Instructions for Use of miRNA

I. Product Overview

Thank you for choosing GenCefe's miRNA-related products. We utilize advanced oligonucleotide synthesis technology and superior purification processes to provide a wide range of oligonucleotide-related products for our customers.

- miRNA mimics are double-stranded RNA molecules that simulate endogenous miRNAs in function.
- miRNA inhibitors are single-stranded oligonucleotides that are reverse complementary to target miRNAs, effectively inhibiting their function.
- miRNA agomirs are specially chemically modified miRNA agonists, purified by HPLC, suitable for both cell-based and animal experiments.
- miRNA antagomirs are chemically modified miRNA antagonists, also purified by HPLC, and are applicable to both in vitro and in vivo 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.

- Systemic administration:
  - Agomir: 5–20 nmol per 20g body weight
  - Antagomir: 50–200 nmol per 20g body weight
- Local administration:
  - Agomir: 1-5 nmol per dose
  - Antagomir: 5–20 nmol per dose
- Frequency: 2-3 times/weeks, or refer to a literature

Example of 20 μM stock preparation

Oligo	Sequence (5'-3')		Modification
hsa-miR-216a-5p mimics	Sense	UAAUCUCAGCUGGCAACUGUGA	/
	Antisense	ACAGUUGCCAGCUGAGAUUAUU	/
Packaging	3.03 nmol/tube 1.0 OD/tube 2 tubes		
Dissolution volume	151.5 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 Transfection Concentration

The optimal working concentration of miRNA products varies depending on the cell type and research purpose. The initial concentration of miRNA mimic is about 50nM, and the concentration of miRNA inhibitor is 100nM. The transfection concentration can be optimized according to the specific conditions of the experiment, and the optimization range is recommended to be 10~200nM.

3.2 Transfection Procedure

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

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

1) Adherent cells: One day before transfection,0.5-2.0×10^5 cells per well were seeded in 500 μL of culture medium containing antibiotics. 60-80% confluency is recommended for transfection.

2) Preparation of Transfection Complex:
   - Place Transfection Reagent at room temperature and gently mix before use;
   - 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;
   - Tube B: Add 50 ul serum-free culture medium or OPTI-MEM to another sterile tube, add 2 ul miRNA mimic, mix gently with a pipette, and stand at room temperature for 5 min;

- 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: miRNA mimic is generally recommended to be stored at a concentration of 20 uM, and a total volume of 2μL is 40 pmol.

- Add 100 μL complex per well and shake gently.
- Replace with complete medium after 6–8 hours.
- 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	
				miRNA mimic 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

Functional testing can be performed 24 to 72 hours after transfection. The detection methods include but are not limited to qRT-PCR, gene chips, high-throughput sequencing, cell function experiments, etc. The optimal detection time is related to the cell type and the miRNA being studied. The following are several commonly used miRNA effect detection methods:

1) Use qRT-PCR, gene chips, high-throughput sequencing and other methods to detect whether the target gene mRNA transcription level or even the whole gene expression spectrum has changed.
2) Use Western Blot, protein chip and other methods to detect whether the protein level of the target gene has changed accordingly.
3) Verify whether the cell function has changed accordingly.
4) To study the effect of miRNA mimic on the target gene, effective mimics can be used as a positive control.
5) Verify the effect of miRNA mimic/inhibitor by co-transfection with the miRNA target gene dual luciferase reporter vector.

IV. Animal Experiment Guidelines

The in vivo environment of miRNA animal experiments is complex and the experimental cycle is long, which puts higher requirements on the stability of miRNA products. GenCefe enhances miRNA stability and uptake via chemical modifications, offering non-viral gene silencing solutions. miRNA animal experimental methods are mainly divided into two categories, cell transplantation and direct administration. We recommend the use of chemically modified agomir and antagomir with better stability and higher reliability 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
agomir	Local administration	1-5 nmol/20 g body weight
	Systemic administration	5-20 nmol/20 g body weight
antagomir	Local administration	5-20 nmol/20g body weight
	Systemic administration	50-200 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 detect the effects of miRNA mimic, inhibitor, agomir, and antagomir? Can qPCR be used to detect the target miRNA?

After using mimic, qPCR can be used to verify the overexpression of miRNA, but inhibitor is not applicable. In principle, inhibitor binds to miRNA and inhibits the function of miRNA, and it does not change the expression of miRNA. Its effect can be detected by detecting the protein expression level of the target gene or by the luciferase reporter system. Agomir and antagomir may have a certain impact on qRT-PCR due to the addition of chemical modifications, and the effect of use cannot be verified by qPCR method. Agomir can detect miRNA expression through Northern Blot, and antagomir can verify its effect by detecting the expression of the target gene of miRNA

2. Can the negative control (NC) of mimic and inhibitor, agomir and antagomir be used universally? Is NC necessary?

It **CANNOT** be used universally because mimic/agomir is double-stranded RNA and inhibitor/antagomir is single-stranded RNA. It is recommended to use NC as a control group to exclude false positive results that may be caused by stress.