Thermo Scientific miRIDIAN shMIMIC
Lentiviral microRNAs

- Stable over-expression of mature microRNA
- Innovative design for consistent processing and function
- Lentiviral particles deliver into difficult-to-transfect cells

Part of Thermo Fisher Scientific
Thermo Scientific miRIDIAN(shMIMIC Lentiviral microRNAs: stable modulation of an endogenous RNAi mechanism

microRNAs are endogenous non-coding ~22mer RNAs that are highly conserved in mammals and that regulate gene expression post-transcriptionally through innate RNA interference mechanisms. As key players in the fine-tuning of biological networks (Figure 1), microRNAs have significant diagnostic and prognostic potential as biomarkers not only of fundamental cellular physiology but also of disease etiology and progression. To study long-term microRNA-induced phenotypes, we have developed a novel system for stable expression of the mature microRNA. miRIDIAN shMIMIC microRNA designs are created for each human microRNA described in the miRBase database.

Figure 1. Borrowing the endogenous pathway that processes host-encoded microRNA transcripts to over-express functional mature microRNA.

(1) The lentiviral particle binds to the host cell and delivers its engineered RNA genome, which includes encoded microRNA, to the cytoplasm. (2) The viral genome is reverse-transcribed in the cytoplasm (i.e. RNA to DNA). The DNA intermediate form is imported into the nucleus and is stably integrated into the host genome. (3) shMIMIC pri-miRNA transcripts are transcribed in the same manner as endogenous microRNA genes. Native and over-expressed pri-miRNA transcripts enter the microRNA processing pathway, are processed by the Drosha complex (4), shuttled out of the nucleus into the cytoplasm (5) and further processed by the Dicer complex into active, mature microRNA sequences (6) which are incorporated into the RNA Induced Silencing Complex (RISC). These microRNA-loaded RISC bind to target mRNA and cause transcript destabilization, resulting in down-regulation of protein expression (7).
miRIDIAN shMIMIC Lentiviral microRNAs were developed using a unique and proprietary design strategy resulting in highly functional microRNA mimics. Each element of the vector was experimentally assessed in a systematic series of studies to identify an efficiently processed microRNA scaffold, from which the mature microRNA is expressed. shMIMIC microRNA constructs incorporate design elements critical for accurate processing and efficient downstream activity of only the mature microRNA of interest.

**These design elements include:**

- Novel, proprietary microRNA expression scaffold for consistent and accurate Drosha and Dicer processing to yield only the mature microRNA.
- MicroRNA expression scaffold empirically confirmed to show minimal activity of the passenger, or star (*), strand.
- Critical native secondary structure of the microRNA scaffold is maintained to promote preferential loading of the active, mature strand of the microRNA of interest.

### Active mature microRNA sequences from miRBase 13.0

![shMIMIC scaffold](image)

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<th>Vector Element</th>
<th>Utility</th>
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<tr>
<td>CMV Promoter</td>
<td>Drives strong transgene expression</td>
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<td>TurboGFP(nuc)</td>
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<td>IRES (internal ribosomal entry site)</td>
<td>Allows expression of TurboGFP and puromycin resistance genes in a single transcript</td>
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### Vector Elements

- **TurboGFP** Marker to visualize transduction
- **SIN-LTR** Self-inactivating long terminal repeat results in replication-incompetent particles
- **Puro'** Puromycin resistance permits antibiotic selective pressure and propagation of stable integrants
- **IRES** Internal ribosomal entry site
- **CMV Promoter** Drives strong transgene expression

**miRIDIAN shMIMIC Lentiviral microRNAs are highly functional at low multiplicity of infection without puromycin selection**

**Figure 2.** shMIMIC lentiviral microRNAs efficiently suppress luciferase expression without the need for puromycin selection. HEK293T cells were transduced in biological triplicate with specific shMIMIC or negative control microRNA at various multiplicities of infection (MOI). After 48 hours, cells were transfected using Thermo Scientific DharmaFECT Duo (0.2 µl/well) with psiCHECK-2™ plasmid (Promega, WI) containing the microRNA target site or no target site. Cells were harvested 48 hours post-transfection and a Dual-Glo® Luciferase Assay (Promega, WI) was performed.
**shMIMIC Lentiviral microRNAs** show consistent and reproducible function

miRIDIAN shMIMIC lentiviral vectors contain functional attributes to yield mature microRNA expression, TurboGFP protein translation and the ability to create and maintain stable cell lines. Expression of a VSVg envelope protein facilitates transduction in a broad range of cells including difficult-to-transfect cell types such as primary cells, stem cells and non-dividing neuronal cells.

**miRIDIAN shMIMIC microRNAs induce repression of endogenous human microRNA targets in difficult-to-transfect cells**

Figure 3a. TurboGFP expression in four different cell lines visualized by epifluorescence microscopy 120 hours post-transduction. Puromycin selection was not used in these experiments.

Figure 3b. HEK293, HUVEC, K562 or SH-SY5Y cells were transduced in biological triplicate with specific microRNA or shMIMIC negative control viral particles. Cells were harvested for RNA isolation 120 hours post-transduction (no puromycin). Repression of endogenous gene targets was determined using either Thermo Scientific Solaris qPCR gene assays (HEK293 and SH-SY5Y data) or Thermo Scientific Verso SYBR Green 2-Step qRT-PCR Kit (HUVEC and K562 data). Data was normalized to a housekeeping gene (PPIB) and further normalized to matched shMIMIC negative control.

**shMIMIC microRNA over-expression does not result in perturbation of global endogenous microRNA expression profiles**

Figure 4. HEK283T cells were transduced with hsa-miR-122 shMIMIC microRNA or negative control viral particles at an MOI of 0.3. After 48 hours, infected cells were selected by treatment with puromycin (5 µg/mL) for six days before total RNA was extracted and global microRNA expression profiling performed (Thermo Scientific microRNA array). Expression data were analyzed by error-weighted ANOVA and visualized as cluster heat maps, where log(2) signals have been mean-centered for each microRNA. Shown here is an enlarged section of a heat map illustrating significant (23-fold) increase in miR-122 expression in shMIMIC microRNA-treated cells. In contrast, only 9 microRNAs (hsa-miR-887, -184, -451, -875-3p, -574-3p, -30d, -15b & -186*) were found to be differentially expressed with fold changes between 1.2 and 1.8 amongst untreated, negative control-treated and miR-122 shMIMIC microRNA-treated conditions.
miRIDIAN shMIMIC microRNAs permit long-term study of microRNA-induced phenotypes

Thermo Scientific shMIMIC Lentiviral microRNAs can be used not only to study microRNA regulation of putative gene targets but also to observe resulting phenotypic responses to target gene down-regulation. Such observations provide insight into microRNA participation in critical biological feedback loops.

CASE STUDY: Effects of hsa-miR-429-mediated regulation of mesenchymal-to-epithelial transition in breast cancer cells

Members of the human miR-200 family are involved in a morphological process indicative of cancer progression called epithelial-to-mesenchymal transition (EMT) as well as in the reverse process (mesenchymal-to-epithelial transition, MET). Here, using RT-qPCR, we show that transduction of a breast cancer cell line, MDA-MB-231, with human shMIMIC miR-429 results in down-regulation of a putative endogenous miR-429 gene target (ZEB1, Figure 5a) by RT-qPCR. Repression of ZEB1, a transcriptional repressor, in turn up-regulates expression of the protein E-cadherin, which can be visualized by immunofluorescent labeling and microscopy (Figure 5b). We can observe a clear change in the cells indicating transition from an invasive, migratory state to a more stationary, epithelial morphology.

Figure 5a and 5b. MDA-MB-231 cells were transduced at multiple MOIs with either miR-429 shMIMIC microRNA or negative control. Transductions were performed in triplicate with no puromycin selection. Figure 5a: Cells were harvested 120 hours post-transduction and silencing of ZEB1 expression was measured using SYBR® Green 2-Step Thermo Scientific Verso QRT-PCR Kit. Data was normalized to a housekeeping gene (PPIB) and further normalized to the matched shMIMIC microRNA negative controls. Figure 5b: Cells were fixed in 2 % paraformaldehyde 96 hours post-transfection. Immunofluorescence was used to visualize E-cadherin expression and cellular localization. E-cadherin protein localization is in red, while nuclei stained with Hoechst 33342 are in blue. Note the increased expression of E-cadherin in cells transduced with miR-429 shMIMIC microRNA (MOI = 20) as well as the localization of E-cadherin to newly formed adherens junctions.
miRIDIAN shMIMIC microRNAs are provided as transduction-ready lentiviral particles.

miRIDIAN shMIMIC microRNAs are provided as high-titer, purified lentiviral particles, eliminating the substantial investment of time, labor and money required in the design, production and quality control of functional microRNA vectors. All proprietary shMIMIC lentiviral microRNA vectors are packaged into lentiviral particles using the newest generation Thermo Scientific Trans-lentiviral Packaging System for enhanced biosafety and consistent titers.

The arrayed miRIDIAN shMIMIC Lentiviral microRNA Library is provided as a triplicate set of all human microRNAs in 96-well plates in transduction-ready lentiviral particle format.

Individual miRIDIAN shMIMIC microRNAs and controls are provided as concentrated, purified lentiviral particles.

Assess gene down-regulation at mRNA level and analyze by phenotypic or biochemical assays.

Formats available:

- Individual miRIDIAN shMIMIC microRNAs and controls are provided as concentrated, purified lentiviral particles.

- The arrayed miRIDIAN shMIMIC Lentiviral microRNA Library is provided as a triplicate set of all human microRNAs in 96-well plates in transduction-ready lentiviral particle format.
miRIDIAN shMIMIC microRNAs are available individually in tubes and as arrayed lentiviral particles in triplicate sets of 96-well plates. The arrayed library format allows high-throughput phenotypic or high-content screening of the entire human microRNA collection in difficult-to-transfect cells. Such screens can be performed with and without drug treatment or in genetically engineered cell backgrounds for microRNA-based drug discovery and development.

**Example of readouts:**
- QPCR for target identification
- High-content (multi-parametric) analysis
- Immunocytochemistry or ELISA
- Fluorescence activated cell sorting
- Live-cell imaging
- Biochemical assays

Arrayed screening-ready microRNA library provided in triplicate

Add required amount of virus to target cells based on transduction efficiency and multiplicity of infection requirement

Assay for gene down-regulation and microRNA-based cellular events

MicroRNA regulation and biology  Therapeutic development  Host-pathogen interactions  Biomarker discovery  Pathway analysis  microRNA target identification
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