Chemically Modified miRNA Mimic

User's Instruction

Description

Chemically Modified miRNA Mimic is double-stranded small RNA molecules designed according to the sequence of mature miRNA *in vivo*, which is used to simulate the sequence of endogenous mature miRNA. Chemically Modified miRNA Mimic includes a sequence consistent with the target mature miRNA sequence and a sequence complementary to the mature miRNA. Specific Chemically Modified miRNA Mimic can be introduced into the corresponding miRNA cells to simulate the effect of miRNA, or combine with the Dual Luciferase Reporter Assay System with miRNA binding sites to verify the regulatory relationship between miRNA and target genes.

Features

- Compared with the common miRNA mimic, Chemically Modified miRNA Mimic shows higher affinity to the cell membrane, thus the amount of transfection reagent required in cell transfection experiments is significantly reduced.
- It is particularly suitable for experiments in animals. Moreover, it has higher stability in *in vivo* experiments, and can be used in many ways such as systemic injection or local injection with easy operation.
- Can be enriched in target cells to achieve high specific and stable activity.
- Active for at least a week and may even extend to 5-6 weeks.

Applications

- Use Chemically Modified miRNA Mimic to study gain-of-function effect
- Screening for miRNAs that regulate gene expression and influence cellular developmental processes
- Study the role of miRNA in biological processes, such as cell development, proliferation, differentiation and apoptosis
- Discovery and validation of endogenous miRNA targets

Analysis

Analysis of mRNA expression level

- In order to analyze the up regulation level of miRNA, Chemically Modified miRNA Mimic can be transfected into cells and detected by Northern Blot and qPCR.

Analysis of target gene expression level

- By qPCR and Western Blot of intracellular mRNA target genes transfected with Chemically Modified miRNA Mimic and negative control sequence, the protein expression levels of target gene can be detected, which can verify the regulatory relationship between miRNAs and target genes.

Analysis of miRNA 3 'UTR target sites

- By constructing one or more predicted miRNA 3' UTR binding sites onto reporter plasmid as well as cotransfecting cells with Chemically Modified miRNA Mimic and reporter plasmid, Chemically Modified miRNA Mimic can repress reporter gene on the plasmid, which allows direct examination of the functional relationship between predicted miRNA binding sites and Chemically Modified miRNA Mimic. We recommend using the Chemically Modified miRNA Mimic negative control as a reference. The concentration and the transfection conditions of the negative control are same as the experimental groups.

Protocol

Transfection efficiency varies for different cell lines and different transfection reagents. The optimal transfection conditions still need to be determined experimentally. We suggest that the concentration range for optimization shall be set between 1-100 nM.

	96 Well Plate	24 Well Plate	12 Well Plate	6 Well Plate
Transfection Reagent	0.3-1.0 μl	1-3 μl	2-4 μl	3–6 μl
Chemically Modified miRNA Mimic	3 pmol	15 pmol	30 pmol	75 pmol
Cell Density	6,000 cells/well	40,000 cells/well	80,000 cells/well	200,000 cells/well

Final Volume/Well	0.1 ml	0.5 ml	1.0 ml	2.5 ml

Note:

- The recommended amount of transfection reagent should be adjusted according to the reagent you are using.
- We recommend you to optimize the amount of miRNA according to your cell type.
- The cell density value depends on the cell size and growth. General we recommend cell confluency at 30-70%.

Optimization

Optimization of transfection efficiency is one of the most critical factors to maximize the activity of Chemically Modified miRNA Mimic. For each transfection reagent, the transfection efficiency can be optimized mainly from the following aspects:

- Quantity of transfection reagent
- Quantity of Chemically Modified miRNA Mimic
- Cell density at transfection
- Operation sequence of transfection
- Contact time of cells with transfection reagent-siRNA complex

Storage

Store at -20°C or -80°C for long term.