









Pre-miR™ miRNA Precursor Molecules— Negative Control #1

	Package Contents	Catalog Number AM17110	Size 5 nmol lyophilized pellet
<ul style="list-style-type: none"> 1.75 mL Nuclease-free Water 			
	Storage Conditions	<ul style="list-style-type: none"> Store at or below -20°C. Do not store in a frost-free freezer. (Dried oligonucleotides are shipped at room temperature.) 12-month shelf life 	
	Required Materials	<ul style="list-style-type: none"> RNase-free reagents Transfection reagent e.g. Lipofectamine® RNAiMAX 	
	Timing	Transfection preparation: 15 minutes Final incubation: 1–3 days	
	Selection Guide	miRNAs Go online to view related products.	
	Product Description	<ul style="list-style-type: none"> Pre-miR™ miRNA Precursors are chemically modified to minimize off target effects and designed to mimic endogenous miRNAs. Pre-miR™ miRNA Precursor—Negative Control #1 is a double-stranded RNA oligonucleotide designed to serve as a negative control for experiments involving Pre-miR™ miRNA Precursors. 	
	Important Guidelines	<ul style="list-style-type: none"> Handling instructions: RNA oligonucleotides are susceptible to degradation by exogenous ribonucleases introduced during handling. Wear gloves when handling this product. Use RNase-free reagents, tubes, and barrier pipette tips. Transfect Pre-miR™ miRNA Precursor—Negative Control #1 using the same methodology as for your experimental Pre-miR™ miRNA Precursors. Then, use the target gene expression from the sample transfected with the Pre-miR™ miRNA Precursor—Negative Control #1 as a baseline for evaluation of the effect of the experimental Pre-miR™ miRNA Precursor on target gene expression. 	
	Online Resources	Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support .	



For Research Use Only. Not for use in diagnostic procedures.


miRNA Resuspension Protocol

We recommend preparing 100 μM miRNA stock solution. Dilute the stock solution to 10 μM for immediate use.

- Briefly centrifuge the tube or plate to ensure that the dried miRNA is at the bottom of the tube.
- Resuspend the 5 nmol miRNA using 50 μL of the nuclease-free water provided for a final concentration of 100 μM .
- Make 10 μM working stock using nuclease-free water for immediate use. A 10- μM stock of miRNA duplex is equivalent to 10 pmol/ μL .
- (Optional) Aliquot miRNAs into one or more daughter tubes or plates to limit the number of freeze-thaw cycles to which the miRNAs are subjected. Solutions at concentrations $>2 \mu\text{M}$ can undergo up to 50 freeze-thaw cycles without significant degradation.
- Store at or below -20°C in a non-frost-free freezer until use.

Once reconstituted in nuclease-free water, the miRNA is ready to transfect and can be used at your choice of final concentration.

RNAi Transfection Protocol

 See page 2 to view guidelines for transfecting miRNAs using Lipofectamine® RNAiMAX Reagent.

Transfection Amounts per Well

Use 10 nM miRNA duplex as a starting point.

	96-well	24-well	6-well
Final miRNA	1 pmol	5 pmol	25 pmol
Final Lipofectamine® RNAiMAX	0.3 μL	1.5 μL	7.5 μL

Reverse Transfection of RNAi

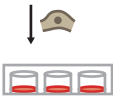


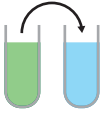

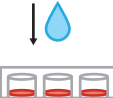

Reverse transfection is faster to perform than forward transfection and is the method of choice for high-throughput transfection. Perform reverse transfection by preparing siRNA or miRNA transfection complexes inside the wells, and then adding cells and medium. Because the cells and miRNA-reagent complexes are prepared on the same day, we recommended using 2.5 \times more cells than for a regular transfection.

 **Limited Product Warranty and Disclaimer Details**

RNAi Transfection Protocol

This procedure is designed for one RNA amount combined with one amount of Lipofectamine® RNAiMAX.

The prepared mix is enough to have triplicates (96-well), duplicates (24-well), and single well (6-well) transfections, and account for pipetting variations.

Timeline			Steps	Procedure Details			
Day 0	1		Seed cells to be 60-80% confluent at transfection	Component	96-well	24-well	6-well
	2		Dilute Lipofectamine® RNAiMAX Reagent in Opti-MEM® Medium	Adherent cells	1–4 × 10 ⁴	0.5–2 × 10 ⁵	0.25–1 × 10 ⁶
	3		Dilute miRNA in Opti-MEM® Medium	Opti-MEM® Medium	25 µL	50 µL	150 µL
Day 1	4		Add diluted miRNA to diluted Lipofectamine® RNAiMAX Reagent (1:1 ratio)	Lipofectamine® RNAiMAX Reagent	1.5 µL	3 µL	9 µL
	5		Incubate	Opti-MEM® Medium	25 µL	50 µL	150 µL
	6		Add miRNA-lipid complex to cells	miRNA (10 µM)	0.5 µL (5 pmol)	1 µL (10 pmol)	3 µL (30 pmol)
				Diluted miRNA	25 µL	50 µL	150 µL
Day 2–4	7		Visualize/analyze transfected cells	Diluted Lipofectamine® RNAiMAX Reagent	25 µL	50 µL	150 µL
				Incubate for 5 minutes at room temperature.			
				Component	96-well	24-well	6-well
				miRNA-reagent complex per well	10 µL	50 µL	250 µL
				Final miRNA used per well	1 pmol	5 pmol	25 pmol
				Final Lipofectamine® RNAiMAX used per well	0.3 µL	1.5 µL	7.5 µL
				Incubate cells for 1–3 days at 37°C. Then, analyze transfected cells.			