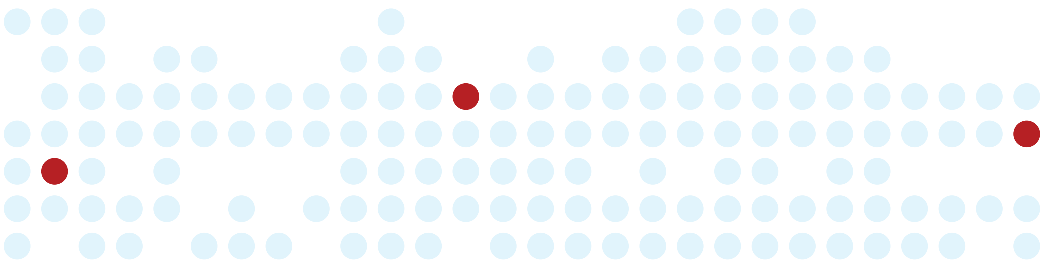

miRCURY LNA™ microRNA Inhibitor Library

Instruction manual

EXIQON

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Literature citations:

Please refer to miRCURY LNA™ microRNA Inhibitor Library, organism and version when describing a procedure for publication using this product.

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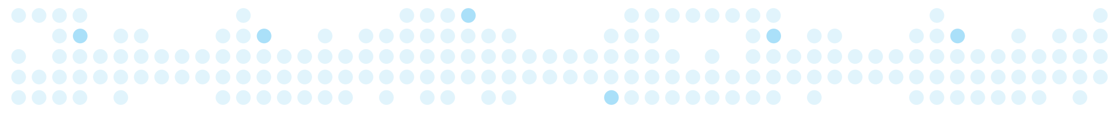

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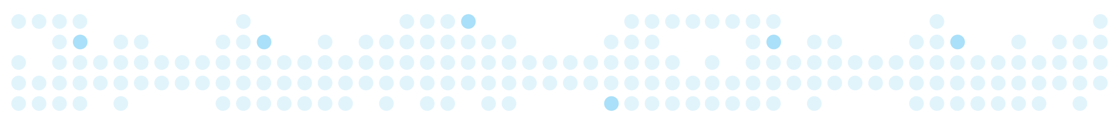


Product summary

Content and Coverage

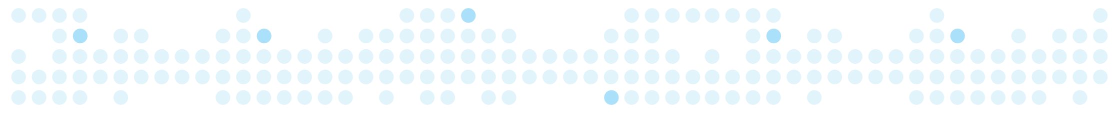
This is a generic manual for our miRCURY LNA™ microRNA Inhibitor Library products. For the content and coverage of any specific library please refer to the accompanying data sheet and the plate layout file that can be downloaded from www.exiqon.com/mirna-inhibitor-library

Amount

0.25 nmol of each oligonucleotide, dried down in 96 well plates (Axygen #P-96-450V; www.axygen.com).

Additional required materials

Nuclease-free water
Microcentrifuge with rotor for microtiter plates
Microtiter plates
Cell culture plates
Cell culture medium
Transfection reagent (with adherent cells)
Electroporation system (with non-adherent cells)



Product description

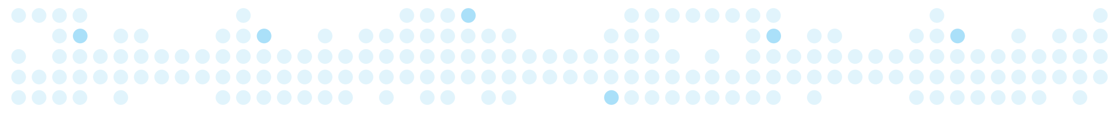
miRCURY LNA™ microRNA Inhibitors are antisense oligonucleotides with perfect sequence complementary to their target. When introduced into cells, they sequester their target microRNA in highly stable heteroduplexes thereby effectively preventing the microRNA from hybridizing with its normal cellular interaction partners. The sequences of the oligonucleotides and their LNA™ spiking patterns have been carefully designed to achieve uniform high potency for all microRNA inhibitors in the library regardless of the GC-content of their target. This was accomplished by ensuring T_m normalization around an optimal temperature while keeping the level of self-complementarity to a minimum.

Applications

The miRCURY LNA™ microRNA Inhibitor Library enables 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 at room temperature. The sealed, unopened library should be stored at -20ºC or below. Shelf life is at least one year after shipment date when stored in this manner. Oligonucleotides are degraded by repeated cycles of thaw and freezing, especially when in solution. For storage at -20ºC, please use a constant temperature freezer. **Do not store in frost-free freezer with automatic freeze-thaw cycles!** Exposure to higher ambient temperatures during shipment does not pose any risk to the stability of the oligonucleotides.



Protocol

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 under a tissue culture hood.

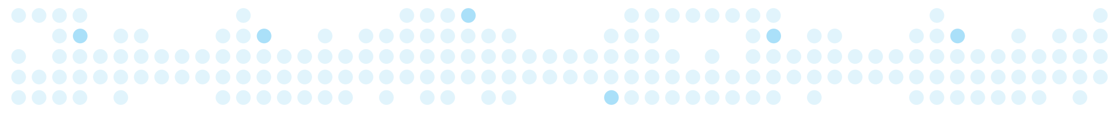
Resuspension

1. Briefly centrifuge each plate 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.
2. Remove seal carefully.
3. Add nuclease-free, sterile water using a multichannel pipette with sterile filter tips to achieve the desired concentration. Stock solutions should not be lower than 10 µM (Adding 25 µl water to 0.25 nmol microRNA inhibitor will make a 10 µM solution).
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 library in sister plates to limit the number of thaw-freeze cycles. Place a new sterile seal (such as Axygen PCR-AS-200) on plates before storing.
8. Store at –20ºC.
9. Avoid thaw-freezing more than 5 times (working solutions can be stored at 4º C for a period of maximum 14 days).

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 microRNA inhibition while minimizing secondary effects.

Expect to spend some time finding the optimal transfection conditions before using the library.



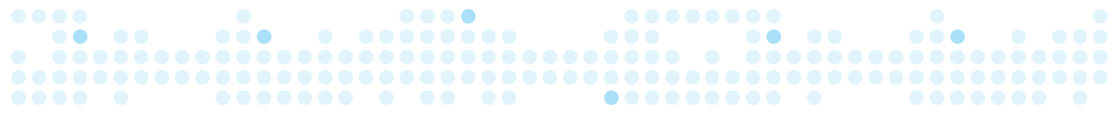
One way of determining the optimal transfection conditions is to use a reporter plasmid with a microRNA target site in the 3'UTR of a reporter gene. Choose the target site for a microRNA strongly expressed in the cell line to be used with the library. The effect of transfection with an inhibitor against this particular microRNA can be assessed by measuring the relief of inhibition of reporter gene expression caused by the endogenous microRNA. Typically, this type of experiment also involves a second plasmid with another reporter gene to normalize for plasmid transfection variation. Reporter plasmids with microRNA target cloning sites in the 3'UTR of reporter genes are commercially available from several companies.

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

Transfection conditions can also be optimized with a well characterized siRNA that induces a quantifiable phenotype. Alternatively siRNA activity can be gauged by qRT-PCR on the corresponding mRNA target.

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 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 the whole library. This is possible because of the unique T_m-normalized probe design that ensures that all probes will have uniform high affinity for their target microRNA.



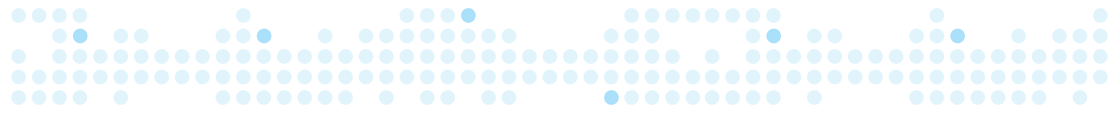
At sufficiently high concentration all oligonucleotides are cytotoxic. The level of toxicity is sequence dependent and the sensitivity of cell lines varies considerably. Screening with a microRNA inhibitor library should therefore only be performed under optimized transfection conditions with the minimal required inhibitor concentration. This is especially important with cell viability screens. Typically, the first signs of toxicity can be observed at 100nM concentrations. Expect a non negligeable amount of false positives. Validate hits with independently synthesized individual inhibitors and perform adequate controls to ensure that the resulting phenotype is due to antisense inhibition of the targeted microRNA.

Cell culture plate	96 well	24 well	12 well
Transfection reagent[A]	0.3 – 1.0 µl	1 – 3 µl	2 – 4 µl
LNA™ miRNA inhibitor[B]	5 pmol	25 pmol	50 pmol
Cell density (cells/well)[C]	6000	40000	80000
Final volume per well	100 µl	500 µl	1000 µl

[A] Refer to the instructions that come with the transfection reagent.
[B] The amount shown yields a microRNA inhibitor concentration of 50 nM. Robotic pipettors may require volumes of 2 – 5 µl for accurate pipetting. We recommend making a plate with an appropriate dilution of the stock so that the pipetting volume is sufficient to ensure accuracy.
[C] Optimal cell density varies with the cell type depending on cell size and growth characteristics. In general, 30 – 70% confluency is recommended.

Electroporation

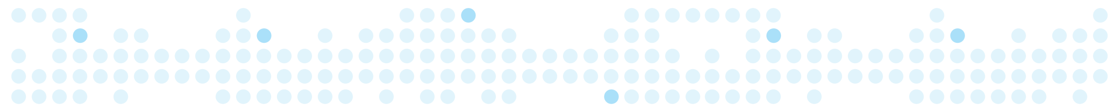
miRCURY LNA™ microRNA Inhibitors can also be introduced into cells by electroporation. This is especially useful with cells that are notoriously difficult to transfect (i.e. non adherent cells such a lymphocytes, bone marrow stem cells and primary cancer cells). Please follow the instructions provided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



Screening in multiwell plates

When growing cells in multiwell culture plates problems of evaporation from wells at the edge of the plates are frequently encountered. This can introduce noise in the results of the screening with our microRNA inhibitor library as cell growth can be affected by concentration of the growth medium. It is a good idea to check the level of evaporation from the edge of the plate and its effect on cell growth and the screening assay before using the library. Problems of this nature are in most cases solved by one the following different simple measures:

1. Use maximum culture volume in each well (e.g. with 96 well plates use 200µl instead of 100µl – this is very effective even upon long incubations lasting up to a week).
2. Use gas permeable seals on the plates. There are many commercially available products of this type on the market.
3. Avoid using the wells at the border of the plates and fill them with water.



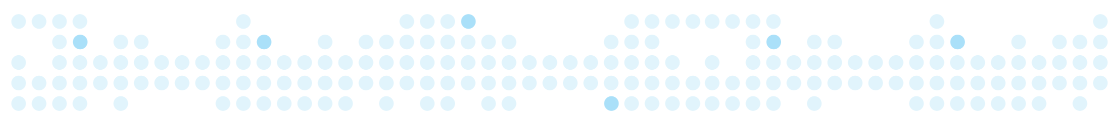
Related products

miRCURY LNA™ microRNA Inhibitor Negative Controls:
These oligonucleotides have the same length and LNA™ content as the oligonucleotides in the library. However, they are designed to have no known microRNA targets in miRBase. The oligonucleotides are provided unlabeled or fluorescence-labeled.

miRCURY LNA™ microRNA inhibitors and Power inhibitors
Inhibitors from the library can be ordered individually. Look up the product number in the plate layout file and order online at our web shop (www.exiqon.com/mirna-inhibitor) or write to order@exiqon.com.
A few of the inhibitors in the library are not regular products (no product number in the plate layout file - only a probe ID). To order these oligonucleotides go to www.exiqon.com/contact and in the inquiry field write: miRCURY LNA™ microRNA Inhibitor Library and specify the probe ID number of the desired oligonucleotide and the required amount. Typically, 5 nmol is enough for most practical purposes.

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

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



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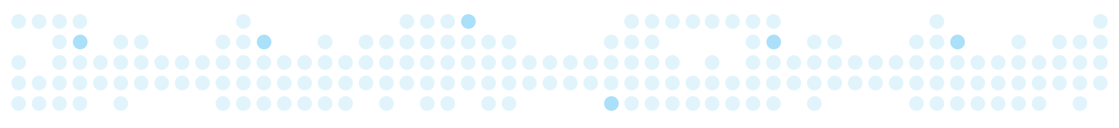
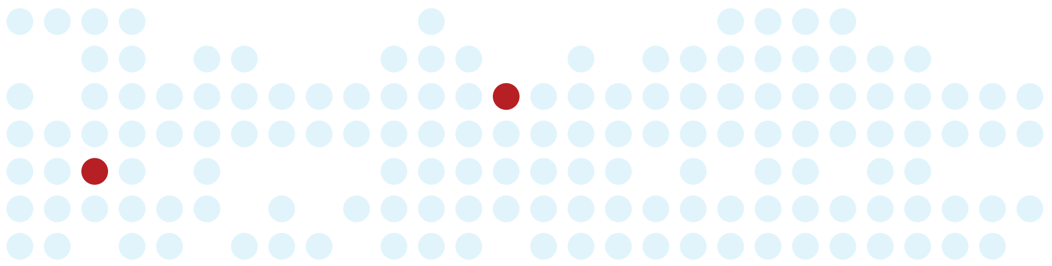
- Griffiths-Jones, S. The microRNA Registry. Nucleic Acids Research 2004, 32, Database Issue, D109-11
- miRBase: www.mirbase.org
- www.exiqon.com/mirna-inhibitor





Outside North America
Exiqon A/S · Skelstedet 16
DK-2950 Vedbaek · Denmark
Phone +45 45 660 888
Fax +45 45 661 888

North America
Exiqon Inc. · 14 F Gill Street
Woburn, MA 01801 · United States
Phone +1 781 376 4150
Fax +1 781 376 4152
Toll free (US) +1 888 miRCURY

www.exiqon.com/contact
www.exiqon.com

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