



microRNA Mimics, Agomirs, Inhibitors and Antagomirs

Store at -20°C		
Cat. No.	Description	Quantity
MCHXXXXX	Human miRNA Mimic	2 x 2.5 nmol
MCMXXXXX	Mouse miRNA Mimic	2 x 2.5 nmol
MCRXXXXX	Rat miRNA Mimic	2 x 2.5 nmol
MAHXXXXX	Human miRNA Agomir	2 x 2.5 nmol
MAMXXXXX	Mouse miRNA Agomir	2 x 2.5 nmol
MARXXXXX	Rat miRNA Agomir	2 x 2.5 nmol
MIHXXXXX	Human miRNA Inhibitor	2 x 5.0 nmol
MIMXXXXX	Mouse miRNA Inhibitor	2 x 5.0 nmol
MIRXXXXX	Rat miRNA Inhibitor	2 x 5.0 nmol
MNHXXXXX	Human miRNA Antagomir	2 x 5.0 nmol
MNMXXXXX	Mouse miRNA Antagomir	2 x 5.0 nmol
MNRXXXXX	Rat miRNA Antagomir	2 x 5.0 nmol

Description

Synthetic microRNA Mimics and Agomirs are double-stranded miRNA-like RNA which are designed to copy the functionality of mature endogenous miRNA upon transfection. Synthetic microRNA Inhibitors and Antagomirs are complementary, antisense single-stranded oligonucleotides to their target endogenous mature miRNA. Inhibitors and Antagomirs effectively prevent the target miRNA to bind to normal cellular binding sites.

Agomirs differ from Mimics in that they are chemically-modified to contain (i) 2'-methoxy throughout the entire antisense strand, (ii) 2 phosphorothioates at the 5' end, and (iii) 4 phosphorothioates plus 4 cholesterol moieties at the 3' end. Antagomirs are single-strand miRNA inhibitors carrying the same chemical modifications. Agomirs and Antagomirs exhibit enhanced transfection efficiency and increased resistance to various RNases.

Transfection of synthetic miRNA followed by downstream gene expression analysis or phenotypic analysis is performed to elucidate the targets and roles of particular miRNAs.

Reconstitution Procedure

We recommend re-suspending the lyophilized synthetic miRNA using DNase and RNase-free ddH₂O. To make a 100 µM stock, dissolve the lyophilized powder using 25 µl of ddH₂O (for mimics and agomirs) or 50 µl of ddH₂O (for inhibitors and antagomirs).

Storage Conditions

Store at -20°C. Avoid freeze-thaw cycles after reconstitution.

Transfection Protocol

- Cell Seeding (Day Before Transfection):** Seed cells in 500 µl of antibiotic-free growth medium 24 hours prior to transfection, targeting 30-50% confluency at the time of transfection. Seeding at lower densities extends the window between transfection and downstream assays while reducing cell viability loss from overgrowth.
- Preparation of siRNA-Lipid Complexes:** Prepare the following for each transfection sample:

Solution A: Dilute 20 pmol of miRNA oligomer into 50 µl of serum-free or reduced serum medium. Mix by gentle pipetting. (Note: final RNA concentration upon addition to cells will be approximately 33 nM.)

Solution B: Combine 1 µl of DNAfectin™ Plus Transfection Reagent (Cat. No. G2500) with 50 µl of serum-free or reduced serum medium. Mix gently and allow to stand at room temperature for 5 minutes. (Note: Gently mix the transfection reagent before use.)

Complex Formation: After the 5-minute incubation, combine Solution A and Solution B. Mix gently and incubate at room temperature for an additional 20 minutes. A slight cloudiness in the mixture is normal.
- Transfection:** Add the prepared siRNA-lipid complexes directly to each well containing cells in media. Gently rock the plate several times to ensure even distribution.
- Incubation and Assay:** Incubate cells at 37°C with CO₂ for 24-96 hours, depending on your downstream application (e.g., gene knockdown analysis). If desired, media may be replaced 4-6 hours post-transfection.

Scaling Transfection Volumes

When working with tissue culture vessels of different sizes, adjust the amounts of transfection reagent, nucleic acid, cells, and media proportionally based on the relative surface area of each format, as outlined in the table below.

For automated or high-throughput workflows using 96-well plates, a complex solution volume of 50 µl per well is recommended.

Table 1: Reagent quantities for different culture vessels

Culture Vessel	Surface Area (cm ²)	Medium Volume	Transfection Complex Vol. (Solution A + B)	miRNA Oligomer	DNAfectin™ Plus (Cat. G2500)
96-well	0.3	100 µl	2 x 25 µl	5 pmol	0.25 µl
24-well	2	500 µl	2 x 50 µl	20 pmol	1.0 µl
12-well	4	1 ml	2 x 100 µl	40 pmol	2.0 µl
6-well	10	2 ml	2 x 250 µl	100 pmol	5.0 µl
6-cm dish	20	5 ml	2 x 500 µl	200 pmol	10 µl
10-cm dish	60	15 ml	2 x 1.5 ml	600 pmol	30 µl