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USER MANUAL for BIONEER's *AccuTarget*™ miRNA Mimics and Inhibitors

MicroRNA overview

MicroRNAs (miRNAs) are 21-25 nucleotide (nt)-long single-stranded RNA molecules that serve as a post-transcriptional regulator of gene expression in eukaryotes. The human genome may encode over 1000 miRNAs, which bind with imperfect complementarity to their target mRNAs, generally within the 3'UTR (untranslated region), and repress protein production by destabilizing the mRNA as well as translational suppression. miNRA-mediated translational repression has important roles in wide range of biological process, including development, cell proliferation and differentiation, apoptosis and metabolism.

Product Description

Bioneer's *AccuTarget*™ miRNA mimics are chemically synthesized, double-stranded RNA oligonucleotides and are available for 875 Human Mature microRNAs in the miRBase Sequence Database. *AccuTarget*™ miRNA inhibitors are the single-stranded synthetic inhibitor targeting all human miRNAs in the miRBase Sequence Database. These miRNA mimics & inhibitors are available at 5, 10 and 20 nmole guaranteed yield. We also offer miRNA mimics and inhibitors library sets consisting of predesigned mimics or inhibitors at various small scaled (0.25, 0.5, 1, or 2 nmole) in a 96-wel plate layout to meet the unique needs of individual customers. In addition, flexible miRNA library sets for customer-specified mimics & inhibitors are also available for the minimum order of 48 ea.

Shipping and storage

AccuTarget™ mimics and inhibitors are shipped as dried pellets and upon arrival should either be stores dry at ≤-20℃, or resuspended immediately per the protocol below.

Handling recommendations

- Wear gloves and a mask when handling *AccuTarget*™ miRNAs mimics and inhibitors.
- Always use RNase free tubes and pipette tips
- Only use DEPC treated (or RNase free) water when diluting *AccuTarget*™ miRNA mimics and inhibitors.

Quality Control

All Bioneer miRNA inhibitors are provided as single-stranded miRNA (antisense strand of target miRNA) and all Bioneer miRNA mimics are provided as double-stranded siRNA. Each sense siRNA and an antisense siRNA are QC'ed by MALDI-TOF analysis. Every annealed double-stranded miRNA is then QC-tested using non-denaturing PAGE to confirm proper annealing.

(1) Resuspention Protocol

1. Briefly centrifuge tubes (or multi-well plates) containing miRNA mimic or inhibitor to ensure that the miRNA pellet is located at the bottom of the tube.

2. Dissolve miRNAs to a convenient stock concentration using the recommended volume of DEPC-DW (or RNase-free water) shown in Table 1.

3. Pipette the solution up and down 3-5 times (or vortex briefly)

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4. Briefly centrifuge tubes (or multi-well plates) containing miRNA to ensure that the solution is collected at the bottom of the tube.

5. Aliquot the miRNAs into small volumes and sotre at ≤20℃. miRNA is stable (for 1 year under the specified storage condition). For best results, limit freeze-thaw events for each tube no more than five times.

Table1. Recommended miRNA resuspension Volumes and Concentrations

miRNA Amount (nmol)	DEPC-DW volume(μl) for desired final concentartion	
	100 μM stock	20 μM stock
1.0	10	50
5.0	50	250
10.0	100	500
20.0	200	1000

(2) Transfection Protocol

*** We used the Lipofectamine™ RNAiMAX (invitrogen; Cat.No. 13778) and Hela cell for our miRNA validation.**

*** This protocol is optimized for transfection in a 6-well culture plate format (To perform transfection in different cell culture formats, refer to Table2 and invitrogene Lipofectamine™ RNAiMAX protocol.**

1. One day before transfection, plate 2.0 X 10^5 Hela cells in each well with 2ml of growth medium without antibiotics so that they will be 50-60% confluent at the time of transfection.

2. Remove the growth medium from the cells.

3. Add the 1ml fresh growth medium without serum.

4. For each well to be transfected, prepare miRNA-Lipofectamine™ RNAiMAX complexes as follows.

4-1. Dilute miRNA in 500μl growth medium (or Opti-MEM® I Reduced Serum medium) without serum to make a final concentration of 5nM-100nM. Mix gently by vortex.

4-2. Mix Lipofectamine™ RNAiMAX gently before use, then dilute 3.5μl in 500μl medium (or Opti-MEM® I Reduced Serum medium) without serum. Incubate for 5 minutes at room temperature.

4-3. Combine the diluted siRNA duplex with the diluted Lipofectamine™ RNAiMAX. Mix and incubate for 20 minutes at room temperature.

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5. Add the mixture to each well containing HeLa cells. The final volume in each well is 2ml. Mix gently by hnad rocking the plate back and forth.

6. Incubate the cells for 5-6 hours at 37℃ in CO_2 incubator.

7. Change the medium with a fresh one containing serum and incubate the cells 24-48 hours until you are ready to assay for miRNA functional studies.

Table2. the relative surface area of in vitro cell culture dish and culture media volume

Culture Vessel	Relative surface area	Volume of plating medium
96-well	0.2	100μl
48-well	0.4	200μl
24-well	1	500μl
6-well	5	2.5ml
60nm	10	5ml
100nm	30	10ml

Support

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