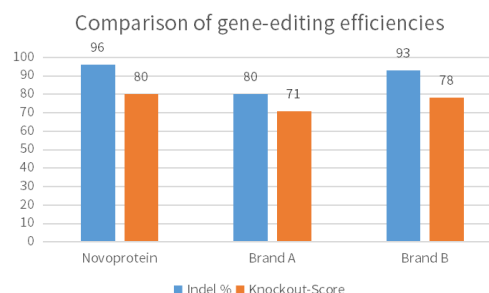


Figure 3: Comparison of editing efficiency of different Cas9 proteins from different companies.

11/ FAQ

1. What are the reasons of incomplete cutting bands?

- 1) It may be caused by the wrong ratio of Cas9 protein, sgRNA and target DNA. We recommend that the molar ratio of Cas9 Nuclease, sgRNA and target DNA is at least 10:10:1.
- 2) It may be related to the sequence of the sgRNA, and the best sgRNA sequence can be selected according to the target DNA.
- 3) It may be caused by sgRNA degradation, and the integrity of sgRNA can be verified by gel electrophoresis.

2. How to determine the cell transfection efficiency of the transfection reagent when CRISPR Cas9/sgRNA RNP system is used for cell gene editing?

High transfection efficiency is a necessary condition for efficient gene editing efficiency, which can be determined by transfection of NLS-Cas9-EGFP Nuclease (Cat.No.: E379) or other tracer proteins. For cells that are difficult to transfer, electroporation, microinjection, and other methods are recommended, and the optimal experimental conditions can be determined by adjustments based on Electrotransfer and microinjection instruments.

3. What are the advantages of CRISPR Cas9/sgRNA RNP system compared with Cas9 protein expressed by plasmids and Cas9 stable expressing cell lines or lentivirus systems?

The CRISPR Cas9/sgRNA RNP system has the following advantages compared to plasmids or stable cell lines:

- 1) Cas9 protein directly interact with the target gene, resulting in low off-target efficiency and no risk of DNA integration and promoter compatibility problems, which greatly improves the application safety.
- 2) The ready-to-use system can complete the editing efficiency detection in 5-6 days by one-step transfection, which is easy to operate and can significantly reduce the experimental time.
- 3) There is no need to construct the plasmid or package the lentivirus, and there is no need for viral-grade laboratory environment.
- 4) The gene editing efficiency is higher than plasmid method in most cells.

4. How to quickly determine the effect of sgRNA?

The Cas9 cutting efficiency is related to the target sequence recognized by the sgRNA, and different sgRNA had different recognition efficiency. It is recommended that the sgRNA with high editing efficiency should be obtained by rapidly screened by in vitro enzyme digestion experiments which cut the target DNA fragments in vitro.

5. What is the reason for the absence of cutting bands detected by T7EI?

- 1) For the low transfection efficiency of cells, it is recommended to optimize the transfection procedure or switch to a cell type that is easy to transfect.
- 2) For Cas9 protein degradation, it can be eliminated by setting up a positive control group for co-transfection.

- 3) If the Cas9 nuclease was unable to access the target site or cut the target site in cells, it is suggested that the sgRNA should be redesigned.
- 4) The denaturation and annealing steps of the edited fragments of DNA product were not performed. The error of the experimental operation can be confirmed by setting up a positive control group, and the specific method is shown in the Instructions for use of Novoprotein T7EI (Cat.No.: M017).

6. There are many non-specific bands when the agarose gel electrophoresis is used to analyze the cutting efficiency. What if the cutting efficiency cannot be analyzed?

Cutting bands and non-target bands can be distinguished by setting a negative control. If necessary, primers should be redesigned or PCR conditions should be optimized to reduce non-specific amplification. Nested PCR is recommended to improve the specificity of the amplified fragments.

7. How to solve the problem of band dispersion during T7EI detection?

T7EI has a low non-specific DNA cutting ability, it is recommended to increase the amount of DNA, reduce the amount of enzyme, reduce the digestion time to reduce the possibility of non-specific cutting.

8. Which CRISPR-Cas9 system should be selected for different cell types such as stem cells, primary cells or suspension cells?

Transfection reagents are usually difficult to achieve ideal transfection effect in cells which are difficult to transfect such as stem cells, primary cells or suspension cells, and electroporation is more suitable for transfection of these cells. Cas9/sgRNA RNP system is recommended for the above cells, and the electroporation conditions can be determined according to the specific experimental conditions.