miRCURY LNA™ microRNA Inhibitor library

Instruction manual v2.0

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EXIQON
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Product summary

Content

This manual addresses the use of Exiqon's microRNA inhibitor libraries:

miRCURY LNA™ Inhibitor libraries for *in vitro* screening:

Product No	Product description	Amount
190104-1	microRNA Inhibitor Library - Human (1972 x 0.25 nmol)	0.25 nmol per well
190104-2	microRNA Inhibitor Library - Human (1972 x 0.125 nmol)	0.125 nmol per well
190204-2	microRNA Inhibitor Library - Mouse (1624 x 0.25 nmol)	0.25 nmol per well
190204-2	microRNA Inhibitor Library - Mouse (1624 x 0.125 nmol)	0,125 nmol per well
190105-1	microRNA Family Inhibitor Library	0.25 nmol per well
190105-2	microRNA Family Inhibitor Library	0,125 nmol per well

Additional required materials

- Nuclease-free TE-buffer (10mM Tris, 0.1mM EDTA, pH 7.5 or 8.0)
- Microcentrifuge with tray for 96 well plates
- DNase-free microtiter plates
- Multi-well cell culture plates
- Cell culture medium
- Transfection reagent

Product description

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

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

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

	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

Empty | Control /miR | Positive Control

Plate layout

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

Well B2 is left empty for a control oligonucleotide of choice. This could for example be one of our negative controls with or without FAM label (for visual inspection of transfection efficiency). This oligonucleotide is purchased separately and added to the B2 well in the plates - for information about our controls see our website: http://www.exiqon.com/mirna-inhibitor-controls).

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 a microRNA 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 amount of supporting scientific data enables smarter screening workflows with a subset of the plates containing inhibitors of the best validated microRNAs without the need for laborious pipetting and reformatting of the library.

Detailed information about library content and plate distribution of the inhibitors can be found in our plate layout files: http://www.exiqon.com/mirna-inhibitor-library-downloads

Applications

The miRCURY LNA™ microRNA Inhibitor Libraries enable genome-wide high-throughput screening of microRNA function. MicroRNA Inhibitors are primarily used to study microRNA function by assessing the biological consequences of inhibiting microRNA activity.

The effect of inhibiting a microRNA 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.

Shipping and storage

This product is shipped dried down at room temperature. The unopened 96 well plates should be stored at -20°C or below. Shelf life is at least 12 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. 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™ oligonucleotides are susceptible to degradation by exogenous nucleases introduced during handling. Wear powder-free gloves when handling this product. Use DNase-free reagents and filter pipette tips. Whenever possible, work should be conducted under a tissue culture hood.

Resuspension

Step 1	Briefly centrifuge each plate of the library 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 seal in step 2.
Step 2	Remove the seal carefully.
Step 3	Add nuclease-free, sterile TE-buffer* using a pipette with a sterile filter tip to achieve the desired concentration. Stock solutions should not be lower than 10 µM (Adding 25 µL TE buffer* to 0,25 nmole microRNA inhibitor will yield a 10 µM solution).
Step 4	Let the plate 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.
Step 7	We recommend aliquoting the library in sister plates to limit the number of thaw-freeze cycles. Place a new sterile seal on plates before storing (such as Nunc™ 96-Well Cap Mats)

*TE-buffer: 10 mM Tris, pH 7.5 or 8.0, 0.1 mM EDTA

Step 8	Store at –20°C or below in constant temperature freezer.
Step 9	Avoid thaw-freezing more than 5 times (working solutions can be stored at 4°C for a period of maximum two weeks).

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 antisense 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. Choose the target site of an abundant microRNA. The effect of transfection can be assessed by measuring the relief of inhibition of reporter gene expression caused by microRNA inhibition. 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, derepression of endogenous microRNA targets (validated or predicted) can be measured at either mRNA or protein level. Transfection conditions can also be optimized with a well characterized siRNA or LNA™ gapmer that induces a quantifiable phenotype. Alternatively, siRNA or gapmer activity can be measured by qRT-PCR on the corresponding RNA target.

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

- Amount of transfection reagent
- Amount of microRNA Inhibitor
- 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

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 mixture of transfection reagent and LNA oligonucleotide with liquid handling robots to cell cultures often result in detachment of cells in the center of the well. For this reason reverse transfection is often the better solution - ie. drying down a mixture of transfection reagent and oligonucleotide in the well first and adding 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, microRNA 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 microRNA 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 microRNA.

The optimal time for analysis of the effect of transfection must be determined experimentally. However, 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.

Electroporation

miRCURY LNA™ microRNA Inhibitors 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 primary cancer cells). Please follow the instructions provided with the electroporation system.

Important note

microRNA qPCR is not a reliable method for measuring the level of microRNA knock down. Even if this method is often cited in literature, it is not recommended. microRNA inhibitors do not degrade their targets instead they form stable complexes with their target causing an accumulation of the microRNA due to reduced turnover. Moreover, failed transfections are often the result of accumulation of oligonucleotides inside vesicles so that the inhibitor and microRNA target are present in different subcellular compartments. Upon cell lysis, liberated vesicular inhibitors will form strong heteroduplexes with their microRNA target and therefore efficient and inefficient transfection cannot be distinguished by microRNA qPCR. In addition, LNA™ oligonucleotides can interfere with PCR primers and give rise to aberrant results.

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™ 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

- Griffiths-Jones, S. The microRNA Registry. Nucleic Acids Research 2004, 32, Database Issue, D109-11

- Torres AG, Fabani MM, Vigorito E, Gait MJ. MicroRNA fate upon targeting with anti-microRNA oligonucleotides as revealed by an improved Northern-blot-based method for microRNA detection. RNA 2011, 17, 933-43

- miRBase: www.mirbase.org

- 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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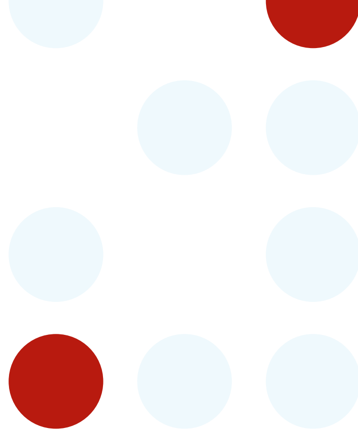

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