

miRCURY LNA™ microRNA Knockdown Library

Instruction manual

Literature citations:

Please refer to miRCURY LNAT[™] microRNA Knockdown Library, organism and version when describing a procedure for publication using this product.

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Product summary

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Content and Coverage

This is a generic manual for our miRCURY LNA™ microRNA Knockdown 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/knockdown

Amount

0.25 nmol of each oligonucleotide, dried down in 96 well plates (Axygen #P-96-450V; www.axxygen.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 Knockdown Probes are antisense microRNA inhibitors 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 knockdown probes and their LNA™ spiking patterns have been carefully designed to achieve uniform high potency of all the microRNA inhibitors in the library. 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 Knockdown library enables genome-wide high throughput screening of microRNA function.

miRCURY LNA™ microRNA Knockdown Probes 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 -20°C 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 -20°C , please use a constant temperature freezer. **Do not store in frost-free freezer with automatic thaw/freezing cycles!** Exposure to higher ambient temperatures during shipment does not pose any risk to the stability of the oligonucleotides.



Protocol

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LNATM oligonucleotides are susceptible to degradation by exogenous ribonucleases introduced during handling. Wear powder-free gloves when handling this product. Use RNase-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 (Adding 50 μ l water to 0.25 nmol knockdown probe will make a 5 μ M 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 -80°C .
9. Avoid thaw/freezing more than 5 times (working solutions can be stored at 4°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 knockdown probe
- 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

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 Knockdown Probes display potent activity at final concentrations of 1-25 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.



Cell culture plate	96 well	24 well	12 well
Transfection reagent ^A	0.3 – 1.0 μ l	1 – 3 μ l	2 – 4 μ l
miRCURY LNA™ KD probe ^B	2.5 μ mol	12.5 μ mol	25 μ mol
Cell density (cells/well) ^C	6000	40000	80000
Final volume per well	100 μ l	500 μ l	1000 μ l

^A Refer to the instructions that come with the transfection reagent.

^B The amount shown yields a miRCURY LNA™ knockdown probe concentration of 25 nmol. Robotic pipettors may require volumes of 2 – 5 μ l 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 Knockdown Probes 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 as 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 KD 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 200 μ l instead of 100 μ l – 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

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Control miRCURY LNA™ microRNA Knockdown Probes:

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 with different types of end labels, for example unlabeled or 3' fluorescence labeled (products # 199002-00, # 199002-02, # 199002-04, # 199002-06, # 199002-08, # 199003-00, # 199003-02, # 199003-04, # 199003-06, # 199003-08).

Individual miRCURY LNA™ microRNA Knockdown Library probes

Inhibitors from the library can be ordered individually. Go to www.exiqon.com/contact and in the inquiry field write: miRCURY LNA™ microRNA Knockdown Library and specify the 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™ microRNA PCR System

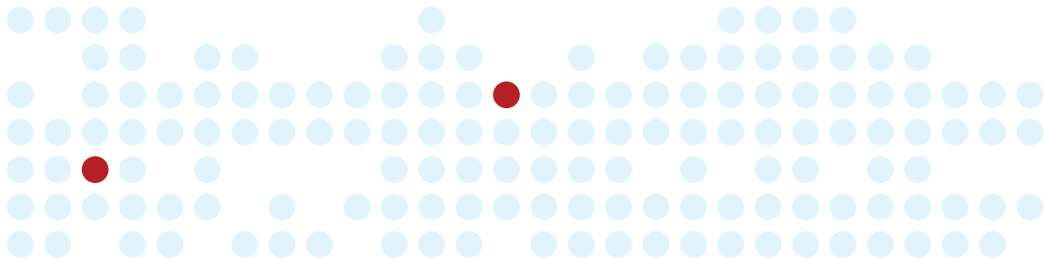
Quick and accurate determination of microRNA expression with real-time PCR.



References

- Griffiths-Jones, S. The microRNA Registry. Nucleic Acids Research 2004, 32, Database Issue, D109-11
- miRBase, Wellcome Trust Sanger Institute: <http://microrna.sanger.ac.uk>
- www.exiqon.com/knockdown





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