Anti-miR™ hsa-let-7c miRNA Inhibitor

Store at or below –20°C.
Do not store in a frost-free freezer.

Catalog # (P/N):	4392431	
Product Description:	A modified RNA oligonucleotide designed to serve as a functional positive control for experiments involving Anti-miR™ miRNA Inhibitors.	
Amount:	5 nmol	
Appearance:	Powder	
Additional Material(s) Included:	1.75 mL Nuclease-free Water	
Anti-miR Target:	Sanger miRNA Accession#:	MI0000064 (human hsa-let-7c), MI0000559 (mouse mmu-let-7c-1), MI0000560 (mouse mmu-let-7c-2)
	Description:	hsa-let-7c, found in *Homo sapiens* and *Mus musculus*
	Mature miRNA Sequence:	UGAGGUAGUAGGUUGUAUGGUU
miRNA Gene Target Information:	Gene symbol:	HMGA2
	Full Gene Name:	High mobility group AT-hook 2
	Organism(s):	Human and Mouse
	RefSeq Number(s):	NM_003484 (human) and NM_010441 (mouse)
	Entrez Gene ID(s):	8091 (human) and 15364 (mouse)
	TaqMan® Assay(s):	HMGA2 TaqMan Gene Expression Assays Hs00171569_m1(Human) and Mm00780304_sH (Mouse) recommended (Applied Biosystems; not included)
Storage Conditions:	Store at or below –20°C. ***Do not store in a frost-free freezer.*** (Dried oligonucleotides are shipped at ambient temperature.)	

USER INFORMATION

General Information:

Ambion Anti-miR™ miRNA Inhibitors (patent pending) are small, chemically modified single-stranded RNA molecules designed to specifically bind to and inhibit the activity of endogenous mature miRNA molecules. They enable detailed study of miRNA biological effects via loss-of-function experiments. Anti-miR miRNA Inhibitors can be introduced into mammalian cells using chemical transfection or electroporation parameters similar to those used for siRNAs or Pre-miR™ miRNA Precursors.

Anti-miR™ hsa-let-7c miRNA Inhibitor provides a convenient, validated positive control for experiments using Anti-miR miRNA Inhibitors. Endogenous let-7 miRNA has been shown to negatively regulate HMGA2 mRNA in cultured cells. HMGA2 is a ubiquitously expressed, nonhistone, chromatin protein that can modulate gene expression through changes in chromatin architecture. Regulation of HMGA2 mRNA by let-7 miRNA is thought to occur through a base-pairing interaction in the 3' untranslated region of the HMGA2 message that results in mRNA cleavage [1, 2]. When transfected into human and mouse cell lines, Anti-miR hsa-let-7c miRNA Inhibitor blocks endogenous let-7c miRNA, resulting in increased levels of HMGA2 mRNA. Thus, Anti-miR hsa-let-7c miRNA Inhibitor activity can be monitored in human or mouse cells by real-time reverse transcription PCR (RT-PCR) targeting HMGA2 mRNA, using the TaqMan® Gene Expression Assays recommended above.

Scientists at Applied Biosystems, Austin, have shown that expression of HMGA2 mRNA increased by 100% following Anti-miR hsa-let-7c miRNA Inhibitor transfection in HeLa cells, compared to transfection with Anti-miR™ miRNA Inhibitors—Negative Control #1 (P/N AM17010), using real-time RT-PCR and the TaqMan Gene Expression Assay indicated above.

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. Upon receipt, store in a non-frost-free freezer at or below –20°C (dried oligonucleotides are shipped at ambient temperature).

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 reconstituted in Nuclease-free Water, the oligonucleotide is ready to transfect and can be used at your choice of final concentration.

Store the resuspended oligonucleotide at or below –20°C. ***Do not store in a frost-free freezer.***

Applications:

Positive Control for Anti-miR miRNA Inhibitor Transfection
Anti-miR hsa-let-7c miRNA Inhibitor can be used as a positive control when monitoring Anti-miR miRNA Inhibitor transfections, to confirm that the transfection procedure and cell cultures support Anti-miR miRNA Inhibitor activity.

We recommend transfection with nontargeting Anti-miR™ miRNA Inhibitors—Negative Control #1 as a baseline reference. The Anti-miR miRNA Inhibitors—Negative Control should be used at the same concentration as Anti-miR hsa-let-7c miRNA Inhibitor positive control and experimental Anti-miR miRNA Inhibitors, because nucleic acid concentrations within cells can affect the activity and specificity of miRNAs.

Anti-miR hsa-let-7c miRNA Inhibitor activity can be monitored in human and mouse cells using real-time RT-PCR and the recommended TaqMan® Gene Expression Assays targeting HMGA2. To calculate the change in HMGA2 gene expression, Applied Biosystems, Austin, scientists typically use the $\Delta\Delta C_T$ method [3], normalizing to an endogenous control RNA, such as 18S rRNA, and comparing to Anti-miR miRNA Inhibitors—Negative Control-transfected samples. We recommend determining Anti-miR hsa-let-7c miRNA Inhibitor activity 24 hr post-transfection when optimizing transfection conditions.

Optimization of Transfection Conditions for Anti-miR miRNA Inhibitors
Anti-miR hsa-let-7c miRNA Inhibitor is designed for use in development and optimization of transfection conditions for Anti-miR miRNA Inhibitors in adherent cultured mammalian cells. 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 hsa-let-7c miRNA Inhibitor into cultured adherent mammalian cells.

General Transfection Starting Points for Anti-miR hsa-let-7c miRNA Inhibitor in Cultured Mammalian Cells

Plate Format	96 wells	24 wells	12 wells	6 wells
Transfection Agent [a]	0.15–1.2 µL	0.5–4 µL	2–4 µL	3–36 µL
Anti-miR miRNA Inhibitor [b]	5 pmol	25 pmol	50 pmol	150 pmol
Cell Density [c]	8,000 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 50 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, 5 pmol of a 5 µM oligonucleotide solution is 1 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™ hsa-let-7c miRNA Inhibitor transfection are determined, they should be kept constant from experiment to experiment for a given cell type. However, your experimental Anti-miR miRNA Inhibitor targets or biological assays may require a post-transfection incubation period longer than the 24 hr recommended for monitoring Anti-miR hsa-let-7c miRNA Inhibitor activity.

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

References:

1. Lee YS, and Dutta A (2007) The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. *Genes & Dev.* **21**:1025–1030.
2. Mayr C, Hemann MT, Bartel DP (2007) Disrupting the pairing between let-7 and Hmga2 enhances oncogenic transformation. *Science* **315**:1576-1579.
3. *Real-Time PCR Systems: Applied Biosystems 7900HT Fast Real-Time PCR System and 7300/7500 Real-Time PCR Systems Chemistry Guide (Part #4348358).* Search by Part # at **www3.appliedbiosystems.com/sup/gl/search.htm** (Applied Biosystems Product & Service Literature).

RELATED PRODUCTS

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.

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.

siPORT™ *Amine* Transfection Agent
P/N AM4502 and AM4503
An easy-to-use blend of polyamines that delivers siRNA into mammalian cells with minimal cytotoxicity.

TaqMan® Gene Expression Assays
www.allgenes.com
A comprehensive collection of over 700,000 probe and primer sets for quantitative gene expression analysis using real-time PCR.

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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