ACCEGEN
BIOTECHNOLOGY

MIRacle™ Agomir Product Manual

Agomir Product Information Sheet

Quality Control

PAGE	MicroRNA Agomir is a special-labeled and chemically modified double-stranded small RNA with accurate molecular weight that mimics the endogenous miRNA to regulate the biological function of the target gene.
HPLC Purification	HPLC purification and analysis of double-stranded miRNA agomir. Purity >95%
Attention	RNA oligo is prone to degrade with the existence of exogenous nuclease. Please wear gloves to do the experiment, not to use RNase contaminated reagents, test tubes, pipettes and tips. Store in -20°C or -80°C environment as soon as possible after receiving the product.
Resuspension	Centrifuge the EP tube at low speed with a maximum speed of 4,000 x g to collect the miRNA Agomir at the bottom of the tube. 1. Gently open the tube cover. 2. Add 125 μL of DEPC water per 1 OD to make a 20 μM stock solution. 3. Gently pipette the stock solution 5 times. 4. Dispense the solution according to specific dosage to avoid multiple freeze-thaw cycles. 5. Seal the EP tube when re-storing. 6. Store at -80°C for later use.

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MicroRNA Agomir Product

Description

MicroRNA Agomir is a special-labeled and chemically modified double-stranded small RNA designed by mature microRNA. One strand is consistent with the target mature miRNA sequence and another one is complementary. Specific microRNA agomir is introduced into the cells which express the corresponding microRNA, and then it simulates the effect of microRNA; Or specific microRNA agomir also combines with the double luciferase reporter system via miRNA binding sites to verify the regulatory relationship between miRNA and target gene.

1. Analysis of miRNA expression level
In order to analyze the up-regulation level of miRNA, miRNA agomir is transfected into cells for functional verification via Northern blotting and real-time quantitative fluorescent PCR.
2. Analysis of target gene expression level
In both miRNA agomir transfected cells and negative control cells, real-time quantitative fluorescent PCR, and Western blotting is used to detect the expression level of the target gene and verify the regulatory relationship between miRNA and target gene.
3. Validation analysis of miRNA 3'UTR target site
By constructing one or more predicted miRNA 3'UTR binding target sites to the reporter plasmid, and co-transfecting miRNA agomir with the reporter plasmid, agomir inhibits the reporter gene on the report plasmid, and thus directly test the relationship between miRNA agomir and miRNA predicted binding site.
We suggest that miRNA agomir negative control is used as a reference. The concentration and transfection conditions of the negative control are the same as those of the experimental group.

Transfection Procedure

Transfection efficiency is different for different cell lines or different transfection reagents. The optimal transfection conditions still need to be determined by experiments. We suggest that the concentration range of miRNA agomir is extended to 1-100 nm.

	96 well plate	24 well plate	12 well plate	6 well plate
Transfection reagent[A]	0.2-0.6 μL	0.5-2 μL	1-3 μL	2-5 μL
MicroRNA agomir	3 pmol	15 pmol	30 pmol	75 pmol
Cell density[C]	6,000 cells/well	40,000 cells/well	80,000 cells/well	200,000 cells/well
Final volume per well	0.1 mL	0.5 mL	1.0 mL	2.5 mL

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A: The recommended amount of transfection reagent depends on the different reagents you ordered

B: The final concentration of miRNA agomir was 30 nM. It could be optimized according to the difference in the amount of maximum miRNA agomir activity in different cell types.

C: The cell density varies in different cell lines. It mainly depends on the cell size and growth of cells. Generally speaking, we recommend 30-70% cell fusion.

Transfection Optimization

Optimization of transfection efficiency is one of the most important factors to maximize the activity of miRNA agomir. For each transfection reagent, you should first determine the most appropriate transfection reagent, mainly depends on the following aspects:

- Amount of transfection reagent
- Amount of MicroRNA agomir
- Cell density during transfection
- Operation sequence during transfection
- Contact time of cells and transfection reagent/siRNA complex

Guidelines- in vivo experiments

Through local injection or tail vein injection in animals, miRNA agomir plays a role in local or systemic high expression of target miRNA. The dose of agomir varies greatly with the way of administration. The dosage regimen is referred to as follows.

miRNA agomir/antagomir system

Recommended dosage every time:

Agomir 5-80μg/g body weight;

Dosage in mouse (body weight 15-20g):

Agomir 200nmol (with about 3 injections)

Route of administration to reach the target site effectively:

Intravenous administration (6-week-old mouse, 45-150 μg, continuous injection for 3 days, test- 24 hours after the last injection) is suitable for tissues with rich blood flow such as heart, liver, kidney, lung, tumor tissues, etc.

Respiratory administration: suitable for the respiratory system.

Intraperitoneal administration: suitable for internal organs in the abdominal cavity such as pancreas, spleen, kidney, ovary, etc.

Intracranial administration: suitable for the central nervous system.

Local administration:

Sites that are difficult to reach by systemic administration, such as epidermis, subcutaneous (tumor), uterine cavity, etc. For example, tumor cell lines are used to establish subcutaneous xenografts in nude mice.

1) Injection when the tumor was up to 4 mm x 4 mm;
2) Agomir was dissolved in high pressure sterilized phosphate buffer, 10% glucose solution, or normal saline;
3) The range of each injection was 1-10 nmol (0.5-4 OD) for 2-4 weeks, twice a week. The detection was performed 2-4 weeks after the first injection.
4) The single injection dose depends on the size of tumor tissue, and the injection volume is about 30-100 μL;
5) Multi-point injection is used in tumor tissue. 3-4 sites are injected evenly in tumor tissue.
6) The experimental results were evaluated after injection.

Modification methods: agomir was chemically modified only on the antisense chain, with 3' end cholesterol modified, 5' end two thio modified, 3' end 4 thio modified, antisense chain full base methylation modified.
mmu-miR-140-3p Agomirs
Sense: 5' UACCACAGGGUAGAACCACGG 3'
Antisense: 5'AsCsGUGGUUCUACCCUGUGGUAUUAsAsUsUs-Chol-3'

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