October 2017

miRCURY® LNA® miRNA Inhibitors and Target Site Blockers Handbook

LNA-optimized antisense oligonucleotides for use in miRNA functional studies

Sample to Insight

QIAGEN

Contents

Kit Contents

The exact product numbers vary, depending on the particular inhibitor or target site blocker ordered and the product specifications.

miRCURY LNA miRNA Inhibitors:

Product	Cat. nos.	Amount supplied	Label
miRCURY LNA miRNA Inhibitor	339120 339121 339122	1 nmol, 5 nmol or 15 nmol oligonucleotide, dried down in tube format	No label or 5′ or 3′ FAM for 5 nmol scale
miRCURY LNA miRNA Power Inhibitor	339130 339131 339132	1 nmol, 5 nmol or 15 nmol oligonucleotide, dried down in tube format	No label or 5′ or 3′ FAM for 5 nmol scale
miRCURY LNA miRNA Inhibitor Control	339125 339126 339127	1 nmol, 5 nmol or 15 nmol oligonucleotide, dried down in tube format	No label or 5′ or 3′ FAM for 5 nmol scale
miRCURY LNA miRNA Power Inhibitor Control	339135 339136 339137	1 nmol, 5 nmol or 15 nmol oligonucleotide, dried down in tube format	No label or 5′ or 3′ FAM for 5 nmol scale
miRCURY LNA miRNA Family Power Inhibitor	339160	5 nmol oligonucleotide set, dried down in tube format	No label

miRCURY LNA miRNA Target Site Blockers:

Product	Cat. nos.	Amount supplied	Label
miRCURY LNA miRNA Power Target Site Blocker	339194 339195	5 nmol or 15 nmol oligonucleotide, dried down in tube format	No label
miRCURY LNA miRNA Power Target Site Blocker, *in vivo* ready	339199 339200	5 nmol or 15 nmol oligonucleotide, dried down in tube format	No label

Storage

The miRCURY LNA miRNA Inhibitors and Target Site Blockers are shipped at room temperature. Unopened vials should be stored at –15 to –30°C or below. Fluorescence-labeled oligonucleotides 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 store the miRCURY LNA miRNA Inhibitors and Target Site Blockers in aliquots 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 Inhibitors and Target Site Blockers 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.

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.

Quality Control

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

Introduction

miRCURY LNA miRNA Inhibitors

miRCURY LNA miRNA Inhibitors are antisense oligonucleotides with perfect sequence complementary to their target. When introduced into cells, they sequester their target miRNA in highly stable heteroduplexes, effectively preventing the miRNA from hybridizing with its normal cellular interaction partners.

Three different categories of miRNA inhibitors are available, in different amounts with different labeling options and nucleotide backbones:

- miRCURY LNA microRNA Inhibitors: Pre-designed LNA-enhanced miRNA inhibitors. These DNA/LNA mixmer antisense oligonucleotides have normal phosphodiester nucleotide bonds.
- miRCURY LNA miRNA Power Inhibitors: Power inhibitors have a fully phosphorothioate (PS) modified backbone, which makes them highly resistant to enzymatic degradation. As a result, they have superior potency and greatly increased stability. Efficient inhibition of miRNA activity can even be achieved by simple addition of Power inhibitors directly to cell cultures. This allows you to study the consequences of miRNA silencing without confounding side effects from the transfection reagents. In all other respects, this class of inhibitors is identical to the regular line of miRNA inhibitors.
- Custom miRCURY LNA miRNA Inhibitors: Custom designed antisense sterical blockers of miRNA and other short ncRNAs not in miRbase.

Four pre-designed negative controls for use with the miRNA Inhibitors are available: two miRCURY LNA miRNA Inhibitor Controls, with normal phosphodiester bonds and two miRCURY LNA miRNA Power Inhibitor Controls, with the phosphorothioate backbone to match the Power Inhibitors.

- Negative Control A (identical to the former Scramble-miR control): No hits of >70% homology to any sequence in any organism in the NCBI and miRBase databases.
- Negative Control B (identical to the former Sense miR-159 control): No hits of >70% homology to any human, mouse or rat sequence in the NCBI and miRBase databases. Not recommended for use in plants.

miRCURY LNA miRNA Power Target Site Blockers

miRCURY LNA miRNA Power Target Site Blockers are antisense oligonucleotides designed to compete with miRNA/RISC for an miRNA target site of a particular mRNA. When introduced into cells, a target site blocker will mask the miRNA target site by hybridizing strongly with it, effectively preventing the miRNA from interacting with the specific target mRNA of interest, without otherwise affecting the activity of the endogenous miRNA.

The sequences of the oligonucleotides and their LNA spiking patterns have been carefully designed to achieve uniform, high potency for all miRCURY LNA miRNA Inhibitors and miRCURY LNA miRNA Power Target Site Blockers, regardless of the GC-content of their target. This is accomplished by ensuring normalization of the melting temperature (T_m) around an optimal temperature, while keeping the level of self-complementarity to a minimum.

Principle and workflow

miRNA Inhibitors are primarily used for studying miRNA function by assessing the biological consequences of inhibiting miRNA activity. By a different mode of action, the miRNA target site blockers are also used to study miRNA function. Target site blockers enable evaluation of the biological consequences of blocking the interaction of an miRNA with a specific mRNA target.

The effect of inhibiting an miRNA (using miRNA inhibitors) or preventing the miRNA from binding to a particular mRNA target (using miRNA target site blockers) 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 miRNA targets.

Important Notes

Assessing miRNA knock-down by miRNA qPCR

miRNA qPCR is not a reliable method for measuring the level of miRNA knock-down. Although this method is often cited in the literature, it is not recommended. miRNA inhibitors do not degrade their targets; instead, they form stable complexes with their target, causing an accumulation of the miRNA due to reduced turnover. Moreover, failed transfections are often the result of accumulation of oligonucleotides inside vesicles such that the inhibitor and its miRNA target are present in different subcellular compartments.

Upon cell lysis, liberated, vesicular inhibitors will form strong heteroduplexes with their miRNA target, and therefore, efficient and inefficient transfections cannot be distinguished by miRNA qPCR. In addition, LNA oligonucleotides can interfere with PCR primers and give rise to aberrant results.

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:

- Nuclease-free TE buffer (10 mM Tris, 0.1 mM EDTA, pH 7.5 or 8.0)
- Microcentrifuge
- DNase-free microcentrifuge tubes or microtiter plate
- Cell culture plates
- Cell culture medium
- Transfection reagent

Protocol: Resuspension and Transfection

Important notes before starting

Oligonucleotides are susceptible to degradation by exogenous nucleases introduced during handling. Wear powder-free gloves when handling the miRCURY LNA miRNA Inhibitors and Target Site Blockers. Use DNase-free reagents and filter pipette tips. Whenever possible, work should be conducted under a tissue culture hood.

Resuspension of miRCURY LNA miRNA Inhibitors and Target Site Blockers

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. Add nuclease-free, sterile TE buffer using a pipette with a sterile filter tip to achieve the desired concentration. To prepare a 50 µM solution, add 100 µL TE buffer (10 mM Tris, pH 7.5 or 8.0, 1 mM EDTA) to 5 nmol miRNA inhibitor or target site blocker.

   Note: Stock solutions should not be lower than 10 µM.
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 inhibitor or target site blocker solution into multiple tubes to limit the number of freeze-thaw cycles. Store at –20°C.

   Note: Avoid freeze-thawing more than 5 times.

   Note: Working solutions can be stored at 4°C for a maximum of two weeks.

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 antisense 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 transfection can be assessed by measuring the relief of inhibition of reporter gene expression caused by miRNA inhibition (or by masking of the miRNA target site in the case of a target site blocker). 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, de-repression of endogenous miRNA targets (validated or predicted) can be measured at either the mRNA or protein level. 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 Inhibitor or Target Site Blocker
- 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 siRNA or an Antisense LNA GapmeR Positive Control. The siRNA or LNA GapmeR activity can be assessed by quantification of the RNA target by qRT-PCR analysis.

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
miRNA Inhibitor /Target Site Blocker†	5 pmol	25 pmol	50 pmol	150 pmo
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 a miRNA Inhibitor /Target Site Blocker concentration of 50 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, miRNA Inhibitors and Target Site Blockers display potent activity at final concentrations of 1–50 nM, but a more extensive range of 1–100 nM can be analyzed in optimization experiments. However, Power Inhibitors are frequently toxic at concentrations >50 nM. Always remember to perform adequate controls to ensure that the resulting phenotype is due to antisense inhibition of the targeted RNA. The optimal time for analysis of the effect of transfection must be determined experimentally. Normally, antisense 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.

At sufficiently high concentrations, all oligonucleotides are cytotoxic. The level of toxicity is sequence dependent, and the sensitivity of cell lines varies considerably. miRNA functional analysis should therefore only be performed under optimized transfection conditions with the minimum required inhibitor concentration. Dose-response experiments are often useful for determining the threshold concentration at which the advantage of increasing the dose is

cancelled out by beginning symptoms of toxicity that negatively affect the phenotypic readout (bell-shaped dose-response curves). Typically, the first signs of toxicity can be observed at concentrations of 50–100 nM. Always perform adequate controls to ensure that the resulting phenotype is due to antisense inhibition of the targeted miRNA.

Electroporation

miRCURY LNA miRNA Inhibitors and Target Site Blockers 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.

Non-assisted uptake

miRCURY LNA miRNA Power Inhibitors and Power Target Site Blockers have a fully phosphorothioate (PS)-modified backbone, which makes them highly resistant to enzymatic degradation. They are so stable and potent that they can be added directly to serum-containing culture medium without the need for transfection reagents. The short oligonucleotides are taken up naturally by cells, and efficient antisense activity can be achieved by unassisted "naked" delivery, also known as "gymnosis" (3, 4). However, higher concentrations are required, and the uptake kinetics are slower than when using transfection reagents. The efficiency of unassisted uptake is highly dependent on the cell line. Normally, oligonucleotide concentrations between 100 nM – 5 µM can product potent antisense activity, with the effects typically observed only after a 48–72 hour incubation. In general, unassisted delivery has no effect on cell viability or cell morphology and therefore, allows you to study the consequences of miRNA silencing without having to worry about confounding side effects from the transfection reagents. Unassisted delivery is a useful alternative when working with difficult-to-transfect cells and when normal transfection procedures (use of transfection reagents or electroporation) have unacceptable phenotypic consequences.

Optimal gymnotic conditions are determined by adjusting the following parameters:

- Amount of miRCURY LNA miRNA Power Inhibitor or Target Site Blocker : the miRNA Power Inhibitor or Target Site Blocker solution should be prepared at the final concentration (100 nM – 5 µM) in the appropriate fresh culture media before plating the cells.
- Cell density: to avoid problems associated with over-confluency of cells, the plating cell density can be appropriately decreased if a long incubation period is anticipated.
- Length of exposure of cells to the miRNA Power Inhibitor or Target Site Blocker: a general guideline for the incubation time is 1–6 days after initial treatment. If an incubation of longer than 6 days is required, it is recommended to replace the culture media with fresh media containing the miRNA Power Inhibitor or Target Site Blocker.

References

1. Griffiths-Jones, S. The miRNA Registry. Nucleic Acids Research 2004, 32, Database Issue, D109–111.
2. Torres, A.G., Fabani, M.M., Vigorito, E., and Gait, M.J. (2011) miRNA fate upon targeting with anti-miRNA oligonucleotides as revealed by an improved Northern-blot-based method for miRNA detection. RNA. **17**:933–943.
3. Stein, C.A. et al. (2010) Efficient gene silencing by delivery of locked nucleic acid antisense oligonucleotides, unassisted by transfection reagents. Nucleic Acids Research **38**(1):e3.
4. Soifer, H.S. et al. (2012) Silencing of gene expression by gymnotic delivery of antisense oligonucleotides. Methods Mol Biol. **815**:333.

Ordering Information

Product	Contents	Cat. no.
Inhibitors and controls		
miRCURY LNA miRNA Inhibitor (1 nmol)	1 nmol oligonucleotide, dried down in tube format; no label; normal phosphodiester bonds	339120*
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 Inhibitor (15 nmol)	15 nmol oligonucleotide, dried down in tube format; no label; normal phosphodiester bonds	339122*
miRCURY LNA miRNA Power Inhibitor (1 nmol)	1 nmol oligonucleotide, dried down in tube format; no label; phosphorothioate-modified backbone	339130*
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 Power Inhibitor (15 nmol)	15 nmol oligonucleotide, dried down in tube format; no label; phosphorothioate-modified backbone	339132*
miRCURY LNA miRNA Inhibitor Control (1 nmol)	1 nmol oligonucleotide, dried down in tube format; no label; normal phosphodiester bonds	339125*
miRCURY LNA miRNA Inhibitor Control (5 nmol)	5 nmol oligonucleotide, dried down in tube format; no label or 5' or 3' FAM; normal phosphodiester bonds	339126*

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

Product	Contents	Cat. no.
miRCURY LNA miRNA Inhibitor Control (15 nmol)	15 nmol oligonucleotide, dried down in tube format; no label; normal phosphodiester bonds	339127*
miRCURY LNA miRNA Power Inhibitor Control (1 nmol)	1 nmol oligonucleotide, dried down in tube format; no label; phosphorothioate-modified backbone	339135*
miRCURY LNA miRNA Power Inhibitor Control (5 nmol)	5 nmol oligonucleotide, dried down in tube format; no label or 5' or 3' FAM; phosphorothioate-modified backbone	339136*
miRCURY LNA miRNA Power Inhibitor Control (15 nmol)	15 nmol oligonucleotide, dried down in tube format; no label; phosphorothioate-modified backbone	339137*
miRCURY LNA miRNA Family Power Inhibitor	5 nmol oligonucleotide set, dried down in tube format; no label; phosphorothioate-modified backbone	339160*
Target site blockers and controls		
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 (15 nmol)	15 nmol oligonucleotide, dried down in tube format; no label; phosphorothioate-modified backbone	339195*
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*

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

Product	Contents	Cat. no.
miRCURY LNA miRNA Power Target Site Blocker, *in vivo* ready (15 nmol)	15 nmol oligonucleotide, dried down in tube format; no label; phosphorothioate-modified backbone	339200*
Related products		
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
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 Dye, Nuclease-free Water; for 200 reactions	339345
miRCURY LNA miRNA Mimic (5 nmol)†	5 nmol oligonucleotide, dried down in tube format	339173*
miRCURY LNA Premium miRNA Mimic (5 nmol)†	5 nmol oligonucleotide, dried down in tube format	339178*

* 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.

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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