Instructions for Use of siRNA

I. Product Overview

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

siRNA mediates gene silencing by binding specifically to target mRNA, guiding the RNA-induced silencing complex (RISC) to cleave the mRNA and inhibit gene expression. This process is initiated by Dicer enzyme cleavage of long dsRNA into short fragments. Owing to its high efficiency and specificity, siRNA is widely used in gene function studies and disease therapy.

GenCefe offers both siRNAs for cell experiments and chemically modified in vivo siRNAs for animal studies.

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 H_2O or sterile ddH_2O 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.

For animal experiments, dissolve in saline to the required injection volume. Dosing method and amount depend on the targeted organ. Suggested dosage:

- Systemic administration: 5–20 nmol per 20g body weight
- Local administration: 1–5 nmol each time. Recommended injection frequency: 2–3 times per week, or refer to relevant literature.

Example of 20 μM stock preparation

Oligo	Sequence (5'-3')		Modification
ATG12-human-27	Sense	GCAGCUUCCUACUUCAAUUTT	/
	Antisense	AAUUGAAGUAGGAAGCUGCTT	/
Packaging	3.14 nmol/tube 1.0 OD/tube 2 tubes		
Dissolution volume	157.0 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 Transfection Concentration

The optimal working concentration of siRNA products varies depending on the cell type and research purpose. The initial concentration of siRNA is ~50 nM. The transfection concentration can be optimized according to the specific conditions of the experiment, and the optimization range is recommended to be 5–150 nM.

3.2 Transfection Procedure

Please note: The following steps are for transfecting siRNA using GenCefe SuperTrans transfection reagent. If you use other transfection reagents, please refer to their instructions.

Taking 24-well plate transfection of siRNA as an example, all reagent amounts and volumes are calculated per well.

1) Adherent cells: One day before transfection, seed 0.5–2.0 × 10^5 cells per well in 500 µL of complete culture medium. At the time of transfection, cells should ideally reach approximately 50% confluence.
   Note: Be sure to use serum-containing complete culture medium at this step, as serum-free medium may increase the risk of cell death.

2) Preparation of Transfection Complex:
   A. Place Transfection Reagent at room temperature and gently mix before use;
   B. Tube A: Add 50 ul serum-free culture medium or OPTI-MEM to a sterile tube, add 2 ul transfection reagent, mix gently with a pipette, and stand at room temperature for 5 min;
   C. Tube B: Add 50 ul serum-free culture medium or OPTI-MEM to another sterile tube, add 2 ul siRNA, mix gently with a pipette, and stand at room temperature for 5 min;
   D. Add the mixture of Tube A to Tube B, mix gently with a pipette, stand at room temperature for 15-20 min, and then transfect immediately.

   Note: The medium used for dilution in Steps B and C must be serum-free. siRNAs are generally recommended to be stored at a concentration of 20 µM. A total volume of 2 µL contains 40 pmol of siRNA.

3) Add 100 µL complex per well and shake gently.
4) Replace with complete medium after 6–8 hours.
5) Culture at 37°C for 24-72 hours to detect mRNA expression, and for 48-96 hours to detect protein expression.

Recommended Reagent Volumes

Plate Type	Well area	Inoculation medium	Opti-MEM for dilution	Transfection	
				siRNA Amount	Transfection Reagent
96-well plate	0.3cm^2	100μL	2×10μL	20 pmol	1μL
24-well plate	2.0cm^2	500μL	2×50μL	40 pmol	2μL
12-well plate	4.0cm^2	1mL	2×100μL	80 pmol	4μL
6-well plate	10.0cm^2	2mL	2×200μL	150 pmol	7.5μL
60 mm plate	20.0cm^2	5mL	2×0.5mL	300 pmol	15μL
10 cm plate	60.0cm^2	15mL	2×1mL	600 pmol	30 μL

3.3 Detection of Knockdown Efficiency

RNA and protein level detections can be performed 24 to 72 hours after transfection. The method is as follows:

1) RNA level: Since siRNA depends on the RISC complex and acts directly on RNA. Therefore, the RNA knockdown level directly reflects the siRNA knockdown efficiency. It is recommended to design 1-2 pairs of efficient primers and perform qRT-PCR detection on RNA.
2) Protein level: For mRNA, because it has coding ability, WB can be used to detect the siRNA knockdown efficiency. However, due to the half-life of the protein and the compensation mechanism of the cell, the protein level may not be obvious. At this time, it should be determined whether the mRNA level is knocked down, and other methods such as CRISPR and viruses should be selected for genetic intervention.

In addition, direct detection of cell functions such as proliferation, edu, apoptosis, cycle, etc. can also verify whether siRNA works.

IV. Animal Experiment Guidelines

The in vivo environment of siRNA animal experiments is complex and the experimental cycle is long, which puts higher requirements on the stability of siRNA products. GenCefe enhances siRNA stability and uptake via chemical modifications, offering non-viral gene silencing solutions. siRNA animal experimental methods are mainly divided into two categories, cell transplantation and direct administration. We recommend the use of chemically modified in vivo siRNA with better stability and higher reliability (default 2oME+5'chol modification, we can also provide other chemical modifications, GalNAc modification, etc.) for animal experiments. Among them, direct administration methods include local administration and systemic administration.

- Local administration: superficial tissues and organs, such as: brain, muscle, eye, nasal cavity, skin, cochlea, tumor, pharynx, genitals, joints, etc.
- Systemic administration: circulatory system, immune system, heart, liver, lung, kidney, spleen, lymphatic system, metastatic tumors, etc.

Recommended dosage for animal injection

Product	Administration Method	Recommended Dose
In vivo siRNA	Local administration	1-5 nmol/20 g body weight
	Systemic administration	5-20 nmol/20 g body weight
Frequency		2-3 times/week

Dosage reference for different sites

Animal	Injection Site	Recommended Dose
Mouse	Brain injection	1-5μL
	Subcutaneous injection	30-50μL
	Ocular injection	2-5μL
	Tail vein injection	100-200μL
	Intraperitoneal injection	100-200μL
	Muscular injection	50-100μL
Rat	Brain injection	5-10μL
	Subcutaneous injection	50-100μL
	Ocular injection	2-5μL
	Tail vein injection	100-200μL
	Intraperitoneal injection	100-300μL
	Intramuscular injection	100-200μL

Frequently Asked Questions

1. How to design siRNA groups, and what is the role of each group?

Group	Operation	Purpose
Blank control group	Cell culture, no treatment	Monitor cell growth
Transfection reagent group	Transfection reagent only	Assess the toxicity of transfection reagent
siRNA NC group	Transfect siRNA NC	Prove the specificity of siRNA
siRNA 1-3 group	Transfect target gene siRNAs	Knockdown of target gene
Positive control group	Transfect positive control si-GAPDH	Validate knockdown process
Fluorescence control group	Transfect fluorescent control	Detect transfection efficiency

2. What colors are there for fluorescent controls? What should I pay attention to when operating?

GenCefe provides fluorescent controls labeled with FAM, Cy3, and Cy5, usually FAM.

- FAM has a maximum absorbance at 495nm and a maximum emission at 520nm.
- Cy3 has a maximum absorbance at 550nm and a maximum emission at 565nm.
- Cy5 has a maximum absorbance at 643nm and a maximum emission at 667nm.

After 24-48 hours of transfection, it can be observed using a fluorescence microscope and a confocal microscope. Pay attention to light protection during the transfection operation and culture process. Other operations are the same as siRNA.

3. What is the reason for cell death after transfection, and how to operate?

In general, it is the toxicity of the transfection reagent to the cells. You can reduce the amount of transfection reagent, or use other transfection reagents or transfection methods; you can also try to reduce the amount of siRNA. If the cell itself is not in good condition, it is recommended to re-experiment and re-recover the cells if necessary.

4. What is an acceptable knockdown efficiency?

No strict benchmark. The interference efficiency varies with different cell types and different genes. The transfection efficiency of different cell types is also different, and the expression levels of different genes also vary greatly, mainly due to the interference effect.

5. Low knockdown efficiency—how to improve?

There are mainly two reasons:

- Low transfection efficiency-It is necessary to optimize the transfection reagent and siRNA dosage, or replace the transfection reagent to improve the transfection efficiency;
- Poor siRNA target design – If it is determined that there is no problem with the transfection efficiency, the siRNA target may need to be redesigned.