Thermo Scientific Dharmacon miRIDIAN microRNA Mimics & Hairpin Inhibitors

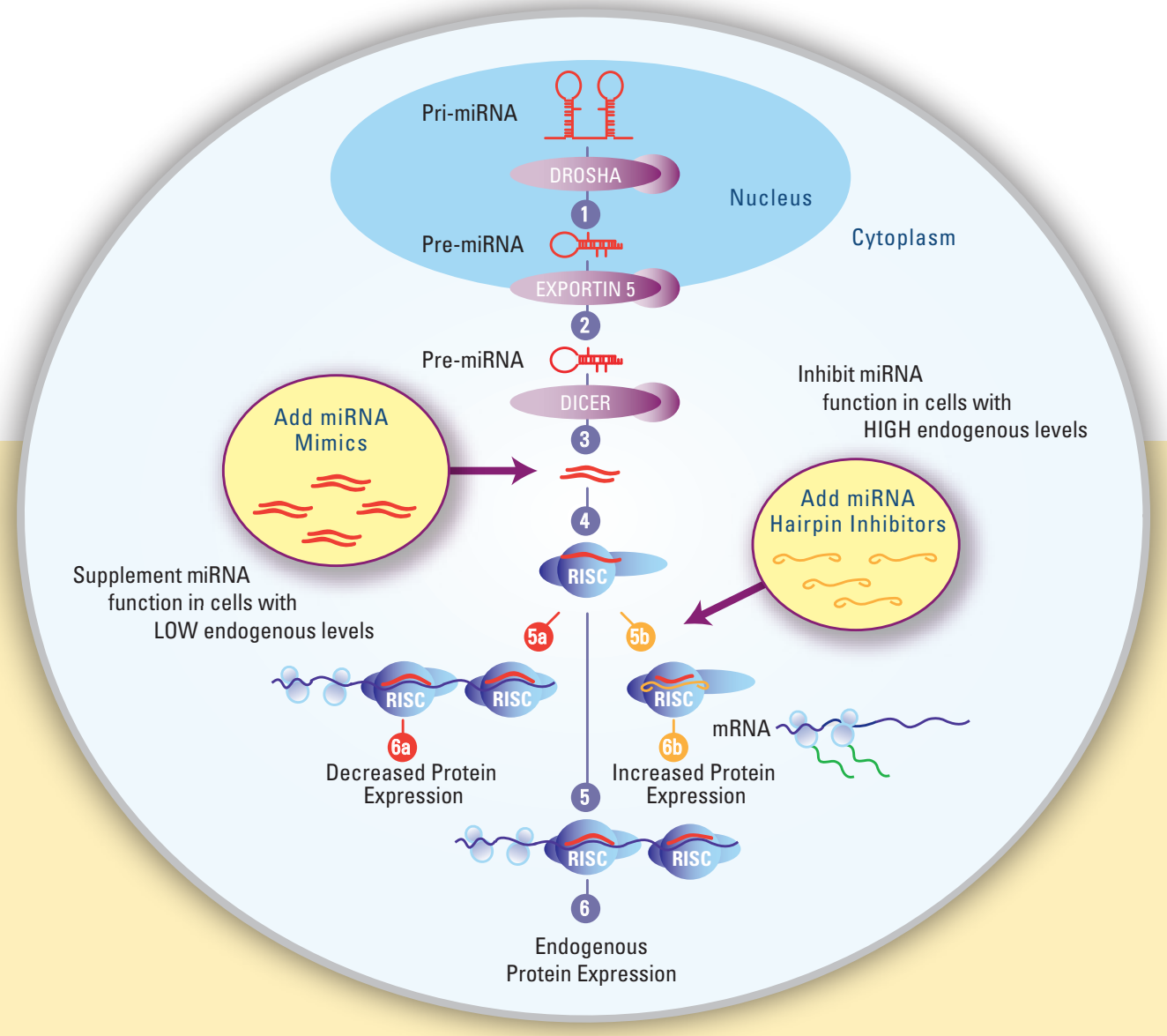
Highly potent oligonucleotides for modulating miRNA function

Reliable reagents for reproducible studies of miRNAs

Advanced design for all human, mouse and rat miRNAs

Thermo Scientific Dharmacon miRIDIAN microRNA Mimics and Hairpin Inhibitors: modulation of an endogenous RNAi mechanism

microRNAs are endogenous non-coding ~22mer RNAs that are highly conserved and regulate gene transcript levels through RNA interference mechanisms. As key players in the fine-tuning of biological networks, microRNAs have significant diagnostic and prognostic potential as biomarkers not only of fundamental cellular physiology but also of disease etiology and progression. To explore microRNA biology, we provide potent and specific tools for dissecting microRNA function.



Effects of miRNA mimics and inhibitors on protein expression

1. *Transcribed pri-miRNA is cleaved by Drosha-DGCR8 protein complex to form a hairpin pre-miRNA.*
2. *Pre-miRNA is transported out of the nucleus by an Exportin 5 protein complex.*
3. *Pre-miRNA is cleaved by Dicer to form a short double-stranded miRNA duplex.*
4. *miRNA duplex is loaded into RISC. Cleavage of inactive strand results in formation of programmed RISC.*
5. *Programmed RISC binds to 3'-UTR of target mRNA (5/5a). miRNA inhibitors compete with mRNA for programmed RISC (5b).*
6. *Target protein expression is decreased when miRNA mimic is added (6a) and increased when miRNA inhibitor is added (6b).*

microRNA experimental workflow

Supplementing or inhibiting microRNA activity and examining the resulting phenotypic effects is crucial for understanding basic microRNA involvement in, for example:

- RNA interference mechanisms
- Fundamental cellular and physiological processes
- Gene regulatory networks and pathways
- Disease etiology and progression
- Response to disease treatment regimens

DISCOVERY

DETECTION

MODULATION

TARGET ID

Product and services for the microRNA experimental workflow

- microRNA Expression Profiling Services
  - Global microRNA expression analysis through the Thermo Scientific RNAi Discovery and Therapeutic Services (RDTS)
- Mimic and Hairpin Inhibitor library screening services
  - End-point and high-content assay read-outs
- miRIDIAN® microRNA Mimics and Hairpin Inhibitors
  - Individual molecules and plated libraries
- miRIDIAN microRNA Mimic and Hairpin Inhibitor Experimental Controls
- Thermo Scientific DharmaFECT Transfection Reagents

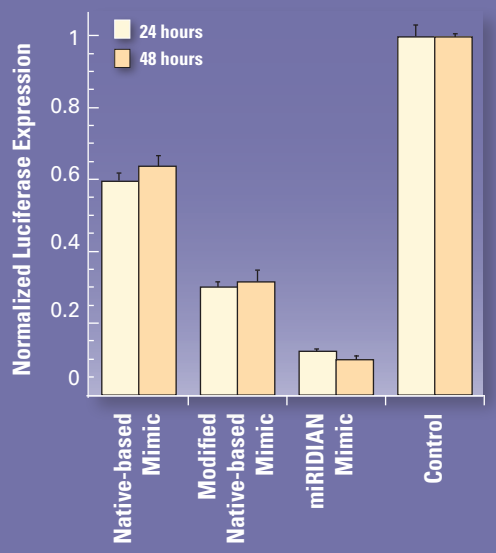
miRIDIAN microRNA Mimics

miRIDIAN microRNA Mimics are double-stranded RNA oligonucleotides, chemically enhanced with a proprietary design and available for all human, mouse and rat miRNAs in the miRBase Sequence Database.[1]

- Supplement miRNA activity with miRIDIAN microRNA Mimics to study gain-of-function effects
- Superior performance in comparison to native-based mimic design
- Effective mimic of endogenous mature miRNA function
- Preferential programming of RISC-like complex with active strand of miRNA and exclusion of passenger strand through a proprietary chemical modification pattern

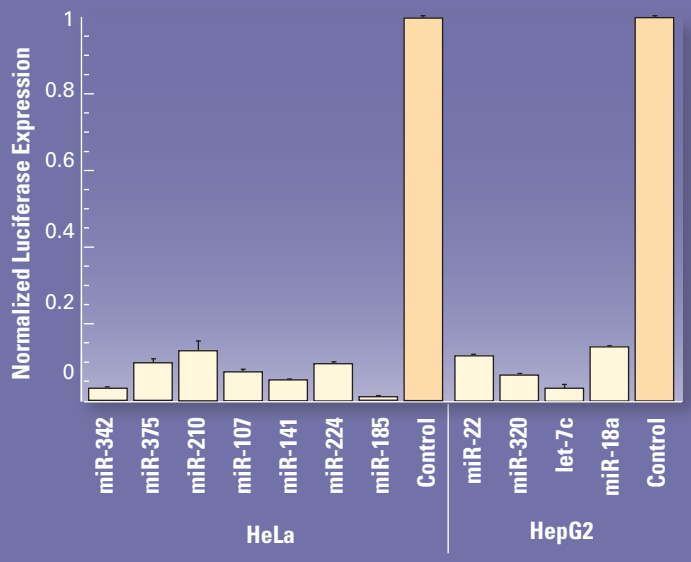
specific programming of RISC

Optimization of miRIDIAN microRNA Mimic design



miRNA mimic function was assayed in HeLa cells 24 and 48 hours after transfection of 10 nM miR-375 native-based mimic, modified native-based mimic, or miRIDIAN microRNA Mimic using a dual luciferase reporter system and normalized to the control (no mimic).

miRIDIAN microRNA Mimics effectively simulate miRNA function



miRIDIAN microRNA Mimic function for 11 human miRNAs was assayed in HeLa and HepG2 cells 48 hours after transfection of 10 nM mimic using a dual luciferase reporter system and normalized to the control (no mimic). Similar results were obtained with 1 nM miRIDIAN microRNA Mimic (data not shown).

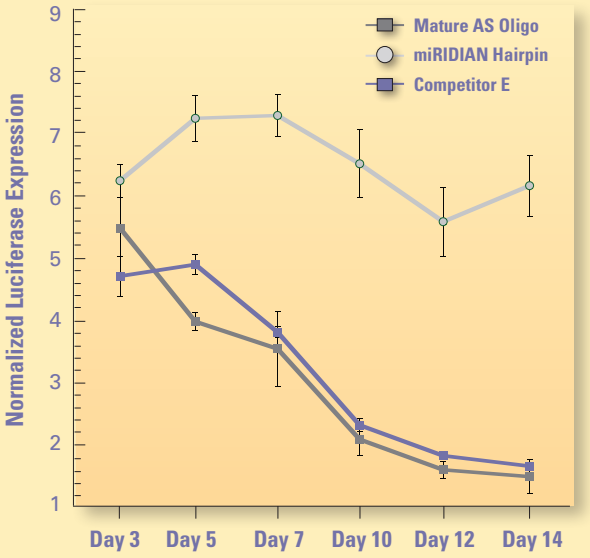
[1] *www.mirbase.org*

miRIDIAN microRNA Hairpin Inhibitors

miRIDIAN microRNA Hairpin Inhibitors[2] are single-stranded, chemically enhanced oligonucleotides available for all human, mouse and rat miRNAs in the miRBase Sequence Database.[1]

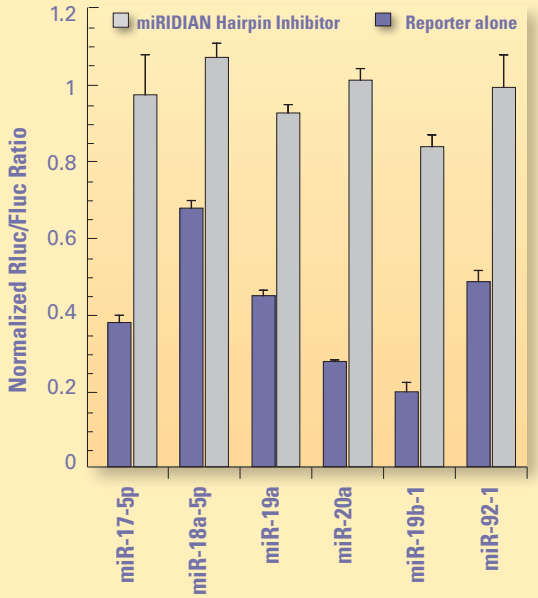
- Most effective inhibition of endogenous mature microRNA function by means of innovative design
- Patent-pending molecule combines chemical modifications and completely novel secondary structure motif
- Superior potency and longevity in comparison to any other synthetic product offered commercially
- Enhanced potency and longevity allows for multiplexed microRNA inhibition at very low nanomolar concentrations with minimal toxicity

miRIDIAN microRNA Hairpin Inhibitors* are potent and long-lived at low concentrations



Time course of inhibition by three different designs targeting endogenous miR-23 in a MCF-7 reporter cell line from a stably-integrated luciferase construct. miRIDIAN Hairpin Inhibitor, mature antisense (AS) and Competitor E inhibitors were transfected at 40 nM using DharmaFECT®1. Data was normalized to cells with no inhibitor, so no inhibition is equal to 1.

miRIDIAN microRNA Hairpin Inhibitors allow combinatorial inhibition of clustered miRNAs



Six co-expressed miRNAs (mir-17-5p, miR-18a-5p, miR-19a, miR-20, miR-19b-1 and miR-92-1) from the "Cancer Cluster" were inhibited by miRIDIAN Hairpin Inhibitors. All six miRIDIAN Hairpin Inhibitors were pooled together for a 0.8 nM total inhibitor concentration. Pools were co-transfected with DharmaFECT Duo into HeLa cells with one of six reporter plasmids specific for each miRNA. Data were normalized to similarly-treated empty reporter plasmid only.

* *Patent pending*

[1] *www.mirbase.org*

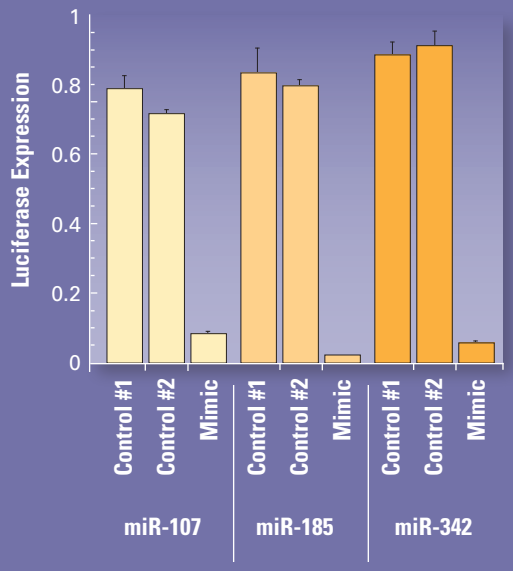
[2] *This novel microRNA inhibitor design is based on findings published by Vermeulen et al. (Double-stranded regions are essential design components of potent inhibitors of RISC function. RNA, May 2007. 13: 723 - 730).*

miRIDIAN microRNA Mimic and Hairpin Inhibitor Negative Controls

miRIDIAN microRNA Negative Control sequences are based on *C. elegans* miRNAs (#1: cel-miR-67, #2: cel-miR-239b) for use as negative experimental controls in human, mouse and rat cells. The Negative Controls have been analyzed by BLAST against all human, mouse and rat genomic sequences and miRNA sequences in the miRBase Sequence Database.[1]

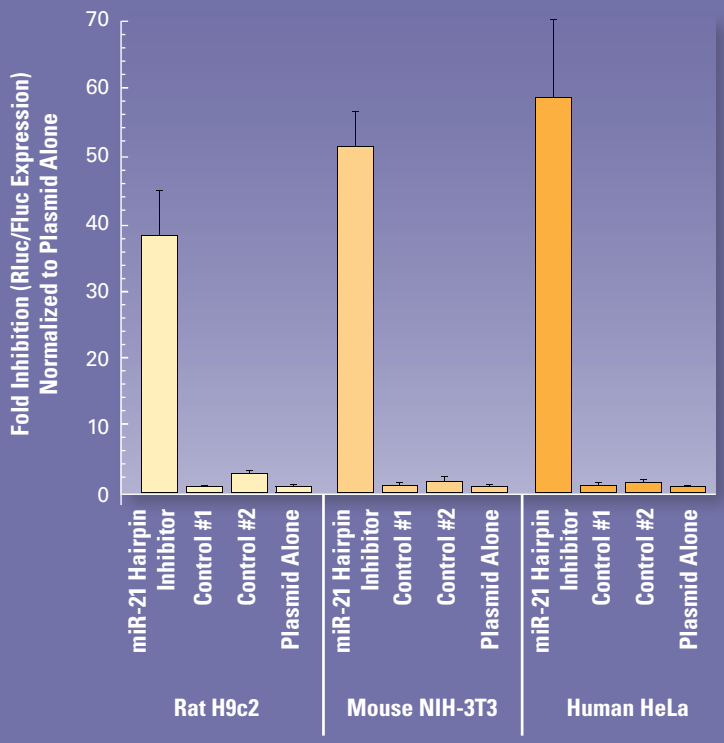
- Non-targeting mimics and inhibitors for use as controls for non-sequence-specific effects in miRNA experiments
- Allow distinction between mimic or inhibitor activity and background effects
- Designs and modifications are identical to experimental miRIDIAN microRNA Mimics and Hairpin Inhibitors
- No identifiable effects on tested miRNA function in multiple cell lines

miRIDIAN microRNA Mimic Negative Controls have no apparent effect on miRNA function



Effects of miRIDIAN microRNA Mimic Negative Controls on the function of three human miRNAs were assayed at 24 hours after transfection of 10 nM mimic or negative control in HeLa cells using a dual luciferase reporter system.

miRIDIAN Hairpin Inhibitor Negative Controls are tools for distinguishing between miRNA-specific and non-specific effects



Effects of miRIDIAN microRNA Hairpin Inhibitor Negative Controls on the function of miR-21 were assayed at 48 hours after transfection of 5 nM inhibitor or negative control in H9c2, NIH3T3 or HeLa cells using DharmaFECT Duo and a dual luciferase reporter system.

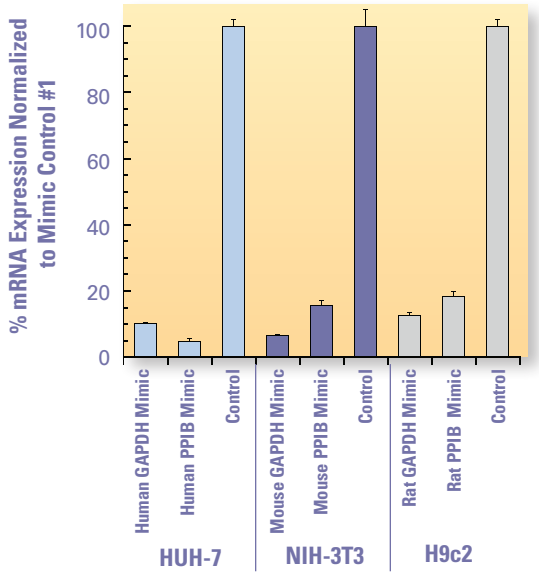
[1] *www.mirbase.org*

miRIDIAN microRNA Mimic Positive Controls

Successful microRNA functional studies begin with optimization of the assay in a cell line and or type of interest. The **miRIDIAN microRNA Mimic Housekeeping (HKG) Positive Controls** provide the ability to monitor function of a mimic molecule at the mRNA level which can be assessed using standard transcript quantification methods such as qRT-PCR.

- Employ the same structure and design as experimental miRIDIAN microRNA Mimics
- Target the 3' UTR (untranslated region) of standard housekeeping genes, PPIB or GAPDH
- Allow a clean, straightforward cleavage-based readout of mimic function

miRIDIAN microRNA Mimics designed to target the 3' UTR of either PPIB or GAPDH were transfected at 50 nM using DharmaFECT 1 into the indicated cell lines and assessed for their ability to decrease target mRNA levels. PPIB or GAPDH down-regulation was determined using the Quantigene branched DNA assay (Panomics) at 48 hours post-transfection.

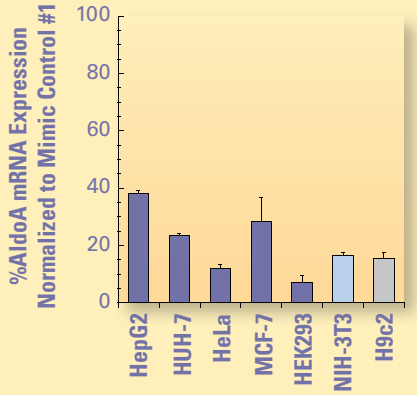


The **miRIDIAN microRNA Mimic Endogenous Positive Control** provides the ability to monitor effects of a specific mimic on target protein levels in a validated endogenous assay. The assay is based upon the targeted activity of miR-122 on Aldolase A mRNA levels in cell lines that express low to moderate levels of miR-122.

- Validated miRIDIAN microRNA Mimic that targets Aldolase A in human, mouse and rat
- Provides the ability to optimize assay conditions by monitoring mimic function on an endogenous gene target (Aldolase A) with a conserved miR-122 binding site

miRIDIAN microRNA Mimic Endogenous Control leads to regulation of Aldolase A in human, mouse and rat cell lines

Many cells lines express low to moderate levels of miR-122. Aldolase A is a predicted target of miR-122 and the 3' UTR is conserved in human, mouse and rat at the 8-mer miR-122 predicted seed site. miRIDIAN microRNA Mimic designed to modulate endogenous miR-122 was transfected at 50 nM (Huh-7 at 40 nM) using DharmaFECT 1 into the indicated cell lines and assessed for their ability to decrease AldoA mRNA levels. AldoA down-regulation was determined using the Quantigene branched DNA assay (Panomics) at 3 days (HepG2 at 5 days) post-transfection.

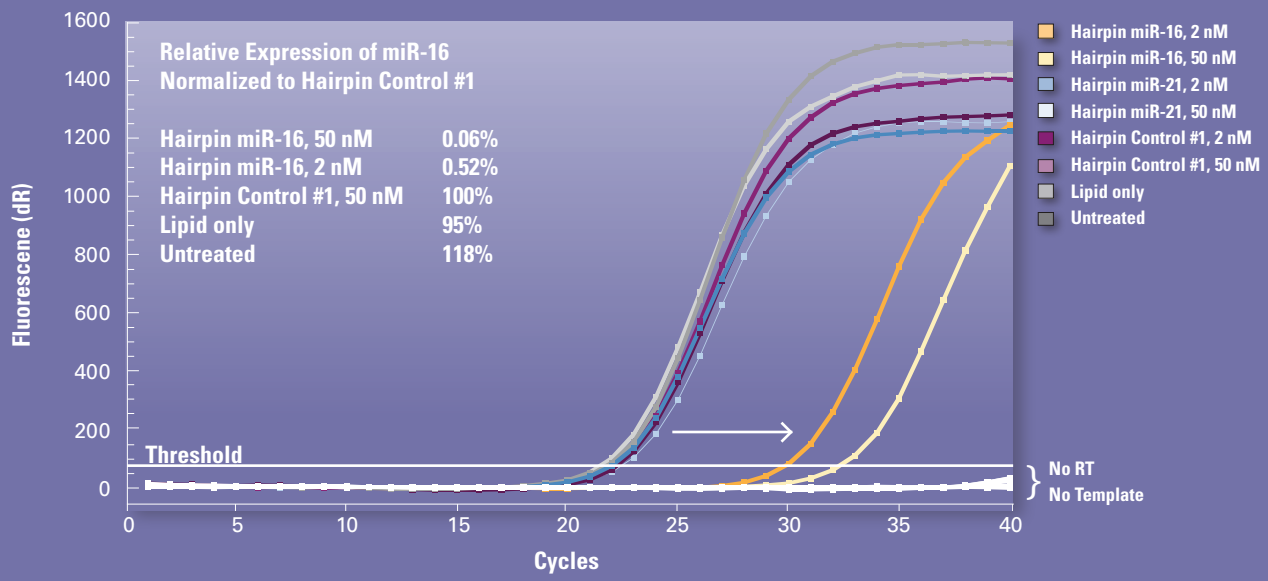


miRIDIAN microRNA Hairpin Inhibitor Positive Controls

The miRIDIAN microRNA Hairpin Inhibitor Positive Control targets a well-conserved microRNA (miR-16) that is broadly expressed in many human, mouse and rat cell lines. This positive control allows the optimization of microRNA functional assays and evaluation of hairpin inhibitor function.

- Targets miR-16 in human, mouse and rat cells resulting in reduced miR-16 activity
- Allows the optimization of microRNA inhibition assays by targeting an endogenous microRNA in a cell line which expresses miR-16
- Demonstrates specific inhibition of miR-16 which can be directly assessed by qRT-PCR and/or Northern blotting

2 nM of miR-16 hairpin inhibitor is sufficient to bind or sequester a majority of endogenous miR-16



Endogenous levels of miR-16 were assayed 48 hours after transfection of either miRIDIAN miR-16 Hairpin Inhibitor, miR-21 Hairpin Inhibitor or Hairpin Inhibitor Control #1. HeLa cells were transfected using DharmaFECT 1.

Detection of miR-16 by qRT-PCR was performed using a poly-A tailing method (Shi R and Chaing VL "Facile means for quantifying microRNA expression by real-time PCR" (Biotechniques 39:519-529 October 2005) and Thermo Scientific Verso SYBR Green 2-Step QRT-PCR Kit (ABgene) according to protocol. Relative expression of miR-16 first normalized by miR-21 expression and then further normalized to miRIDIAN Hairpin Inhibitor Control #1 levels.

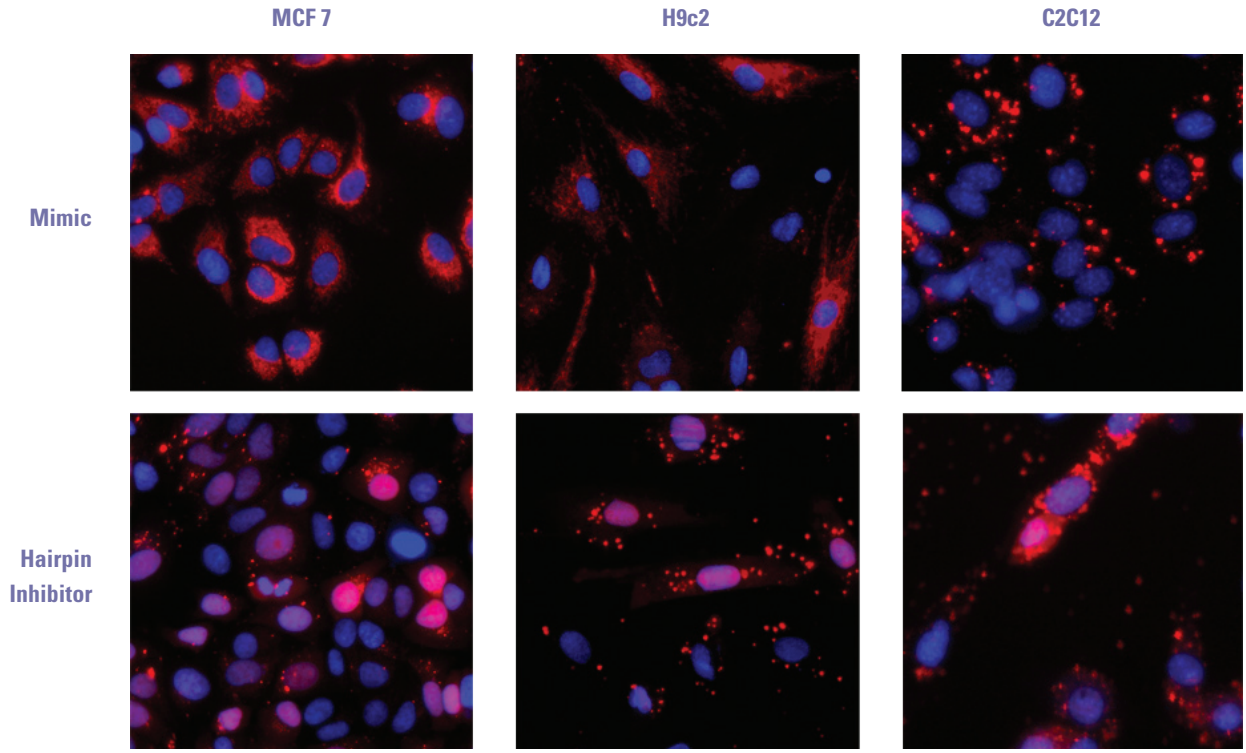
miRIDIAN microRNA Mimic and Hairpin Inhibitor Transfection Controls

The miRIDIAN microRNA Mimic and Hairpin Inhibitor Transfection Controls are Dy547-labeled Mimic and Hairpin Inhibitor that are based on *C. elegans* miRNA (#1: cel-miR-67) for monitoring delivery into human, mouse and rat cells.

- Non-targeting miRIDIAN Mimic and Hairpin Inhibitor labeled with Thermo Scientific Pierce Dy547 (absorbance/emission max: 557/570 nm)
- Transfection can be monitored and visualized by epifluorescence
- Control designs and modifications are identical to experimental miRIDIAN microRNA Mimics and Hairpin Inhibitors
- No identifiable effects on tested miRNA function in multiple cell lines

validated experimental controls

Dye-labeled miRIDIAN Mimic and Hairpin Inhibitor Transfection Controls allow for qualitative evaluation of transfection



Images shown are of human (left), rat (middle) and mouse (right) cells 48 hours after transfection with Pierce Dy547-labeled miRIDIAN Mimic Negative Control #1 (top panels) and miRIDIAN Hairpin Inhibitor Negative Control #1 (bottom panels). Cells were counterstained with Hoechst 33342 (blue, nuclei).

Potent and long-lived tools to validate microRNA targets

miRIDIAN microRNA Mimic and Hairpin Inhibitors can serve as complementary molecular tools providing critical phenotypic information and rapid confirmation of putative miRNA-target interactions. The combination of gain-of-function (mimic-induced down regulation) and loss-of function (inhibitor-induced up regulation) experiments provide the necessary support to demonstrate microRNA-target relationships.

miRIDIAN microRNA Mimics

- Specific microRNA activity through use of chemical modifications to induce strand-bias

miRIDIAN microRNA Hairpin Inhibitors

- Potent and long-lasting microRNA inhibition demonstrated up to 14 days or longer

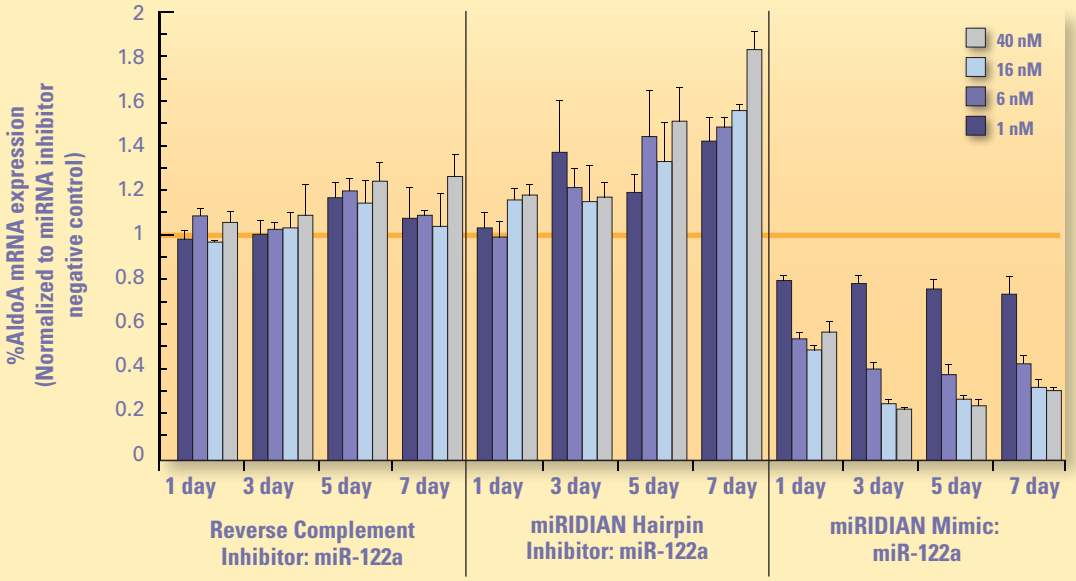
miRIDIAN microRNA Mimic and Hairpin Inhibitor Negative Controls

- Validated negative controls to distinguish non-specific effects

miRIDIAN microRNA Mimic and Hairpin Inhibitor Positive Controls

- Validated positive controls for assay development and optimization

Extended duration of effect enables microRNA target analysis



Aldolase A is a putative target of miR-122 with a conserved 8-mer miR-122 predicted seed site in the 3' UTR. microRNA mimics and inhibitors designed to modulate endogenous miR-122 were transfected at several concentrations using DharmaFECT 1 into the human liver cell line, Huh-7 (10K cells), and assessed for their ability to alter Aldo A mRNA levels. Aldo A up- or down-regulation was determined using the Quantigene branched DNA assay (Panomics) at 1, 3, 5 and 7 days post-transfection.

Modulation of AldoA mRNA levels in Huh-7 cell line using miRNA miRIDIAN Mimics & Inhibitors. Orange line indicates AldoA expression level as a result of endogenous miR-122 regulation. AldoA mRNA expression values >1 indicates inhibition of miR-122, while values < 1 indicates additional down regulation of AldoA by miRNA. The miRIDIAN microRNA Inhibitor resulted in an increase in Aldo A mRNA levels even at 7 days, whereas a standard 2'-O-methyl-RNA oligo complementary to mature miR-122 showed a small effect. The miRIDIAN microRNA Mimic immediately reduced Aldo A mRNA levels and sustained suppression throughout the experiment.

High-throughput Phenotypic Screening: miRIDIAN microRNA Mimic and Hairpin Inhibitor Libraries

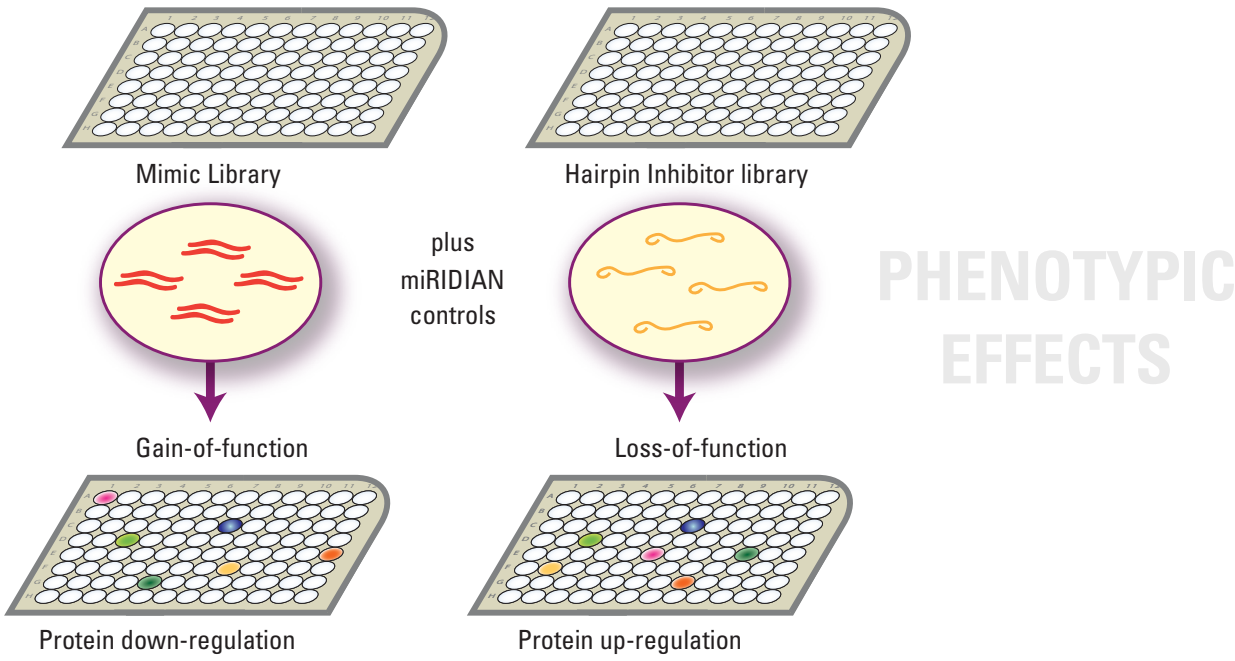
miRIDIAN microRNA Mimic and Hairpin Inhibitor Libraries are complete collections of mimics and inhibitors in 96-well plates which enable classical genetic approaches to:

- Perform phenotypic high-throughput screening
- Find potential biomarkers of normal or diseased cellular processes
- Couple microRNA screening with high-content analysis for multi-parametric data
- Identify microRNAs that synergize with drugs of interest for increased therapeutic benefit

Trust your screening results to the leader in RNAi technologies

Why screen with miRIDIAN Libraries? Successful microRNA phenotypic screening requires best-in-class reagents to minimize false or misleading effects and achieve high-confidence data. miRIDIAN Mimics and Hairpin Inhibitors are designed for robust and reproducible results.

- Mimics are modified to prevent sense-strand uptake and ensure phenotypes resulting from only the mature microRNA
- Hairpin inhibitors have long-lasting and potent inhibition, allowing enough time for full phenotypes to develop
- Libraries are offered as complete collections for human, mouse and rat or as custom user-defined collections



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