

DharmaconTM miRIDIANTM microRNA Mimics, Hairpin Inhibitors and negative controls

miRNA overview

MicroRNAs (miRNAs) are small endogenous RNA molecules (~ 21-25 nt) that regulate gene expression by targeting one or more mRNAs for translational repression or cleavage. Several thousand miRNAs have been identified in organisms as diverse as viruses, worms, and primates through cloning or computational prediction. Information on miRNAs is currently available in the miRBase Sequence Database hosted by the Faculty of Life Science at the University of Manchester (mirbase.org).

To promote research on the biochemistry and biological function of miRNAs, we offer the miRIDIANTM microRNA product line. miRIDIAN microRNA Mimics, Hairpin Inhibitors and Negative Controls are designed based on known or predicted miRNA sequences in miRBase for human, mouse and rat. miRNA Mimics facilitate gain-of-function studies, while miRNA Inhibitors facilitate loss-of-function studies.

In experiments using miRIDIAN microRNA Mimics and Hairpin Inhibitors, we recommend including a sample treated with an equal concentration of a negative control to confirm that the effects observed with the mimic or inhibitor is specific. We offer two universal negative controls for both Mimics and Inhibitors based on two *C. elegans* miRNA sequences. These miRNAs

have been confirmed to have minimal sequence identity with miRNAs in human, mouse and rat. Table 1. provides the identity, accession numbers and sequences of the two miRNAs that the controls are based upon. We recommend using the mature miRNA sequences to determine which control would be most appropriate for the miRNA being studied.

Product description

miRIDIAN microRNA Mimics

- Double-stranded oligonucleotides designed to mimic the function of endogenous mature miRNA
- Chemically enhanced to preferentially program RISC with active miRNA strand

miRIDIAN microRNA mimic negative controls

Chemically modified double-stranded oligonucleotides designed to serve as negative controls for miRIDIAN microRNA Mimic experiments

Table 1. miRIDIAN microRNA negative controls

| | Mature miRNA | Mimic negative controls* | inhibitor negative controls* |
|---------------|--------------------------|---|---|
| ID | cel-miR-67 | | |
| Accession No. | MIMAT0000039 | Cat. #CN-001000-01-XX Mol. Wt. 14074 g/mol | Cat. #IN-001005-01-XX Mol. Wt. 18379 g/mol |
| Sequence | UCACAACCUCCUAGAAAGAGUAGA | | |
| ID | cel-miR-239b | | |
| Accession No. | MIMAT0000295 | Cat. #CN-002000-01-XX Mol. Wt. 14084 g/mol | Cat. #IN-002005-01-XX Mol. Wt. 18394 g/mol |
| Sequence | UUGUACUACACAAAAGUACUG | | |

*Cat. = Catalog; Mol. Wt. = Molecular weight
XX=05,20, or 50 for 5, 20, and 50 nmol amounts

miRIDIAN microRNA Hairpin Inhibitors

- RNA oligonucleotides with novel secondary structure designed to inhibit the function of endogenous miRNA
- Chemically enhanced to improve efficacy and longevity

miRIDIAN microRNA Hairpin Inhibitor negative controls

Chemically modified oligonucleotides designed to serve as negative controls for miRIDIAN microRNA Inhibitor experiments.

Quality control

Mass of each oligonucleotide strand is confirmed by mass spectrometry.

Disclaimer

We designed the miRIDIAN reagents based on publicly available information, in-house design, and applied proprietary algorithms to improve efficacy and specificity. We presently do not offer functional guarantees for these products. For some suggested experimental parameters on the use of Mimics and Inhibitors, please review the publications listed in (Table 3.).

Shipping and storage

- miRIDIAN microRNA reagents are shipped as dried pellets at room temperature (23 °C). Under these conditions, they are stable for at least four weeks.
- Upon receipt, miRIDIAN microRNA reagents should be stored at -20 °C to -80 °C. Under these conditions, they are stable for at least one year.

Resuspension

- Resuspend miRIDIAN microRNA reagents in an appropriately buffered, RNase-free solution to give a stock concentration of 20 µM (Table 2.).
- Upon resuspension, aliquot the miRIDIAN microRNA reagents into small volumes and store at -20 °C to -80 °C. For best results, limit freeze-thawing of each tube to five events. Under these conditions, the reagents are stable for at least one year.

Table 2. Recommended volumes for preparing 20 µM miRIDIAN microRNA reagents

| Amount per tube (nmol) | Amount of buffer to be added (mL) |
|------------------------|-----------------------------------|
| 5 | 0.25 |
| 20 | 1.0 |
| 50 | 2.5* |

*Volume recommended exceeds capacity of tube provided.

Handling precautions

Oligonucleotides are susceptible to enzymatic degradation by nucleases and to chemical degradation by extreme pH and temperatures. We recommend wearing gloves and maintaining nuclease-free conditions when handling the oligonucleotides.

Table 3. Applications of synthetic miRNA Mimics and Hairpin Inhibitors: The concentration of miRNA Mimics and Inhibitors used in these publications range from 5 pM to 20 µM. Although the Mimics and Inhibitors reported here are not miRIDIAN microRNA reagents, the concentrations used serve as good starting points for experimental optimizations. Detection of miRNA mimic or inhibitor function was conducted by reporter assays, unless indicated by an asterisk (*).

| Reference | miRNA | Cells | [Mimic] | [Inhibitor] |
|---|----------------|------------------------|------------|-------------|
| Meister, G., M. Landthaler, <i>et al. RNA</i> , 2004. 10 544-550. | miR-21 | HeLa S3 | 40 nM | 120 nM |
| Poy, M., L. Eliasson, <i>et al. Nature</i> , 2004. 432 226-30. | miR-375 | MIN6 | 200 nM | 200 nM |
| Yekta, S., I.H. Shih, <i>et al. Science</i> , 2004. 304 594-596. | miR-196a | HeLa | 5 or 20 pM | N/A |
| Esau, C., X. Kang, <i>et al. J. Biol. Chem.</i> , 2004. 279 52361-52365. | miR-143 | HeLa | N/A | 20 nM |
| | | Primary pre-adipocytes | N/A | 250 nM* |
| Yu, Z., T. Raabe, <i>et al. Biol. Reprod.</i> , 2005. 73 427-433. | miR-122a | NIH 3T3 | 15-20 nM | N/A |
| Lee, Y.S., H.K. Kim, <i>et al. J. Biol. Chem.</i> , 2005. 280 16635-16641. | miR-125b | PC3, HeLa | N/A | 160 nM* |
| Lim, L., N. Lau, <i>et al. Nature</i> , 2005. 433 769-73. | miR-1, miR-124 | HeLa | 100 nM | N/A |
| Chen, J-F., E. Mandel, <i>et al. Nat. Gen.</i> , 2006. 38 228-33. | miR-1, miR-133 | C2C12 | 200 nM | 200 nM* |
| Schratt, G., F. Tuebing, <i>et al. Nature</i> , 2006. 439 283-89. | miR-134 | Primary neurons | 10 µM | 20 µM |

If you have any questions, contact

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