October 2017

miRCURY® LNA® miRNA Mimics Handbook

LNA-optimized oligonucleotides for use in miRNA functional studies

Sample to Insight

QIAGEN

Contents

Kit Contents

Product	Cat. no.	Amount supplied
miRCURY LNA miRNA Mimic (5 nmol)	339173	5 nmol oligonucleotide, dried down in tube format
miRCURY LNA miRNA Mimic (20 nmol)	339174	20 nmol oligonucleotide, dried down in tube format
miRCURY LNA Premium miRNA Mimic (5 nmol)	339178	5 nmol oligonucleotide, dried down in tube format
miRCURY LNA Premium miRNA Mimic (20 nmol)	339179	20 nmol oligonucleotide, dried down in tube format
miRCURY LNA miRNA Mimic Negative Control Cel-miR-39-3p (5 nmol)	Varies YM00479902–	5 nmol oligonucleotide, dried down in tube format
miRCURY LNA miRNA Mimic Negative Control Cel-miR-39-3p (20 nmol)	Varies YM00479902–	20 nmol oligonucleotide, dried down in tube format
miRCURY LNA miRNA Mimic Negative Control 4 (5 nmol)	Varies YM00479903–	5 nmol oligonucleotide, dried down in tube format
miRCURY LNA miRNA Mimic Negative Control 4 (20 nmol)	Varies YM00479903–	20 nmol oligonucleotide, dried down in tube format
miRCURY LNA miRNA Mimic Negative Control 5 (5 nmol)	Varies YM00479904–	5 nmol oligonucleotide, dried down in tube format
miRCURY LNA miRNA Mimic Negative Control 5 (20 nmol)	Varies YM00479904–	20 nmol oligonucleotide, dried down in tube format

Storage

The miRCURY LNA miRNA Mimics are shipped at room temperature. Unopened vials should be stored at –15 to –30°C or below. Mimics with fluorescent labels should be protected from light to avoid bleaching. When stored in this manner, they will remain stable at least 6 months after the shipping date. 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. It is recommended to aliquot and store the product at –15 to –30°C or below in a constant-temperature freezer after re-suspension to avoid repeated freeze-thaw cycles. Do not store in frost-free freezers with automatic thaw-freeze cycles.

Intended Use

The miRCURY LNA miRNA Mimics are intended for molecular biology applications. These products are not intended for the diagnosis, prevention or treatment of a disease.

All due care and attention should be exercised in the handling of the product. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of miRCURY LNA miRNA Mimics is tested against predetermined specifications to ensure consistent product quality.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at **www.qiagen.com/safety** where you can find, view and print the SDS for each QIAGEN kit and kit component.

Introduction

miRCURY LNA miRNA Mimics are designed to simulate naturally occurring, mature miRNAs. Introduction of an miRNA mimic into cells will increase the proportion of RNA-induced silencing complexes (RISC) containing that particular miRNA. By studying the phenotypical consequences of this increased miRNA activity, it is possible to discover miRNA functions.

miRCURY LNA miRNA 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 miRNA (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 into two LNA-enhanced RNA strands (see Figure 1).

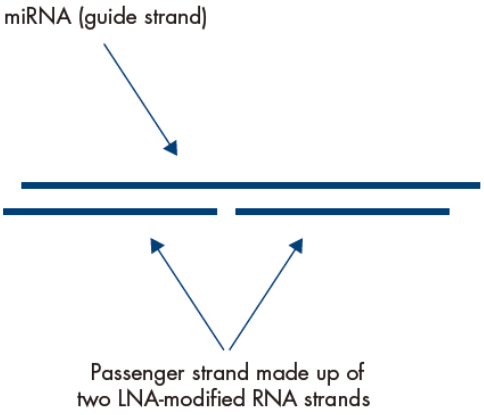

Figure 1. miRCURY LNA miRNA Mimics consists of three RNA strands. An unmodified miRNA (guide) strand has sequence exactly according to the miRBase annotation. The passenger strand is split into two LNA-modified RNA strands that are complementary to the miRNA strand.

When 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 miRNA strand is loaded into the RNA-induced silencing complex (RISC) with no resulting miRNA activity from the passenger strand. Phenotypical changes observed with miRCURY LNA miRNA Mimics can therefore be safely ascribed to the miRNA simulated by the mimic (see Figure 2 and Figure 3).

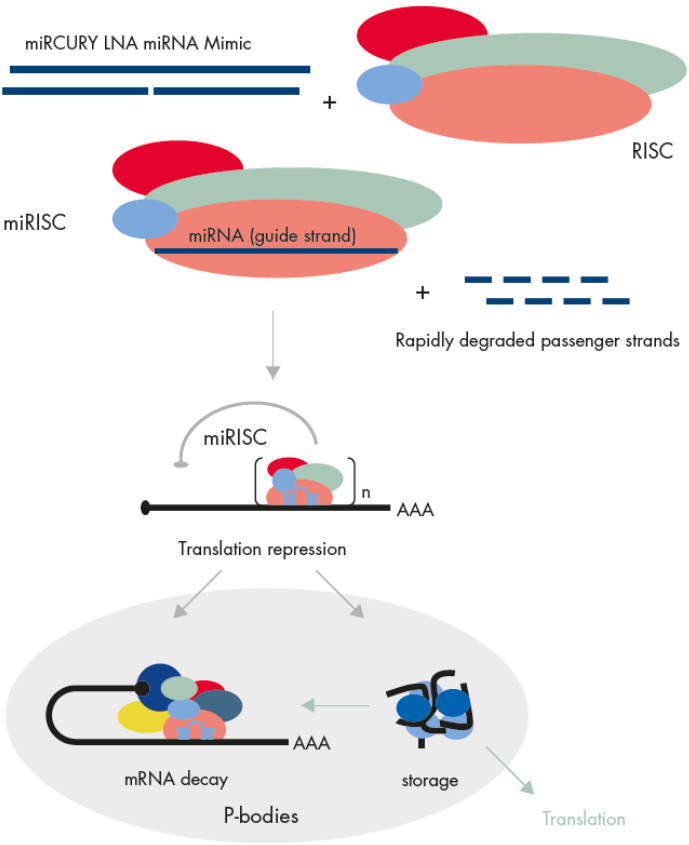

Figure 2. The distinct triple RNA strand design ensures specific miRNA mimicry. Only the miRNA strand is incorporated by the RISC. The two passenger strands are too short to act as miRNAs and are rapidly degraded after displacement from the miRNA strand. Off-target effects from the passenger strands are therefore minimal with miRCURY LNA miRNA 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 using a sophisticated, empirically derived design algorithm.

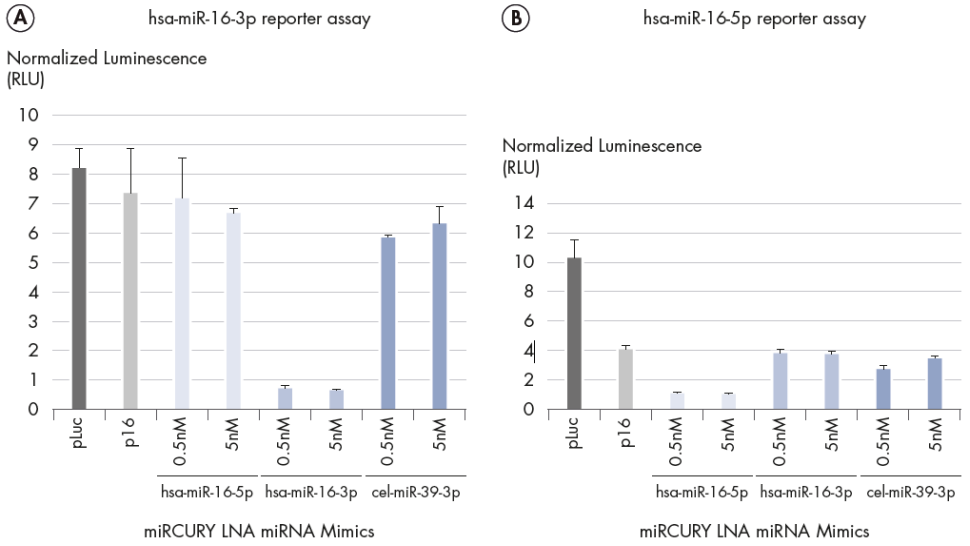

Figure 3. Specific miRNA strand activity with miRCURY LNA miRNA Mimics. HeLa cells harboring hsa-miR-16-3p (A) and hsa-miR-16-5p (B) luciferase reporter plasmids were transfected with hsa-miR-16-3p and hsa-miR-16-5p mimics, respectively, and the cel-miR-39-3p negative mimic control. The results demonstrate that suppression of luciferase activity is only achieved with the miRCURY LNA miRNA Mimic corresponding to the reporter assay.

We currently offer three different negative miRCURY LNA miRNA Mimic Controls (cat. nos. YM00479902–, YM00479903– and YM00479904–) with the same design features as our predesigned miRNA mimics. The guide strands of these controls have no homology to any known miRNA or mRNA sequences in mouse, rat or human and have been tested for adverse effect in multiple cell lines.

Principle and workflow

miRNA mimics simulate the natural functions of endogenous miRNAs and are primarily used in gain-of-function studies, enabling the assessment of the biological consequences of increasing miRNA activity. The effect of increasing the cellular content of an miRNA (by using miRNA 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 of putative miRNA targets can also be measured at the mRNA or protein level.

miRNA mimics are also frequently used for validating miRNA targets in combination with miRNA inhibitors and target site blockers. Typically, plasmid-based assays are used in which the 3′ UTR of the mRNA under investigation has been cloned downstream of a reporter gene. Introducing the mimic into cells harboring the reporter plasmid will reduce reporter gene expression, while miRNA inhibitors and target site blockers masking the miRNA binding site in the 3′ UTR will cause de-repression.

LNA technology

Locked nucleic acids (LNA) are a class of high-affinity RNA analogs in which the ribose ring is "locked" in the ideal conformation for Watson-Crick binding. As a result, LNA oligonucleotides exhibit unprecedented thermal stability when hybridized to a complementary DNA or RNA strand. For each incorporated LNA monomer, the melting temperature (T_m) of the duplex increases by 2–8°C. In addition, LNA oligonucleotides can be made shorter than traditional DNA or RNA oligonucleotides and still retain a high T_m. This is important when the oligonucleotide is used to detect small or highly similar targets.

Since LNA oligonucleotides typically consist of a mixture of LNA and DNA or RNA, it is possible to optimize the sensitivity and specificity by varying the LNA content of the oligonucleotide. Incorporation of LNA into oligonucleotides has been shown to improve sensitivity and specificity for many hybridization-based technologies including PCR, microarray and *in situ* hybridization.

Robust detection of all miRNA sequences, regardless of GC content.

The small sizes and widely varying GC content (5–95%) of miRNAs make them challenging to analyze using traditional methods. DNA- or RNA-based methods for miRNA analysis can introduce high uncertainty and low robustness, because the T_m of the oligonucleotide/miRNA

duplex will vary greatly depending on the GC content of the sequences. This is especially problematic in applications such as microarray profiling and high-throughput experiments in which many miRNA targets are analyzed under the same experimental conditions.

Use of LNA-enhanced oligonucleotides overcomes these challenges. By simply varying the LNA content, oligonucleotides with specific duplex melting temperatures can be designed, regardless of the GC content of the miRNA. T_m-normalized primers, probes and inhibitors all perform well under the same experimental conditions.

Specific discrimination of highly similar targets

Another challenge of studying miRNAs is the high degree of similarity between the sequences. Some miRNA family members vary by only a single nucleotide. LNA can be used to enhance the discriminatory power of primers and probes to allow excellent discrimination of closely related miRNA sequences. LNA offers significant improvement in sensitivity and specificity and ensures optimal performance for all miRNA targets.

An LNA oligonucleotide offers substantially increased affinity for its complementary strand, compared to traditional DNA or RNA oligonucleotides. This results in unprecedented sensitivity and specificity and makes LNA oligonucleotides ideal for the detection of small or highly similar DNA or RNA targets.

Important Notes

Optimal concentration of miRNA mimics

When using elevated concentrations of miRNA mimics (as is also true for siRNA), it is possible to saturate RISC, thereby affecting normal endogenous miRNA regulation (4). In addition, at sufficiently high concentrations, all oligonucleotides are cytotoxic. miRNA functional analysis using miRCURY LNA miRNA Mimics should therefore only be performed under optimized transfection conditions with the minimum required mimic concentration. We recommend that you carry out careful dose response experiments to determine the threshold concentration at which the advantage of increasing the dose is cancelled out by the occurrence of adverse effects that negatively impact the phenotypic readout (e.g., bell-shaped dose-response curves). One way to assess undesired effects due to RISC saturation is to analyze the activity of a different endogenous miRNA in parallel, for example, by using a reporter plasmid for another miRNA. De-repression of targets that are regulated by other endogenous miRNAs 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 miRNA.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. For more information, consult the appropriate safety data sheets (SDSs) available from the product supplier.

Additional required materials:

- DNase- and RNase-free water
- 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

Protocol: Resuspension and Transfection

Important notes before starting

miRCURY LNA miRNA Mimics are made of RNA and are therefore particularly susceptible to degradation by exogenous nucleases introduced during handling. Wear powder-free gloves when handling the miRCURY LNA miRNA Mimics. Use DNase- and RNase-free reagents and filter pipette tips. Whenever possible, work should be conducted under a tissue culture hood.

Resuspension of miRCURY LNA miRNA Mimics

1. Briefly centrifuge the screw cap vial at low speed (maximum 4000 *x g*) to ensure that all material is collected at the bottom of the vial before opening it.
2. Carefully remove the screw cap from the vial.
3. For 5 nmol miRNA mimics, add 75 µl nuclease-free, sterile water. For 20 nmol miRNA mimics, add 300 µl nuclease-free, sterile water. These volumes yield a concentration of 66.67 µM.

   Important: Do not resuspend the mimics in other volumes, and do not use resuspension buffers other than nuclease-free, sterile water. The dried-down product contains salts that must be diluted to the optimal concentration (30 mM HEPES, 100 mM Potassium Acetate pH 7.5) for stabilizing the triple RNA strand complex.
4. Let the vial stand for a few minutes at ambient temperature.
5. Gently pipette up and down 5 times to resuspend.
6. Repeat steps 4 and 5.
7. Aliquot the resuspended miRNA mimics into multiple tubes to limit the number of freeze-thaw cycles. Store at –20°C.

   Note: Avoid freeze-thawing more than 5 times.

Transfection guidelines

Transfection efficiency varies according to the cell type and 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 miRNA mimic activity, while minimizing secondary effects. Expect to spend some time finding the optimal transfection conditions.

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

Alternatively, if they are known, endogenous miRNA targets can be used as miRNA activity assays (e.g., 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 the following parameters:

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

Transfection conditions can also be optimized with a well-characterized miRNA mimic that induces a quantifiable phenotype.

Table 1. General transfection guidelines.

Cell culture plate	96 well	24 well	12 well	6 well
Transfection reagent*	0.3–1.0 µl	1–3 µl	2–4 µl	3–36 µl
miRCURY LNA miRNA Mimic†	0.5 pmol	2.5 pmol	5 pmol	15 pmol
Cell density (cells/well)‡	6,000	40,000	80,000	240,000
Final volume per well	100 µl	500 µl	1000 µl	3000 µl

* Refer to the instructions provided by the transfection reagent supplier.

† The amount shown yields an miRNA mimic concentration of 5 nM.

‡ 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 miRNA Mimics display potent activity at final concentrations of 0.05–5 nM, but a more extensive range of 0.005–50 nM can be analyzed in optimization experiments. The optimal time for analyzing the effect of transfection must be determined experimentally. Normally, miRNA mimic effects are 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 miRNA Mimics can also be introduced into cells by electroporation. This is especially useful with cells that are notoriously difficult to transfect (e.g., non-adherent cells such a lymphocytes, bone marrow stem cells and primary cancer cells). Follow the instructions provided with your electroporation system.

References

1. Bramsen, J.B., et al. (2007) Improved silencing properties using small internally segmented interfering RNAs. Nucleic Acids Research **35**:5886–5897. PMID: 17726057.
2. Griffiths-Jones, S. (2004) The miRNA Registry. Nucleic Acids Research Database Issue **32**:D109–111.
3. miRBase: www.mirbase.org
4. Kahn, A.A., et al. (2009) Transfection of small RNAs globally perturbs gene regulation by endogenous miRNAs. Nature Biotechnology **27(6)**:549–555. doi: 10.1038/nbt.1543.

Ordering Information

Product	Contents	Cat. no.
miRCURY LNA miRNA Mimic (5 nmol)	5 nmol oligonucleotide, dried down in tube format	339173*
miRCURY LNA miRNA Mimic (20 nmol)	20 nmol oligonucleotide, dried down in tube format	339174*
miRCURY LNA Premium miRNA Mimic (5 nmol)	5 nmol oligonucleotide, dried down in tube format	339178*
miRCURY LNA Premium miRNA Mimic (20 nmol)	20 nmol oligonucleotide, dried down in tube format	339179*
Negative control mimics		
miRCURY LNA miRNA Mimic Negative Control Cel-miR-39-3p (5 nmol)	5 nmol oligonucleotide, dried down in tube format	Varies YM00479902-
miRCURY LNA miRNA Mimic Negative Control Cel-miR-39-3p (20 nmol)	20 nmol oligonucleotide, dried down in tube format	Varies YM00479902-
miRCURY LNA miRNA Mimic Negative Control 4 (5 nmol)	5 nmol oligonucleotide, dried down in tube format	Varies YM00479903-
miRCURY LNA miRNA Mimic Negative Control 4 (20 nmol)	20 nmol oligonucleotide, dried down in tube format	Varies YM00479903-
miRCURY LNA miRNA Mimic Negative Control 5 (5 nmol)	5 nmol oligonucleotide, dried down in tube format	Varies YM00479904-

Product	Contents	Cat. no.
miRCURY LNA miRNA Mimic Negative Control 5 (20 nmol)	20 nmol oligonucleotide, dried down in tube format	Varies YM00479904-
Related products		
miRCURY LNA miRNA Inhibitor (5 nmol)†	5 nmol oligonucleotide, dried down in tube format; no label or 5' or 3' FAM; normal phosphodiester bonds	339121*
miRCURY LNA miRNA Power Inhibitor (5 nmol)†	5 nmol oligonucleotide, dried down in tube format; no label or 5' or 3' FAM; phosphorothioate-modified backbone	339131*
miRCURY LNA miRNA Family Power Inhibitor	5 nmol oligonucleotide set, dried down in tube format; no label; phosphorothioate-modified backbone	339160*
miRCURY LNA miRNA Power Target Site Blocker (5 nmol)†	5 nmol oligonucleotide, dried down in tube format; no label; phosphorothioate-modified backbone	339194*
miRCURY LNA miRNA Power Target Site Blocker, *in vivo* ready (5 nmol)†	5 nmol oligonucleotide, dried down in tube format; no label; phosphorothioate-modified backbone	339199*
miRCURY LNA miRNA PCR Assay	LNA-optimized PCR assay for miRNA quantification; for 200 reactions	339306*
miRCURY LNA miRNA Custom PCR Assay	Custom-designed and LNA-optimized PCR assay for miRNA quantification; for 200 reactions	339317*
miRCURY LNA RT Kit	5x RT Reaction Buffer, 10x RT Enzyme Mix, UniSp6, RNA Spike-in template, RNase-free water; for 8–64 reactions	339340

* The exact product numbers vary, depending on the particular product ordered and its specifications.

† Other product sizes available; visit **www.qiagen.com** for more details.

Product	Contents	Cat. no.
miRCURY LNA miRNA PCR Starter Kit	2 miRCURY LNA PCR Assays of your choice, UniSp6 Spike-in control assay, miR-103-3p endogenous control assay, 5x RT Reaction Buffer, 10x RT Enzyme Mix, UniSp6 RNA Spike-in template, RNase-free water, 2x miRCURY SYBR Green Master Mix; for 20 RT reactions and 100 PCR amplifications	339320
miRCURY LNA SYBR Green PCR Kit (200)†	2x miRCURY SYBR Green PCR Master Mix, miRCURY SYBR Green PCR Buffer and dNTP mix (dATP, dCTP, dGTP, dTTP), ROX Reference Dye, Nuclease-free Water; for 200 reactions	339345

* The exact product numbers vary, depending on the particular product ordered and its specifications.
† Other product sizes available; visit **www.qiagen.com** for more details.

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Notes

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