Functional Analysis

# miRCURY LNA™ microRNA Mimics

## Instruction manual v1.0

April 2014

EXIQON

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# Table of Contents

# Product summary

## Content

miRCURY LNA™ microRNA mimics for *in vitro* studies:

**Table 1.**

| Product number | Product name | Product description |
|---|---|---|
| 470000-001 - 479000-001 | miRBase name | miRCURY LNA™ microRNA Mimic, 5 nmol |
| 470000-004 - 479000-004 | miRBase name | miRCURY LNA™ microRNA Mimic, 20 nmol |
| 479902-001 | Cel-miR-39-3p | miRCURY LNA™ microRNA Mimic Negative Control, 5 nmol |
| 479902-004 | Cel-miR-39-3p | miRCURY LNA™ microRNA Mimic Negative Control, 20 nmol |
| 479903-001 | Negative Control 4 | miRCURY LNA™ microRNA Mimic Negative Control, 5 nmol |
| 479903-004 | Negative Control 4 | miRCURY LNA™ microRNA Mimic Negative Control, 20 nmol |
| 479904-001 | Negative Control 5 | miRCURY LNA™ microRNA Mimic Negative Control, 5 nmol |
| 479904-004 | Negative Control 5 | miRCURY LNA™ microRNA Mimic Negative Control, 20 nmol |

## Additional required material

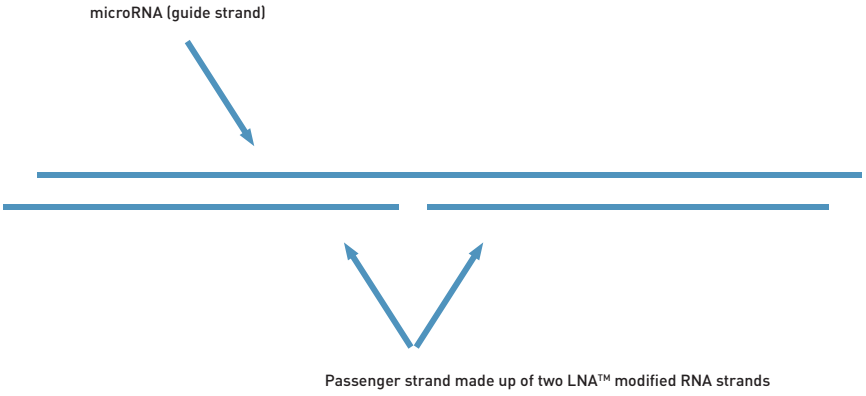
- DNase- and RNase-free $H_2O$
- Microcentrifuge
- DNase- and RNase-free microcentrifuge tubes or microtiter plate
- DNase- and RNase-free sterile filtered pipette tips
- Cell culture plates
- Cell culture medium
- Transfection reagent

## Product description

miRCURY LNA™ microRNA Mimics are designed to simulate naturally occuring mature microRNAs. Introduction of a microRNA mimic into cells will increase the proportion of RNA induced silencing complexes (RISC) containing this particular miRNA. By studying the phenotypical consequences of this increased microRNA activity it is possible to discover microRNA functions.

miRCURY LNA™ microRNA mimics have a unique and novel innovative design[1]. They are based on three RNA strands rather than the two RNA strands that characterize traditional miRNA mimics. The microRNA (guide) strand is a non-modified RNA strand with a sequence corresponding exactly to the annotation in miRBase[2,3]. However the passenger strand is split in two LNA™ enhanced RNA strands (see figure 1).

**Figure 1. miRCURY LNA™ microRNA Mimics consists of three RNA strands.** An unmodified microRNA (guide) strand with sequence exactly according to miRBase annotation. The passenger strand is split in two LNA™ modified RNA strands complementary to the microRNA strand.



Correctly designed such triple RNA strand RNA mimics are as potent as traditional double strand RNA mimics. The great advantage is that the segmented nature of the passenger strand ensures that only the microRNA strand is loaded into the RNA induced silencing complex (RISC) with no resulting microRNA activity from the passenger strand. Phenotypical changes observed with miRCURY LNA™ microRNA Mimics can therefore be safely ascribed to the microRNA simulated by the mimic (see figures 2-3).

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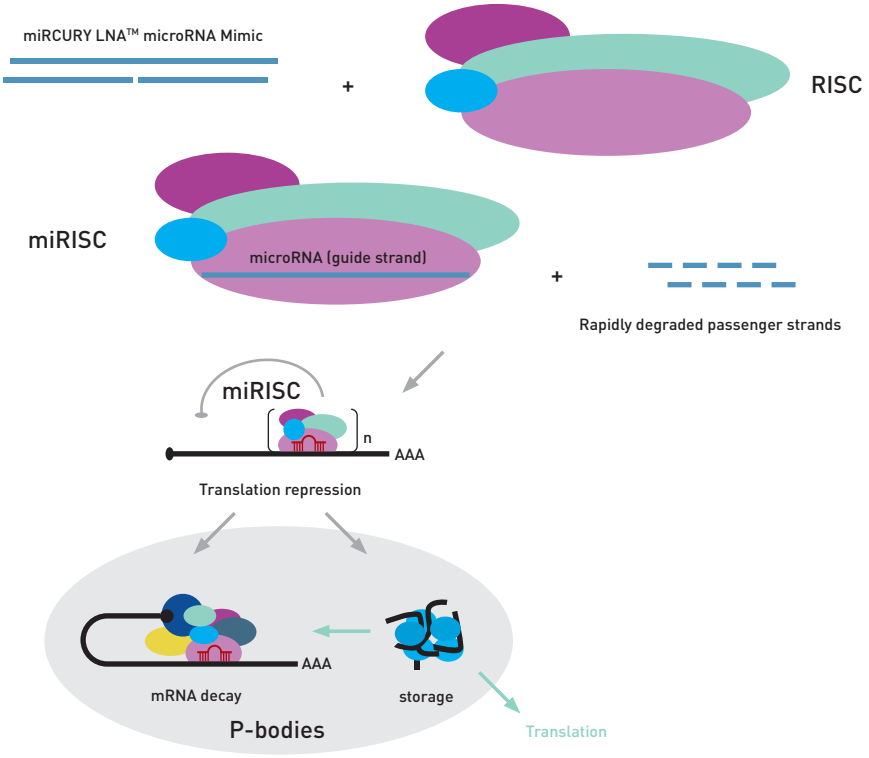
**Figure 2. The distinct RNA triple RNA strand design ensures completely specific microRNA mimicry.** Only the microRNA strand is incorporated by RISC. The two passenger strands are too short to act as microRNAs and are rapidly degraded after displacement from the microRNA strand. Off-target effects from the passenger strands are therefore minimal with miRCURY LNA microRNA Mimics.



The distinct triple RNA strand design is enabled by incorporation of high affinity LNA™ nucleotides into the two passenger strands. The sequence, length and LNA™ spiking pattern of the two segmented passenger strands has been optimized by a sophisticated empirically derived design algorithm.

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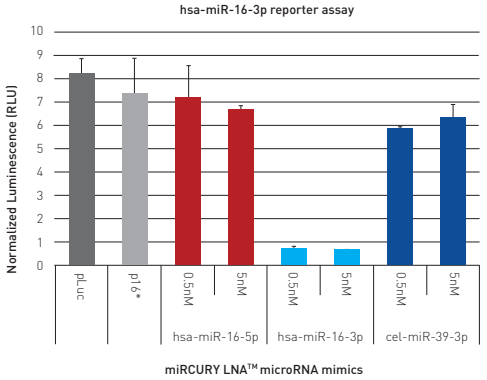
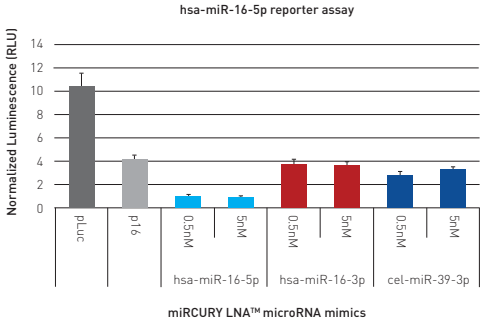
**Figure 3. Perfect microRNA strand specific activity with miRCURY LNA™microRNA Mimics.** Hela cells harboring hsa-miR-16-3p (top panel) and hsa-miR-16-5p (bottom panel) luciferase reporter plasmids respectively were transfected with hsa-miR-16-3p and hsa-miR-16-5p mimics and Cel-miR-39-3p negative mimic control. The results demonstrate that suppression of luciferase actity is only achieved with the miRCURY LNA™ microRNA Mimic corresponding to the reporter assay.



## Applications

MicroRNA mimics serve to simulate the natural functions of endogenous microRNAs and are primarily used in gain-of-function studies by assessing the biological consequences of increasing microRNA activity.

The effect of increasing the cellular content of a microRNA (by using microRNA mimics) can be studied in numerous ways, such as using cellular assays to monitor cell proliferation, cell differentiation, or apoptosis. The effect on gene expression can also be measured at the mRNA or protein level of putative microRNA targets.

microRNA mimics are also frequently used for validating microRNA targets in combination with microRNA inhibitors and target site blockers. Typically plasmid based assays are used where the 3'UTR of the mRNA under investigation has been cloned downstream of a reporter gene. Introduction of the mimic into cells harboring the reporter plasmid will reduce reporter gene expression, while microRNA inhibitors and target site blockers masking the microRNA binding site in the 3'UTR will cause derepression.

## Shipping and storage

This product is shipped dried down at room temperature. The unopened vial should be stored at -20°C or below. Shelf life is at least 6 months after shipping date when stored in this manner. Exposure to higher ambient temperatures during shipment does not pose any risk to the stability of the oligonucleotides.

Oligonucleotides are degraded by repeated freeze-thaw cycles, especially when in solution. After resuspension, it is recommended to aliquot the product before storage at -20°C to -80°C. For storage at -20°C, please use a constant temperature freezer.

**Do not store in frost-free freezer with automatic thaw-freeze cycles.**

# Protocol

**Important note**

LNA™-containing RNA oligonucleotides are susceptible to degradation by exogenous nucleases introduced during handling. Wear powder-free gloves when handling this product. Use DNase- and RNase-free reagents and filter pipette tips. Whenever possible, work should be conducted under a tissue culture hood.

## Resuspension

| | |
|---|---|
| **Step 1** | Briefly centrifuge the screw cap vial at low speed (maximum 4,000 x g) to make sure that all material is collected at the bottom of the wells before removing the cap in step 2. |
| **Step 2** | Remove screw cap carefully. |
| **Step 3** | With 5nmol and 20nmol product add 75µL and 300µL of nuclease free, sterile H2O respectively which will yield a concentration of 66,67 uM* |
| **Step 4** | Let the vial stand for a few minutes at ambient temperature. |
| **Step 5** | Gently pipette up and down 5 times to resuspend. |
| **Step 6** | Repeat steps 4 and 5. |

*It is important to use RNase-free H2O, and not any other kind of buffer and to respect the indicated volumes. This is because the dried down product contains salts that in the correct concentrations (30 mM HEPES 100 mM Potassium Acetate pH 7.5) are ideal for stabilizing the triple RNA strand complex.

| | |
|---|---|
| **Step 7** | We recommend aliquoting the mimics solution into sister DNase- and RNase-free tubes to limit the number of thaw-freeze cycles. |
| **Step 8** | Store at –20°C. |
| **Step 9** | Avoid thaw-freezing more than 5 times. |

## Transfection guidelines

Transfection efficiency varies according to cell type and the transfection reagent used. The optimal combination of cell type, transfection reagent and transfection conditions must be determined empirically. Optimizing transfection efficiencies is crucial for maximizing intended microRNA mimic activity while minimizing secondary effects. Expect to spend some time finding the optimal transfection conditions.

One way of determining the optimal transfection conditions is to use a reporter plasmid in which expression of a reporter gene is regulated by the endogenous microRNA level in the chosen cell line through a microRNA target site in the 3'UTR. The effect of transfecting a microRNA mimic can be assessed by measuring the inhibition of reporter gene expression caused by increased microRNA activity. Typically, this type of experiment also involves a second reporter gene for normalization of variation in plasmid transfection efficiency. Reporter plasmids with microRNA target cloning sites in the 3'UTR of reporter genes are commercially available from several companies.

Alternatively, if they are known, endogenous microRNA targets can be used as microRNA activity assays (Western blot or qRT-PCR) without the need for reporter plasmids.

Optimal transfection conditions are found by identifying efficient transfection reagents for each cell line and by adjusting:

- Amount of transfection reagent
- Amount of microRNA mimics (and reporter plasmid, if relevant)
- Optimized condition for co-transfection with a reporter plasmid
- Cell density at time of transfection
- Order of transfection (plating cells before transfection or plating cells at the moment of transfection)
- Length of exposure of cells to transfection reagent/oligonucleotide complex

Transfection conditions can also be optimized with a well characterized microRNA mimic or siRNA that induces a quantifiable phenotype.

Table 2.

| Cell culture plate | 96 well | 24 well | 12 well | 6 well |
|---|---|---|---|---|
| Transfection reagent [A] | 0.3 – 1.0 µL | 0.3 – 1.0 µL | 0.3 – 1.0 µL | 0.3 – 1.0 µL |
| microRNA mimic [B] | 0.5 pmole | 2.5 pmole | 5 pmole | 15 pmole |
| Cell density (cells/well) [C] | 6000 | 40000 | 80000 | 240000 |
| Final volume per well | 100 µL | 500 µL | 1000 µL | 3000 µL |

[A] Refer to the instructions provided by the transfection reagent supplier.
[B] The amount shown yields a microRNA mimic concentration of 5 nM.
[C] Optimal cell density varies with the cell type depending on cell size and growth characteristics. In general, 30 – 70% confluency is recommended.

Most protocols recommend maintaining mammalian cells in the medium used for transfection for 24 hours. The transfection medium should then be replaced with fresh medium to maximize viability of the cell culture. Normally, miRCURY LNA™ microRNA Mimics display potent activity at final concentrations between 0.05-5 nM, but a more extensive range of 0.005-50 nM can be analyzed in optimization experiments. The optimal time for analysis of the effect of transfection

must be determined experimentally. However, microRNA mimic effects are normally assessed 24-72 hours after transfection. For some applications, such as cell differentiation assays the phenotypic readout may take place 7-10 days after transfection.

## Electroporation

miRCURY LNA™ microRNA mimics can also be introduced into cells by electroporation. This is especially useful with cells that are notoriously difficult to transfect (i.e. non adherent cells such a lymphocytes, bone marrow stem cells and primar y cancer cells). Please follow the instructions provided with the electroporation system.

**Important note**

When using elevated concentrations of microRNA mimics (as is true also for siRNA) it is possible to saturate RISC and thereby affect normal endogenous microRNA regulation[4]. In addition at sufficiently high concentrations all oligonucleotides are cytotoxic. MicroRNA functional analysis using miRCURY LNA™ microRNA mimics should therefore only be performed under optimized transfection conditions with the minimal required mimic concentration. It is recommended to carry out careful dose response experiments in order to determine the threshold concentration where the advantage of increasing the dose is cancelled out by beginning symptoms adverse effects with a negative impact on the phenotypic readout (bell shaped dose response curves). A way of assessing undesired effects due to saturation of RISC is to analyse in parallel the activity of a different endogenous miRNA – (for example by using a reporter plasmid for another miRNA). Derepression of targets that are regulated by other endogenous microRNAs is an indication of RISC saturation. Always remember to perform adequate controls to ensure that the resulting phenotype is due increased activity of the mimicked microRNA.

# Related products

Exiqon offers a broad variety of tools enabling new discoveries concerning the expression, function and spatial distribution of microRNAs:

**miRCURY LNA™ microRNA Inhibitors, Power Inhibitors and Family Inhibitors**
Unravel the function of microRNAs by microRNA inhibition. Sophisticated LNA™ design ensures potent inhibition of all microRNAs regardless of their GC content. Chemically modified, highly stable Power Inhibitors for unrivalled potency. Available for in vitro and in vivo studies.

**miRCURY LNA™ microRNA Inhibitor Negative Controls**
These oligonucleotides are designed to have no known microRNA targets in miRBase. The oligonucleotides are provided unlabeled or fluorescence labeled.

**miRCURY LNA™ microRNA Inhibitor Library**
For genome-wide high throughput screening of microRNA function.

**miRCURY LNA™ Target Site Blockers**
For inhibition of microRNA binding to a specific mRNA target.

**LNA™ longRNA GapmeR**
LNA™ gapmers are potent antisense oligonucleotides used for highly efficient inhibition of mRNA and lncRNA function. Designed using advanced algorithms, the RNase H-activating LNA™ gapmers offer excellent performance and high success rate.

**miRCURY LNA™ Universal RT microRNA PCR**
Fast and accurate determination of microRNA expression with real-time PCR.

**miRCURY LNA™ microRNA Array, microarray kit and Power labeling kit**
Pre-printed miRCURY LNA™ microRNA Array microarray slides, available for hsa, mmu & rno and other species. Kit includes hybridization and wash buffers as well as synthetic spike-in microRNAs. Power Labeling kit for fluorescent labeling of microRNAs from total RNA samples.

**miRCURY LNA™ microRNA Detection Probes**
For in situ hybridization and northern blotting of all annotated microRNAs.

# References

1. Bramsen JB, Laursen MB, Damgaard CK, et al. Improved silencing properties using small internally segmented interfering RNAs. Nucleic Acids Research 2007, 35, 5886-5897, PubMed PMID: 17726057
2. Griffiths-Jones, S. The microRNA Registry. Nucleic Acids Research 2004, 32, Database Issue, D109-11
3. miRBase: w w w.mirbase.org
4. Kahn AA, et al.Transfection of small RNAs globally perturbs gene regulation by endogenous microRNAs, Nature Biotechnology, 2009 Jun;27(6):549-55. doi: 10.1038/nbt.1543

**www.exiqon.com**

**Literature citations**
Please refer to the relevant product: miRCURY LNA™ microRNA Inhibitor, miRCURY LNA™ microRNA Power Inhibitor, miRCURY LNA™ microRNA Family Inhibitor, miRCURY LNA™ microRNA Power Family Inhibitor, miRCURY LNA™ microRNA Target Site Blocker or miRCURY LNA™ microRNA Power Target Site Blocker when describing a procedure for publication using one or more of these products when describing a procedure for publication using this product.

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