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miRCURY® LNA® miRNA Inhibitor Libraries Handbook

LNA-optimized antisense oligonucleotides for use in genomewide, high-throughput screening of miRNA function

Sample to Insight

QIAGEN

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Kit Contents

Product	Cat. no.	Amount supplied
miRCURY LNA miRNA Inhibitor Library (0.125 nmol)	339168	0.125 nmol oligonucleotide per well, dried down in 96-well plates
miRCURY LNA miRNA Inhibitor Library (0.25 nmol)	339169	0.25 nmol oligonucleotide per well, dried down in 96-well plates
miRCURY LNA miRNA Family Power Inhibitor Library (0.125 nmol)	339165	0.125 nmol oligonucleotide per well, dried down in 96-well plate
miRCURY LNA miRNA Family Power Inhibitor Library (0.25 nmol)	339166	0.25 nmol oligonucleotide per well, dried down in 96-well plate

Storage

The miRCURY LNA miRNA Inhibitor Libraries are shipped at room temperature. Unopened 96-well plates should be stored at –15 to –30ºC or below. 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. After resuspension, it is recommended to store the miRCURY LNA miRNA Inhibitor Libraries in aliquots at –15 to –30ºC or below in a constant-temperature freezer to avoid repeated freeze-thaw cycles. Do not store in frost-free freezers with automatic thaw-freeze cycles.

Intended Use

The miRCURY LNA miRNA Inhibitor Libraries 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 Inhibitor Libraries is tested against predetermined specifications to ensure consistent product quality.

Introduction

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.

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

Principle and workflow

The miRCURY LNA miRNA Inhibitor Libraries enable genomewide, high-throughput screening of miRNA function. miRNA Inhibitors are primarily used to study miRNA function by assessing the biological consequences of inhibiting miRNA activity. The effect of inhibiting an miRNA 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.

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.

Coverage

The miRCURY LNA miRNA Inhibitor Libraries provide a high coverage of miRNAs listed in miRBase v. 20. However, we have excluded a number of miRNAs that have either no or

very limited direct experimental evidence. This significantly reduces your cost of screening and time wasted on potentially false-positive results that have very little impact on the "true" coverage of the screen.

- Human miRNA Inhibitor Library: 1,972 inhibitors of human miRNAs listed in miRBase v. 20, corresponding to a coverage of 78%.
- Mouse miRNA Inhibitor Library: 1,624 inhibitors of mouse miRNAs listed in miRBase v. 20, corresponding to a coverage of 86%.
- miRNA Family Power Inhibitor Library: A collection of all our 43 miRCURY LNA miRNA Family Power Inhibitors. The Power inhibitors address miRNA families that are conserved in human and mouse.

Plate layout

The inhibitor libraries are provided in 96-well plates. The plates are all organized as shown in Figure 1. The empty outer rows and columns facilitate easy pipetting into 96-well culture plates in a setup that avoids edge effects caused by evaporation of culture medium.

Well B2 is left empty for a control oligonucleotide of choice. This could, for example, be one of our miRCURY LNA miRNA Inhibitor negative controls with or without a FAM label (for visual inspection of transfection efficiency). This oligonucleotide must be purchased separately and added to the B2 well in the plates manually. See Ordering Information on page 14 for more information.

A positive transfection control is provided in well B3. This control is a toxic oligonucleotide with a significant effect on proliferation in a broad range of cell lines.

We have generated an miRNA ranking based on number of publications, type of experimental evidence, number of sequencing experiments and number of reads, etc. We have used this ranking to position the inhibitors in the plates in a descending order. The

inhibitors with the highest score (the best characterized) are positioned in the first plate, and the inhibitors with the lowest score are positioned in the last plate.

Organizing the inhibitors in the plates according to the amount of supporting scientific data enables smarter screening workflows with a subset of the plates containing inhibitors of the best-validated miRNAs without the need for laborious pipetting and reformatting of the library. Detailed information about the library content and plate distribution of the inhibitors can be found in the plate layout files.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty
B	Empty	Empty	Positive control	hsa-miR-1233-3p	hsa-miR-641	hsa-miR-1204	hsa-miR-1468-5p	hsa-miR-647	hsa-miR-200b-5p	hsa-miR-302c-5p	hsa-miR-1185-5p	Empty
C	Empty	hsa-miR-944	hsa-miR-659-3p	hsa-miR-520f-3p	hsa-miR-1225-3p	hsa-miR-524-5p	hsa-miR-518a-3p	hsa-miR-1286	hsa-miR-3065-3p	hsa-miR-92b-5p	hsa-miR-380-3p	Empty
D	Empty	hsa-miR-569	hsa-miR-20b-3p	hsa-miR-488-5p	hsa-miR-2110	hsa-miR-487a-3p	hsa-miR-1293	hsa-miR-614	hsa-miR-628-3p	hsa-miR-508-5p	hsa-miR-513b-5p	Empty
E	Empty	hsa-miR-412-3p	hsa-miR-938	hsa-miR-501-3p	hsa-miR-548b-3p	hsa-miR-514a-3p	hsa-miR-101-5p	hsa-miR-365a-5p	hsa-miR-1247-5p	hsa-miR-1226-3p	hsa-miR-633	Empty
F	Empty	hsa-miR-575	hsa-miR-890	hsa-miR-1471	hsa-miR-526b-5p	hsa-miR-1255b-5p	hsa-miR-452-5p	hsa-miR-1226-5p	hsa-miR-548d-3p	hsa-miR-1284	hsa-miR-520d-3p	Empty
G	Empty	hsa-miR-1184	hsa-miR-613	hsa-miR-198	hsa-miR-933	hsa-miR-431-3p	hsa-miR-138-1-3p	hsa-miR-617	hsa-miR-523-3p	hsa-miR-1273c	hsa-miR-548p	Empty
H	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty	Empty

Figure 1. Example of a miRCURY LNA miRNA Inhibitor Library plate. This is the layout of panel 10 of the human miRNA inhibitor library. Well B2 is left empty for a control oligonucleotide A positive transfection control is provided in well B3.

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 with rotor for 96-well plates
- DNase-free microtiter plates
- Multi-well 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 Inhibitor Libraries. Use DNase-free reagents and filter pipette tips. Whenever possible, work should be conducted under a tissue culture hood.

Resuspension of miRCURY LNA miRNA Inhibitor Libraries

1. Briefly centrifuge each plate of the library (maximum 4000 *x g*) to ensure that all material is collected at the bottom of the wells before removing the plate seal.
2. Carefully remove the plate seal.
3. Add nuclease-free, sterile TE buffer using a pipette with a sterile filter tip to achieve the desired concentration. To prepare a 10 µM solution, add 25 µl TE buffer to 0.25 nmol miRNA inhibitor.

   Note: Stock solutions should not be lower than 10 µM.
4. Let the plate 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. We recommend aliquoting the resuspended library into multiple plates to limit the number of freeze-thaw cycles. Place a new, sterile seal on each plate before storing. 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.

Transfection conditions can also be optimized using a well-characterized siRNA or an Antisense LNA Positive Control GapmeR. siRNA or LNA GapmeR activity can be assessed by quantification of the RNA target by qRT-PCR analysis.

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
- 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

Liquid handling robots often require volumes of 2–5 µl for accurate pipetting. We recommend making a plate with an appropriate dilution of the library stock solution, so that the pipetting volume is sufficient to ensure accuracy. Dispensing a mixture of transfection reagent and LNA oligonucleotide with liquid handling robots to cell cultures often results in detachment of cells in the center of the well.

For this reason, reverse transfection is often the better solution – first dry down a mixture of transfection reagent and oligonucleotide in the well, and add the suspension of adherent cells afterwards. Reverse transfection protocols are available from several suppliers of transfection reagents. 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 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. Once optimal transfection conditions have been established for a strongly expressed miRNA, they can be adopted with confidence to screening of the whole library. This is possible because of the unique T_m-normalized design that ensures that all inhibitors have uniform high affinity for their target miRNA.

The optimal time for analyzing the effect of transfection must be determined experimentally. However, antisense 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 miRNA Inhibitor Libraries 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. 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.

Ordering Information

Product	Contents	Cat. no.
miRCURY LNA miRNA Inhibitor Library (0.125)	0.125 nmol oligonucleotide per well, dried down in 96-well plates; normal phosphodiester bonds	339168*
miRCURY LNA miRNA Inhibitor Library (0.25)	0.25 nmol oligonucleotide per well, dried down in 96-well plates; normal phosphodiester bonds	339169*
miRCURY LNA miRNA Family Power Inhibitor Library (0.125)	0.125 nmol oligonucleotide per well, dried down in 96-well plate; phosphorothioate-modified backbone	339165*
miRCURY LNA miRNA Family Power Inhibitor Library (0.25)	0.25 nmol oligonucleotide per well, dried down in 96-well plate; phosphorothioate-modified backbone	339166*
Related products		
miRCURY LNA miRNA Inhibitor Control (1)	1 nmol oligonucleotide, dried down in tube format; ready-to-label; normal phosphodiester bonds	339125*
miRCURY LNA miRNA Inhibitor Control (5)	5 nmol oligonucleotide, dried down in tube format; ready-to-label or 5' or 3' FAM; normal phosphodiester bonds	339126*
miRCURY LNA miRNA Inhibitor Control (15)	15 nmol oligonucleotide, dried down in tube format; ready-to-label; normal phosphodiester bonds	339127*

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

Product	Contents	Cat. no.
miRCURY LNA miRNA Power Inhibitor Control (1)	1 nmol oligonucleotide, dried down in tube format; ready-to-label; phosphorothioate-modified backbone	339135*
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*
Antisense LNA GapmeR Standard (5 nmol)†	5 nmol oligonucleotide, dried down in tube format; *in vitro* screening grade	339511*
Antisense LNA GapmeR Premium (5 nmol)†	5 nmol oligonucleotide, dried down in tube format; premium cell-culture grade	339517*
Antisense LNA GapmeR *in vivo* Large Scale	Varies from 5 mg – 1 kg oligonucleotide; option of grades	339532*
Antisense LNA GapmeR Controls (5 nmol)†	5 nmol oligonucleotide, dried down in tube format; *in vitro* screening grade	339515*

* 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.
Antisense LNA GapmeR, custom plate (5 nmol)†	5 nmol oligonucleotide, dried down in 96-well plate; option of grades	339530*
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*
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 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.

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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Notes

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