Cy™3-labeled Pre-miR™ Negative Control #1

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

Catalog # (P/N):	AM17120
Product Description:	A Cy™3-labeled double-stranded RNA oligonucleotide designed for monitoring uptake of Pre-miR™ miRNA Precursors by fluorescence microscopy or other fluorescence-based technique.
Amount:	5 nmol
Purity:	HPLC purified to ≥95% purity
Appearance:	Powder
Additional Material(s) Included:	1.75 mL Nuclease-free Water
Molecular Weight:	13806.8
Spectral Information:	**Dye-conjugated Pre-miR Negative Control**
	1 OD_{260} = 40 µg/mL
	Unconjugated dye
	Excitation max (λ_{max}) 547 nm
	Emission max (λ_{max}) 563 nm
	ε_{260} [L/(mol·cm)] 4,930 L/(mol·cm)
	ε_{547} [L/(mol·cm)] 136,000 L/(mol·cm)
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® Pre-miR™ miRNA Precursors (P/N AM17100, AM17101, AM17103; patent pending) are designed to mimic endogenous mature miRNAs. Cy™3-labeled Pre-miR™ Negative Control #1 is a nontargeting negative control that has the same sequence as Pre-miR™ miRNA Precursor—Negative Control #1 (P/N AM17110). It has a fluorescent moiety on the 5' end of one strand, and is designed for monitoring delivery efficiency in transfection experiments using Pre-miR miRNA Precursors.

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

Transfect Cy3-labeled Pre-miR Negative Control #1 using the same methodology as for your experimental Pre-miR miRNA Precursors.

Cells transfected with labeled Pre-miR Negative Controls can be examined by methods such as fluorescence microscopy, confocal microscopy, or flow cytometry. For observation of Cy3-labeled oligonucleotides by fluorescence microscopy, a Cy3 or tetramethyl rhodamine isothiocyanate (TRITC) 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.)

An online calculator for suspension of dry oligonucleotides is available at
www4.appliedbiosystems.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 of Pre-miR Precursors 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 Pre-miR miRNA Precursor and sequester it, resulting in poor Pre-miR miRNA Precursor activity, despite good delivery of the Pre-miR miRNA Precursor to the cell. Ambion Pre-miR hsa-miR-1 miRNA Precursor (P/N AM17150) is designed for use as a positive control for miRNA-mediated gene silencing.

Transfection Starting Points for Mammalian Cells

As with other small nucleic acids, such as siRNAs and antisense oligonucleotides, the efficiency with which mammalian cells are transfected with Pre-miR™ miRNA Precursors 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 Pre-miR miRNA Precursors typically work best when transfected at a final concentration of 3–30 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 Pre-miR miRNA Precursors into cultured mammalian cells.

General Transfection Starting Points for Pre-miR miRNA Precursors in Cultured Mammalian Cells

Plate Format	96 wells	24 wells	6 wells
Transfection Agent [a]	0.3–1.0 µL	1–3 µL	3–6 µL
Pre-miR miRNA Precursor [b]	3 pmol	15 pmol	75 pmol
Cell Density [c]	6,000 cells/well	40,000 cells/well	200,000 cells/well
Final Volume per Well	0.1mL	0.5 mL	2.5 mL

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

b The amount shown results in a final Pre-miR miRNA Precursor concentration of 30 nM. The amount of Pre-miR miRNA Precursor required for maximal Pre-miR miRNA Precursor 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 Pre-miR miRNA Precursor 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 and type of RNA oligonucleotide
- 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/Pre-miR miRNA Precursor 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 Pre-miR miRNA Precursor.

Once the conditions for optimal transfection efficiency are determined, they should be kept constant from experiment to experiment for a given cell type.

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/techlib/resources/miRNA/index.html.

RELATED PRODUCTS

FAM™-labeled Pre-miR™ Negative Control #1
P/N AM17121
Monitor cellular uptake, distribution, and localization in transfection experiments that use Pre-miR™ miRNA Precursors.

Pre-miR™ hsa-miR-1 miRNA Precursor
P/N AM17150
A positive control for miRNA gain-of-function experiments using Pre-miR™ miRNA Precursors.

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.

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.

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.

QUALITY CONTROL

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

Purity: Analytical HPLC of purified unlabeled and labeled single-stranded RNA oligonucleotides is used to confirm >95% purity and, where applicable, coupling of dye to nucleic acid.

Annealing: The annealed Pre-miR miRNA Precursor is analyzed by nondenaturing gel electrophoresis.

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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