FAM™-labeled Anti-miR™ Negative Control

Store at or below –70°C.
Do not store in a frost-free freezer.

Catalog # (P/N):	AM17012
Product Description:	A carboxyfluorescein (FAM™)-labeled RNA oligonucleotide designed for monitoring uptake of Anti-miR™ miRNA Inhibitors by fluorescence microscopy or other fluorescence-based technique.
Amount:	5 nmol
Purity:	HPLC purified
Appearance:	Powder
Additional Material(s) Included:	1.75 mL Nuclease-free Water
Molecular Weight:	7709.2
Spectral Information:	**Dye-conjugated Anti-miR Negative Control**
	ε_{260} 239,160 L/(mol·cm)
	ε_{494} 75,000 L/(mol·cm)
	Excitation max (λ_{max}) 494 nm
	Emission max (λ_{max}) 520 nm
Storage Conditions:	Store at or below –70°C. ***Do not store in a frost-free freezer.*** The dried product is guaranteed for 1 year from the date of shipment, if properly stored.

USER INFORMATION

General Information:

Ambion® Anti-miR™ miRNA Inhibitors are RNA oligonucleotides designed to inhibit endogenous miRNAs. FAM™-labeled Anti-miR Negative Control is a nontargeting negative control that has the same sequence as Ambion Anti-miR miRNA Inhibitors—Negative Control #1 (P/N AM17010). It has a fluorescent moiety on the 5' end of the oligonucleotide, and is designed for monitoring transfection efficiency in transfection experiments using Anti-miR miRNA Inhibitors.

The fluorescent label enables direct observation of cellular uptake, distribution, and localization of labeled Anti-miR Negative Controls. Their most common application is to monitor transfection efficiency during optimization of transfection conditions.

Transfect the FAM-labeled Anti-miR Negative Control using the same methodology as for your experimental Anti-miR miRNA Inhibitors.

Cells transfected with labeled Anti-miR Negative Controls can be examined by methods such as fluorescence microscopy, confocal microscopy, or flow cytometry. For observation of FAM-labeled oligonucleotides by fluorescence microscopy, a fluorescein or GFP filter can be used.

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.

Dried dye-labeled RNA oligonucleotides may be safely stored at –70°C for 1 year. ***Do not store in a frost-free freezer.***

Resuspension Instructions
Briefly centrifuge the tube to ensure that the dried oligonucleotide is at the bottom of the tube. Resuspend the oligonucleotide at a convenient concentration. To minimize freeze-thaw cycles, we recommend preparing a concentrated stock, such as 50 µM, and then further diluting to a practical working stock concentration. (Resuspend 5 nmol of oligonucleotide in 100 µL of Nuclease-free Water to to obtain a 50 µM solution.)

Ambion provides an online calculator for suspension of dry oligonucleotides on its web site at
www.ambion.com/techlib/append/oligo_dilution.html

Once resuspended in Nuclease-free Water, the oligonucleotide is ready to transfect and can be used at your choice of final concentration (e.g., 1–100 nM).

Store the resuspended dye-labeled oligonucleotides at or below –70°C.

Applications:

Transfection Anti-miR Inhibitors into Mammalian Cells

General Transfection Information

In general, RNA oligonucleotides are taken up by cells via transfection and are distributed throughout the cell. Under optimal transfection conditions, cellular uptake of labeled RNA oligonucleotides is observable at final concentrations in the low nanomolar range, and uptake increases as final concentration increases, up to approximately 50–100 nM.

After chemical (e.g., lipid-mediated) transfection, it appears that labeled RNA oligonucleotides are actively taken up by endosomes. After several hours, a dotted perinuclear localization of the labeled oligonucleotide can be observed. In contrast, labeled RNA oligonucleotides delivered to cells by electroporation, a passive uptake process, produce an even, but less intense, glow throughout the cytoplasm. Because of its brighter signal, Cy3 dye is recommended over fluorescein derivatives such as FAM™ dye for use in electroporation applications.

Different transfection agents interact with RNA oligonucleotides in different ways. Some agents may associate strongly with the Anti-miR miRNA Inhibitor and sequester it, resulting in poor Anti-miR Inhibitor activity, despite good delivery of the Anti-miR miRNA Inhibitor to the cell.

General Transfection Starting Points for Anti-miR miRNA Inhibitors in Cultured Mammalian Cells

Optimization of Transfection with Anti-miR miRNA Inhibitors

As with other small nucleic acids, such as siRNAs and antisense oligonucleotides, the efficiency with which mammalian cells are transfected with Anti-miR miRNA Inhibitors will vary according to cell type and the transfection agent used. The optimal concentration used for transfections should be determined empirically. We have found that Anti-miR miRNA Inhibitors typically work best when transfected at a final concentration of 15–100 nM. However, a more extensive concentration range from 1–100 nM can be analyzed in optimization experiments. The following chart provides general starting points for transfection of Anti-miR Inhibitors into cultured adherent mammalian cells.

Plate Format	96 wells	24 wells	12 wells	6 wells
Transfection Agent [a]	0.3–1.0 µL	1–3 µL	2–4 µL	3–36 µL
Anti-miR miRNA Inhibitor [b]	3 pmol	15 pmol	30 pmol	90 pmol
Cell Density [c]	6,000 cells/well	40,000 cells/well	80,000 cells/well	240,000 cells/well
Final Volume per Well	100 µL	0.5 mL	1.0 mL	3.0 mL

a Refer to the instructions provided with your transfection agent for the recommended volume.

b The Anti-miR miRNA Inhibitor amount indicated results in a final Anti-miR miRNA Inhibitor concentration of 30 nM. The amount of Anti-miR miRNA Inhibitor required for maximal Anti-miR miRNA Inhibitor activity will vary among cell types. For a 96-well plate and 100 µL final transfection volume, 3 pmol of a 5 µM oligonucleotide solution is 0.6 µL. Robotic pipettors may require volumes of 2–5 µL for accurate pipetting. To increase pipetting volumes and accuracy when preparing transfection complexes, we recommend first making a plate with a dilution of your stock oligonucleotide.

c Optimal cell density will vary among cell types, depending on cell size and growth characteristics. In general, we recommend 30–70% confluency.

Transfection Optimization

Optimizing transfection efficiency is crucial for maximizing Anti-miR miRNA Inhibitor-mediated activity while minimizing cytotoxicity. Optimal transfection efficiencies are achieved by identifying an effective transfection agent for each cell type and by adjusting (in order of importance):

- Amount of transfection agent
- Amount of Anti-miR miRNA Inhibitor
- Cell density at the time of transfection
- Order of transfection (pre-plating cells or plating cells/transfecting in tandem)
- Length of exposure of cells to transfection agent/Anti-miR miRNA Inhibitor complexes

Most protocols recommend maintaining mammalian cells in the medium used for transfection; this avoids dilution or removal of RNA oligonucleotides from the cells by adding medium or washing the cells with new medium too soon after transfection. We have found that cells typically exhibit greater viability when existing medium is replaced with fresh medium 24 hours after transfection. Replacing medium after 24 hours generally does not change the activity of the transfected Anti-miR miRNA Inhibitor.

Once the conditions for optimal Anti-miR miRNA Inhibitor transfection are determined, they should be kept constant from experiment to experiment for a given cell type. Include controls in all plates for each experiment to ensure consistency.

For additional information about small RNA transfection, including transfection conditions for many cell types and optimization protocols, see the Ambion siRNA Delivery Resource at:
www.ambion.com/techlib/resources/delivery

Additional Information:

For protocols, background information, a reference, list, and miRNA research tools, see the Ambion miRNA information resource and product guide:
www.ambion.com/miRNA

RELATED PRODUCTS

Cy™3-labeled Anti-miR™ Negative Control
P/N AM17011
Monitor cellular uptake, distribution, and localization in transfection experiments that use Anti-miR™ miRNA Inhibitors.

siPORT™ *NeoFX*™ Transfection Agent
P/N AM4510 and AM4511
A versatile lipid-based agent for efficient and reproducible transfection of adherent cells while subculturing, without increased cytotoxicity.

Anti-miR™ miRNA Inhibitors and Controls
P/N AM17000, AM17001, AM17003, AM17010, AM17120, AM17121
Chemically modified and optimized nucleic acids designed to specifically inhibit microRNA (miRNA) molecules in cells.

Anti-miR™ miRNA Inhibitor Library - Human V3
P/N 4385914
An extensive collection of Anti-miR™ miRNA Inhibitors targeting human miRNAs listed in miRBase Sequence Database Version 9.2.

Pre-miR™ miRNA Precursors (patent pending), Controls, and Library
P/N AM17100, AM17101, AM17103, AM17110, AM17111, AM17150, 4385830
Chemically modified and optimized nucleic acids designed to mimic microRNA (miRNA) molecules in cells.

QUALITY CONTROL

Identity: The mass of a sample of the RNA oligonucleotide is analyzed using MALDI-TOF mass spectrometry and compared to the calculated mass.

Purity: Analytical HPLC of a sample of the purified RNA oligonucleotide is used to confirm ≥90% purity.

OTHER INFORMATION

Material Safety Data Sheets: Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: www.ambion.com/techlib/msds. Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery. For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

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