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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/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 ddH_2O. To make a 100 µM stock, dissolve the lyophilized powder using 25 µl of ddH_2O (for mimics and agomirs) or 50 µl of ddH_2O (for inhibitors and antagomirs).

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

Transfection protocol
Use the following conditions as guidelines to transfect mammalian cells in a 6-well or 35mm dish format. For other culture vessels, please refer to Table 1. The optimal concentration for synthetic miRNA is cell-line dependent and should be determined experimentally.

1. Eighteen to twenty-four hours prior to transfection, seed cells at a density of 1-3 x 10^5 cells per well in 2.0 ml of appropriate growth medium (with serum and antibiotics if normally required). Incubate the cells at 37˚C in a CO_2 incubator until cells are 70% to 90% confluent at the time of transfection.

   Suspension Cells: Just prior to preparing complexes, plate 3-5 x10^5 cells in 0.8 ml of serum-free medium without antibiotics.

2. For each transfection sample, prepare the complexes in sterile micro-centrifuge tubes as follows:

   Solution A: For a 6 well plate, in 125 µl of serum free, antibiotic-free medium, dilute synthetic miRNA to a concentration of 42-840 nM for each well. For other dish formats, refer to Table 1. The final miRNA concentration in each well is generally 5–100 nM.

   Solution B: Mix RNAifectin™ Transfection Reagent (**abm** Cat. No. G073) thoroughly prior to use, then dilute 4-10 µl of reagent in 125 µl serum-free, antibiotic free medium.

   Incubate solution A and B separately at room temperature for 5 minutes.

3. Combine solutions A and B, mix thoroughly to ensure uniform distribution and incubate for 20 minutes at room temperature to allow RNA/oligo-liposome complexes to form.

4. ***Adherent cells ONLY*** *(For suspension cells, go directly to step 5b):*
   Add 0.8 ml of serum-free, antibiotic-free medium to the RNAifectin™ -RNA/oligo complex. Mix solution gently.

5a. *Adherent cells:* Remove growth medium from the cells and add 1.0 ml of RNAifectin™ -RNA/oligo solution to the each well containing cells. Incubate at 37˚C.

5b. *Suspension cells:* Add 0.2 ml of the RNAifectin™ -RNA/oligo solution into each well containing suspension cells in 0.8 ml serum-free, antibiotic-free medium. Incubate at 37˚C.

6. After 5-8 hours, remove transfection solution and add 2.0 ml of the appropriate plating medium (with serum and antibiotics) or add 0.1 ml of FBS directly into each well. Incubate the cells at 37˚C.

7. After 6-72 hours, monitor and assay gene expression (optimal incubation time may vary).

Table 1: Reagent quantities for different culture vessels

Culture Vessel	Surface area per well (cm^2)	Synthetic miRNA (nM) in serum-free medium (µl) per well	RNAfectin™ in serum-free medium (µl)	Transfection medium vol.	Volume of plating medium
24-well	2	90-1800 nM in 25 µl	2-4 µl in 25 µl	0.4 ml	500 µl
12-well	4	40-800 nM in 100 µl	5-8 µl in 100 µl	0.6 ml	1 ml
6-well	10	42-840 nM in 125 µl	4-10 µl in 125 ul	0.8 ml	2 ml
35mm	10	42-840 nM in 125 µl	4-10 µl in 125 µl	0.8 ml	2 ml
60mm	20	34-680 nM in 500 µl	12-30 µl in 500 µl	2.4 ml	5 ml
100mm	60	31.3-3133 nM in 1.5 ml	32-80 µl in 1.5 ml	6.4 ml	10 ml