









# mirVana™ miRNA Inhibitors

	<b>Package Contents</b>	<b>Catalog Number</b> 4464066 <b>Size</b> 5 nmol lyophilized pellet <ul style="list-style-type: none"> <li>1.75 mL Nuclease-free Water</li> </ul>
	<b>Storage Conditions</b>	<ul style="list-style-type: none"> <li>Store at or below <math>-20^{\circ}\text{C}</math>. Do not store in a frost-free freezer. (Dried oligonucleotides are shipped at room temperature.)</li> <li>12-month shelf life</li> </ul>
	<b>Required Materials</b>	<ul style="list-style-type: none"> <li>RNase-free reagents</li> <li>Transfection reagent e.g. Lipofectamine<sup>®</sup> RNAiMAX</li> </ul>
	<b>Timing</b>	Transfection preparation: 15 minutes Final incubation: 1–3 days
	<b>Selection Guide</b>	<a href="#">miRNAs</a> Go online to view related products.
	<b>Product Description</b>	<ul style="list-style-type: none"> <li>mirVana™ miRNA Inhibitors are small, chemically modified, single-stranded RNA molecules designed to specifically bind to and inhibit endogenous miRNA molecules and enable miRNA functional analysis by down-regulation of miRNA activity.</li> <li>mirVana™ miRNA Inhibitors display better potency than any other competitor product. They offers maximum and consistent effect in vitro at low miRNA inhibitor concentration, and can be used in vitro and in vivo, offering consistency throughout your entire research project</li> </ul>
	<b>Important Guidelines</b>	<ul style="list-style-type: none"> <li>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.</li> <li>Transfection efficiency varies according to the cell type and transfection agent used. Determine the optimal transfection conditions that result in maximum miRNA inhibitor-mediated activity with minimal cytotoxicity. Maintain optimal transfection conditions for your cell type across experiments, and include controls in all plates.</li> <li>Transfect mirVana™ miRNA Inhibitors using the same methodology as for your experimental miRNA inhibitors.</li> </ul>
	<b>Online Resources</b>	Visit our <a href="#">product page</a> for additional information and protocols. For support, visit <a href="http://www.lifetechnologies.com/support">www.lifetechnologies.com/support</a> .




## miRNA Resuspension Protocol

We recommend preparing 100  $\mu\text{M}$  miRNA stock solution. Dilute the stock solution to 10  $\mu\text{M}$  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  $\mu\text{L}$  of the nuclease-free water provided for a final concentration of 100  $\mu\text{M}$ .
- Make 10  $\mu\text{M}$  working stock using nuclease-free water for immediate use. A 10- $\mu\text{M}$  stock of miRNA duplex is equivalent to 10 pmol/ $\mu\text{L}$ .
- (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  $-20^{\circ}\text{C}$  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<sup>®</sup> 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 <sup>®</sup> RNAiMAX	0.3 $\mu\text{L}$	1.5 $\mu\text{L}$	7.5 $\mu\text{L}$

## Reverse Transfection of RNAi

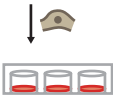


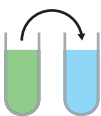

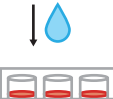

Reverse transfection is faster to perform than forward transfection and is the method of choice for high-throughput transfection. Perform reverse transfection by preparing siRNA or miRNA transfection complexes inside the wells, and then adding cells and medium. Because the cells and miRNA-reagent complexes 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
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	$1-4 \times 10^4$	$0.5-2 \times 10^5$	$0.25-1 \times 10^6$
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-lipid 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.			