

Instructions for Use of CRISPR sgRNA

I. Product Overview

Thank you for choosing GenCefe's sgRNA-related products. We utilize advanced oligonucleotide synthesis technologies and superior purification processes to provide a broad range of oligonucleotide products.

Single guide RNA (sgRNA) is the core component of the CRISPR-Cas9 genome editing system. It guides the Cas9 nuclease to accurately recognize and cut a specific DNA sequence, thereby achieving precise genome modification.

At GenCefe, we use advanced chemical synthesis technology to efficiently produce high-quality sgRNA, providing customers with simpler and more effective genome editing solutions.

II. Product Information, Shipping & Storage

This product series is synthesized at a scale of 1 OD or more and delivered as lyophilized powder. Due to variations in crystallization, the appearance may differ or may not be visible to the naked eye — this is normal.

RNA is sensitive to environmental conditions such as temperature, extreme pH, and enzymes. Please store the product properly upon receipt at -20°C to -80°C. The lyophilized powder remains stable for approximately 6 months. Centrifuge briefly before use to collect the powder at the bottom.

For cell experiments, dissolve in RNase-free H₂O or sterile ddH₂O to make a 20 µM stock solution (refer to the shipping report for exact volumes). Aliquot to avoid repeated freeze-thaw cycles (no more than 3) and use within 3 months. During use, keep samples on ice.

Example of 20 µM stock preparation

Oligo	Sequence (5'-3')	Modification
sgRNA1	ACGAACAGACACGAUCCGGUGUUUUAG AGCUAGAAAUAGCAAGUAAAAUAAGGC UAGUCCGUUAUCAACUUGAAAAAGUGG CACCGAGUCGGUGC	3*OME, 3*S
Packaging	1.01 nmol/tube 1.0 OD/tube	2 tubes
Dissolution volume	50.5 µl/tube	

III. Cell Experiment Protocol

Please note:

- 1) Set at least 3 replicates per transfection condition to reduce experimental error.
- 2) Keep cell density consistent across wells; ensure even distribution on the plate.
- 3) For plates with small well diameters, resuspend cells in medium before plating.

3.1 sgRNA + cas9 mRNA system (24-well plate)

- 1) Add the corresponding volume of DEPC water to the sgRNA according to the delivery report to prepare a 20 μ M stock solution. Before use, it needs to be further diluted to 10 μ M and placed on ice for later use.
- 2) Cas9 mRNA (500ng/ μ L), placed on ice for later use.
- 3) 18-24h before transfection, take cells in the logarithmic growth phase and inoculate them in a 24-well plate. The cell density should reach 50%~70% before the experiment.
- 4) Take a 1.5mL centrifuge tube, add 25 μ L Opti-MEM™ reduced serum medium, and then add 2 μ L mRNA transfection reagent, and incubate at room temperature for 10min.
- 5) Take a 1.5mL centrifuge tube, add 25 μ L Opti-MEM™ reduced serum medium, and then add 2 μ L Cas9 mRNA (500ng/ μ L).
- 6) Then add 1 μ L sgRNA (10 μ M) to the above tube (optimize the amount according to actual needs).
- 7) Add the sgRNA and Cas9 mRNA mixture to the diluted transfection reagent and incubate at room temperature for 5min.
- 8) Add the prepared transfection complex to the cell culture medium and continue to culture for 48~72h.
- 9) Extract the genome and use T7E1 enzyme to detect gene editing efficiency (refer to step 3.3).

3.2 sgRNA + cas9 protein system (24-well plate)

- 1) Add the corresponding volume of DEPC water to the sgRNA according to the delivery report to prepare a 20 μ M stock solution. Before use, further dilute to 5 μ M and place on ice for later use.
- 2) Dilute Cas9 Protein (20 μ M) to 6 μ M with Opti-MEM™ reduced serum medium or PBS and place on ice for later use.
- 3) 18-24h before transfection, take cells in the logarithmic growth phase and inoculate them in 24 plates. The cell density should reach 50%~70% before the experiment.

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- 4) In a 1.5mL centrifuge tube, add 25μL Opti-MEM™ serum medium, and then add 1μL Cas9 Protein (6 μM).
- 5) Add 1.2μL sgRNA (5μM) to the above centrifuge tube and incubate at room temperature for 10 minutes to form Cas9 RNP complex. (The ratio of sgRNA:Cas9 Protein is 1:1-5:1, which can be optimized by yourself).
- 6) Dilute 3μL Lipo3000 transfection reagent with 25 μL Opti-MEM™ reduced serum medium.
- 7) Add Cas9 RNP complex to transfection reagent and incubate at room temperature for 15 minutes.
- 8) After incubation, add the prepared transfection complex to the cell culture medium and continue to culture for 48~72h.
- 9) Extract the genome and use T7E1 enzyme to detect gene editing efficiency (refer to step 3.3).

3.3 T7E1 detection of gene editing efficiency

T7E1 enzyme (T7 endonuclease I) can specifically identify mutations (such as base substitutions, insertions or deletions) and certain DNA high-level structures (such as cross-shaped, Holiday connectors, etc.) in DNA double helices. In gene editing detection, its mismatch cutting characteristics are mainly used to identify the mismatched regions formed. Mix the mutant and wild-type PCR products, heat denature them, and slowly anneal them. Mutant DNA and wild-type DNA will form heteroduplexes, and the mismatched regions will form local single strands or twisted structures due to base mismatches. After the T7E1 enzyme is added to the enzyme cutting system, the mismatched sites in the heteroduplexes are cut, while the completely matched wild-type or homozygous mutant double strands remain intact. The cut products are separated by agarose gel electrophoresis. The wild-type sample only shows the original band, while the sample with mutations will additionally show the cut fragments (such as 300bp+200bp). The mutation rate can be calculated by the band grayscale ratio.

The detection steps are as follows:

- 1) Collect the edited cells after transfection, extract gDNA, and perform absorbance detection.
- 2) Purify gDNA, dilute the DNA concentration to 100-500 ng/μL, and use it for high-fidelity PCR amplification of DNA fragments containing mutation sites. Please note that a small amount of sampling should be performed after electrophoresis to detect band specificity.
- 3) Product purification, purify the DNA containing the target fragment obtained by the above PCR to ensure that the band is clean, single and free of impurities. Then quantify it to 50 ng/μL using a UV spectrophotometer.

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- 4) Prepare a DNA double-strand annealing system according to the following system. And anneal according to the recommended conditions.
- 5) After annealing, add 0.5 μL of T7E1 enzyme to each tube, incubate at 37°C for 30min, and complete enzyme digestion.
- 6) Purify the product and analyze the product by 2% agarose gel electrophoresis. Detect the editing efficiency and quantify the gray value using software such as image J.

Enzyme digestion fragment ratio: $f_{\text{cut}} = (b+c) / (a+b+c)$

Mutation Efficiency: indel (%) = $100 \times (1 - \sqrt{1 - f_{\text{cut}}})$

Components	Volume
10×T7E1 buffer	2 μL
Purified DNA fragment (50ng/ μL)	4 μL
ddH ₂ O	14 μL
Annealing after mixing	95°C 5 min; 95°C 2 s; 95°C-75°C -0.1°C/cycle, 200 times; 75°C 1 s; 75°C-15°C -0.1°C/cycle, 600 times; 16°C 2 min

Disclaimer

Since CRISPR editing efficiency is affected by many factors, including genomic locus of the target, chromatin accessibility, nucleosomes, other components around crRNA-binding sites, etc., the effectiveness of gene targeting at different gene loci is different and depends on the transfection efficiency of each cell. GenCefe Biotech does not promise that if cells are effective, then the editing level of animals will definitely be effective. We solemnly remind that gene editing experiments must be carried out carefully under the premise of strictly following technical standards and ethical norms, and must strictly abide by relevant laws and regulations on scientific ethics.