Pre-miR™ miRNA Precursor Molecules—Negative Control #2

Package Contents

Catalog Number	Size
AM17111	5 nmol lyophilized pellet

- 1.75 mL Nuclease-free Water

Storage Conditions

- 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

- 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

- Pre-miR™ miRNA Precursors are chemically modified to minimize off target effects and designed to mimic endogenous miRNAs.
- Pre-miR™ miRNA Precursor—Negative Control #2 is a double-stranded RNA oligonucleotide designed to serve as a negative control for experiments involving Pre-miR™ miRNA Precursors.

Important Guidelines

- 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 #2 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 #2 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.

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Print Options

miRNA Resuspension Protocol

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

1. Briefly centrifuge the tube or plate to ensure that the dried miRNA is at the bottom of the tube.
2. Resuspend the 5 nmol miRNA using 50 µL of the nuclease-free water provided for a final concentration of 100 µM.
3. 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.
4. (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 µM can undergo up to 50 freeze-thaw cycles without significant degradation.
5. 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× more cells than for a regular transfection.

Limited Product Warranty and Disclaimer Details

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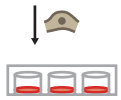
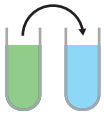
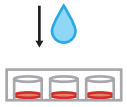
RNAi Transfection Protocol

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
Day 0	1 	**Seed cells to be 60-80% confluent at transfection**
	2 	**Dilute Lipofectamine® RNAiMAX Reagent in Opti-MEM® Medium**
	3 	**Dilute miRNA in Opti-MEM® Medium**
Day 1	4 	**Add diluted miRNA to diluted Lipofectamine® RNAiMAX Reagent (1:1 ratio)**
	5 	**Incubate**
	6 	**Add miRNA-lipid complex to cells**
Day 2–4	7 	**Visualize/analyze transfected cells**

Procedure Details

Component	96-well	24-well	6-well
Adherent cells	1–4 × 10^4	0.5–2 × 10^5	0.25–1 × 10^6
Opti-MEM® Medium	25 µL	50 µL	150 µL
Lipofectamine® RNAiMAX Reagent	1.5 µL	3 µL	9 µL
Opti-MEM® Medium	25 µL	50 µL	150 µL
miRNA (10 µM)	0.5 µL (5 pmol)	1 µL (10 pmol)	3 µL (30 pmol)
Diluted miRNA	25 µL	50 µL	150 µL
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.			