

Anti-miR™ miRNA Inhibitors

Insert PN 4457176 Rev. A

Note: For all reagents, read the Safety Data Sheet (SDS) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product information

Ambion® Anti-miR™ miRNA Inhibitors are designed to bind to, and inhibit the activity of, endogenous miRNAs when introduced into cells. Endogenous mature miRNAs function as part of the RNA-induced silencing complex (RISC), which has the capacity to post-transcriptionally regulate mRNAs that have sequences with partial complementarity to the bound miRNA. Anti-miR miRNA Inhibitors are single-stranded RNA-based inhibitors that are chemically modified to improve their activity and to increase their stability. Use Anti-miR miRNA Inhibitors to enable detailed study of miRNA biological effects through loss-of-function experiments.

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.

Storage of dried oligonucleotides: Store at 4 °C, or in a non-frost-free freezer at or below –20 °C (dried oligonucleotides are shipped at ambient temperature). For long-term storage, store at or below –20 °C in a non-frost-free freezer.

Resuspension instructions

For best results, prepare the Anti-miR miRNA Inhibitor stock solution at the highest concentration that is workable for your experiments. As needed, dilute the stock for immediate use.

1. Briefly centrifuge the tube to ensure that the dried oligonucleotide is at the bottom of the tube.
2. Resuspend the Anti-miR miRNA Inhibitor at a convenient concentration, for example 50 µM. A calculator for suspension of dry oligonucleotides is available at:

www4.appliedbiosystems.com/techlib/append/oligo_dilution.html

Storage of resuspended Anti-miR miRNA Inhibitor: Store at or below –20 °C. Storage in a frost-free freezer is not recommended. Stock solutions at concentrations ≥2 µM can be stored at –20 °C for extended periods (up to 1 year).

Using Anti-miR miRNA Inhibitors to analyze endogenous miRNA function

For the following applications, use a cell line that expresses relatively high levels of the mature miRNA of interest.

Using endogenous miRNA targets

To analyze the effects of the miRNA on a biological process or on an endogenous target, transfect an Anti-miR miRNA Inhibitor into a cell line expressing an miRNA target. See “[Guidelines for transfection](#)” below for general transfection procedure information.

Using an miRNA target reporter plasmid

Using a reporter plasmid with predicted binding sites for an miRNA of interest, assess the ability of an Anti-miR miRNA Inhibitor to relieve inhibition of reporter gene expression by an endogenous miRNA.

1. Clone the binding site for an miRNA of interest into the 3' UTR of a reporter gene, for example, using the pMIR-REPORT™ Kit vector, PN AM5795.
2. Using a cell line that expresses the corresponding miRNA, cotransfect the reporter plasmid and the Anti-miR miRNA Inhibitor. To normalize for transfection variation, cotransfect a second plasmid expressing a different reporter, such as the pMIR-REPORT β-gal Control Plasmid included in the pMIR-REPORT Kit.
3. Evaluate the expression of the reporter gene 24–48 hr post-transfection.

General cotransfection conditions are described in [Table 2](#). See the pMIR_REPORT Kit protocol for a detailed procedure:

www4.appliedbiosystems.com/catalog/CatNum.php?5795

Guidelines for transfection

As with other small nucleic acids, such as siRNAs, the efficiency with which mammalian cells are transfected with Anti-miR miRNA Inhibitors depends on the cell type and transfection agent used. Determine the optimal transfection conditions that result in maximum Anti-miR miRNA Inhibitor-mediated activity with minimal cytotoxicity. Then:

- Maintain optimal transfection conditions from experiment to experiment for a given cell type.
- Include controls in all plates for each experiment to ensure consistency.

Determine optimal transfection conditions

Use your experimental cell line and appropriate positive and negative control Anti-miR miRNA Inhibitors.

1. Identify an effective transfection agent for your cell type.
2. Adjust, in order of importance:
 - a. Amount of transfection agent.
 - b. Amount of Anti-miR miRNA Inhibitor (and reporter plasmid, if applicable).
 - c. Cell density at the time of transfection. In general, 30 to 70% confluency is recommended.
 - d. Transfection method (traditional or reverse transfection).
 - e. Length of exposure of cells to transfection agent-RNA oligonucleotide complexes.

Many protocols recommend maintaining mammalian cells in the medium used for transfection for 48 hours. For many Anti-miR miRNA Inhibitors, maximal activity is achieved after 24 hours, and the existing medium can be replaced with fresh medium 24 hours after transfection, resulting in greater viability of the cells.

General transfection starting points

Anti-miR miRNA Inhibitors typically work best when transfected at a final concentration of 10 to 100 nM. To increase accuracy and reproducibility when preparing transfection complexes, prepare a dilution of your stock Anti-miR miRNA Inhibitor, and pipet a higher volume of diluted stock.

Table 1 provides general starting points for lipid-mediated reverse transfection of easily transfected mammalian cell lines, such as HeLa, with Anti-miR miRNA Inhibitors.

Table 1 General reverse transfection starting points

Plate format	Transfection agent (μL) [†]	Oligo. (pmol) [‡]	Cells/well	Total volume/well
96 wells	0.15–0.5	3	6,000	100 μL
24 wells	0.75–3	15	30,000	500 μL
12 wells	2–4	30	60,000	1.0 mL
6 wells	3–15	90	200,000	3.0 mL

[†] Lipofectamine® RNAiMAX Transfection Reagent recommended. Refer to the instructions provided with your transfection agent for the recommended volume.

[‡] Anti-miR miRNA Inhibitor. The amounts indicated result in a final oligonucleotide concentration of 30 nM.

Table 2 provides general starting points for lipid-mediated forward cotransfection of easily transfected cell lines, such as HeLa with Anti-miR miRNA Inhibitor, and target and control reporter plasmids.

Table 2 General cotransfection starting points

Plate format	Transfect. agent (μL) [†]	Oligo. (pmol) [‡]	Each reporter plasmid (ng)	Cells/well	Total volume/well
96 wells	0.2–0.5	4.2	40	10,000	140 μL
24 wells	1–3	16.5	200	40,000	550 μL
12 wells	2–4	45	400	80,000	1.5 mL
6 wells	5–15	105	1,000	250,000	3.5 mL

[†] Lipofectamine® 2000 Transfection Reagent recommended. Refer to the instructions provided with your transfection agent for the recommended volume.

[‡] Anti-miR miRNA Inhibitor. The amounts indicated result in a final oligonucleotide concentration of 30 nM.

Additional information

The siRNA Delivery Resource:

www4.appliedbiosystems.com/techlib/resources/delivery

The miRNA information resource and product guide:

www4.appliedbiosystems.com/miRNA

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