# mirVana™ miRNA Mimics

## Package Contents

| Catalog Number | Size |
|---|---|
| 4464070 | 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

- mirVana™ miRNA mimics are small, chemically modified, double-stranded RNAs that mimic endogenous miRNAs and enable miRNA functional analysis by up-regulation of miRNA activity.
- mirVana™ miRNA Mimics exhibit maximum and consistent effect in vitro at low concentration. They offer superior specificity due to unique Star strand modification, and can be used in vitro and in vivo, offering consistency throughout your entire research project.

## 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.
- Transfection efficiency varies according to the cell type and transfection agent used. Determine the optimal transfection conditions that result in maximum miRNA mimic-mediated activity with minimal cytotoxicity. Maintain optimal transfection conditions across experiments for your cell type, and include controls in all plates for each experiment to ensure consistency.
- Transfect mirVana™ miRNA Mimics using the same methodology as for your experimental miRNA duplexes.

## Online Resources

Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.

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## 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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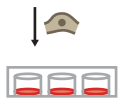
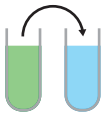
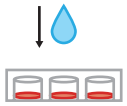
# RNAi Transfection Protocol

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-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. | | | |