Pre-miR™ hsa-miR-1 miRNA Precursor

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

Catalog # (P/N):	AM17150	
Product Description:	A double-stranded RNA oligonucleotide designed for use as a positive control in experiments using Pre-miR™ miRNA Precursors.	
Amount:	5 nmol	
Appearance:	Powder	
Additional Material(s) Included:	1.75 mL Nuclease-free Water	
Product Mimics Precursor for:	Sanger miRNA Registry ID#:	MI0000651 (human mir-1-1), MI0000437 (human mir-1-2), MI0000139 (mouse mir-1-1), MI0000652 (mouse mir-1-2)
	Description:	hsa-miR-1, found in *Homo sapiens* and *Mus musculus*
	Mature miRNA Sequence:	UGGAAUGUAAAGAAGUAUGUA
miRNA Target Information:	Gene Symbol:	PTK9
	Full Gene Name:	Protein tyrosine kinase 9
	Organism(s):	Human and Mouse
	RefSeq Number(s):	NM_002822 (human) and NM_008971.3 (mouse)
	Entrez Gene ID(s):	5756 (human) and 19230 (mouse)
	TaqMan® Assay(s):	PTK9 TaqMan® Gene Expression Assays Hs00702289_s1(Human) and Mm01598981_g1 (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® Pre-miR™ miRNA Precursors (P/N AM17100, AM17101, AM17103; patent pending) are designed to mimic endogenous mature microRNAs (miRNAs). Each Pre-miR miRNA Precursor includes one strand that is identical to a known mature miRNA. The design of Pre-miR miRNA Precursors ensures that the portion of the miRNA precursor identical to the mature miRNA is selected for uptake and activation by the miRNA processing pathway.

Pre-miR™ hsa-miR-1 miRNA Precursor is an miRNA precursor mimic designed to be processed to mature miR-1 miRNA after delivery to mammalian cells. miR-1 is associated with downregulation of many genes [1]. In particular, the gene for Protein Tyrosine Kinase 9 (PTK9) has two predicted binding sites for miR-1 in its 3' UTR, and miR-1-mediated downregulation of PTK9 gene expression has been shown to occur at the mRNA level [1].

miRNAs have been shown to regulate gene expression through three mechanisms: inhibition of translation without concomitant decrease in mRNA levels [2, 3], mRNA degradation [1, 4–6], and transcriptional silencing mediated through changes in chromatin state [7]. miRNA-mediated gene silencing is dependent on complementarity of the target messenger RNAs with critical sequences in the miRNA.

Scientists at Applied Biosystems, Austin, have confirmed the finding by Lim et al [1], showing that expression of PTK9 mRNA decreased by 80% following Pre-miR hsa-miR-1 miRNA Precursor transfection, compared to transfection with Pre-miR™ miRNA Precursors—Negative Control #1 (P/N AM17110), using real-time PCR and TaqMan® Gene Expression Assays indicated above. Thus Pre-miR hsa miR 1 miRNA Precursor provides a convenient positive control for miRNA-mediated gene silencing of PTK9, when monitored using the appropriate TaqMan® Gene Expression Assays and real-time PCR.

Pre-miR™ miRNA Precursors can be delivered to mammalian cells by chemical transfection or electroporation.

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:

Pre-miR™ hsa-miR-1 miRNA Precursor is designed for use as a positive control in miRNA experiments to confirm that the transfection procedure and cell cultures support miRNA-mediated gene silencing. It can also be used in development and optimization of miRNA transfection conditions. Expression of miR-1 is typically restricted to muscle cells and, thus, is not expressed in most other cell lines. Analysis of miR-1 overexpression is straightforward, since expression of miR-1 causes a profound decrease in PTK9 mRNA.

Optimization of transfection conditions is critical to ensure that oligonucleotides are delivered inside the cells and are capable of having biological activity once inside. Transfection optimization involves identifying conditions for maximal activity of the transfected oligonucleotides with minimal amounts of cellular toxicity. The delivery procedure itself can induce changes in gene expression profiles, making a nontransfected control meaningless for evaluation of silencing in delivery optimization. We recommend transfection with nontargeting Pre-miR™ miRNA Precursors–Negative Controls (P/N AM17110, AM17111) as a baseline reference. Nontargeting Pre-miR miRNA Precursors–Negative Controls should be used at the same concentration as positive control and experimental Pre-miR miRNA Precursors, because nucleic acid concentrations within cells can affect the activity and specificity of miRNAs.

To calculate the amount of reduction in target gene expression during transfection optimization or to analyze knockdown of a target gene by real-time PCR, Applied Biosystems Austin scientists typically use the $\Delta\Delta C_t$ method [8], normalizing to 18S rRNA, and comparing to Pre-miR miRNA Precursor–Negative Control-transfected samples.

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	12 wells	6 wells
Transfection Agent [a]	0.3–1.0 µL	1–3 µL	2–4 µL	3–6 µL
Pre-miR miRNA Precursor [b]	3 pmol	15 pmol	30 pmol	75 pmol
Cell Density [c]	6,000 cells/well	40,000 cells/well	80,000 cells/well	200,000 cells/well
Final Volume per Well	0.1 mL	0.5 mL	1.0 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.

References:

1. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, Bartel DP, Linsley PS, Johnson JM. (2005) Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. *Nature* **433(7027)**:769–773. Epub 2005 Jan 30.
2. Lee RC, Feinbaum RL, Ambros V (1993) The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* **75(5)**:843–854.
3. Wightman B, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. *Cell* **75(5)**:855–862.
4. Yekta S, Shih IH, Bartel DP. 2004. MicroRNA-directed cleavage of HOXB8 mRNA. *Science* **304(5670)**:594–596.
5. Bagga S, Bracht J, Hunter S, Massirer K, Holtz J, Eachus R, Pasquinelli AE (2005) Regulation by let-7 and lin-4 miRNAs results in target mRNA degradation. *Cell* **122(4)**:553–563.
6. Jing Q, Huang S, Guth S, Zarubin T, Motoyama A, Chen J, Di Padova F, Lin SC, Gram H, Han J (2005) Involvement of microRNA in AU-rich element-mediated mRNA instability. *Cell* **120(5)**:623–634.
7. Schramke V, Sheedy DM, Denli AM, Bonila C, Ekwall K, Hannon GJ, Allshire RC. 2005. RNA-interference-directed chromatin modification coupled to RNA polymerase II transcription. *Nature* **435(7046)**:1275–1279.
8. Bustin SA (2002) Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. *J Mol Endocrinol*. **29(1)**:23–39.

RELATED PRODUCTS

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

Pre-miR™ miRNA Precursors—Negative Controls
P/N AM17110, AM17111
Designed to serve as a negative control for experiments involving Pre-miR™ miRNA Precursors.

Pre-miR™ miRNA Precursor Molecule Library
P/N 4385830
An extensive collection of Pre-miR miRNA Precursors mimicking precursors to human miRNAs in miRBase Sequence Database Version 9.2.

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.

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.

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 a sample of each purified single-stranded RNA oligonucleotide is used to confirm ≥90% purity.

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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