

miRCURY LNA™ microRNA Inhibitors

Instruction manual

Literature citations:

Please refer to miRCURY LNA™ microRNA Inhibitor or miRCURY LNA™ microRNA Power Inhibitor when describing a procedure for publication using this product.

Patents and Trademarks

Exiqon, LNA™, miRCURY™ and miRPlus™ are trademarks of Exiqon A/S, Vedbaek, Denmark. Locked Nucleic Acids (LNA™) are covered by patents and patent applications owned by Exiqon A/S.

Disclaimer

Products are for research use only and not for diagnostic or therapeutic use. The products may be used only for the buyer's internal research purposes and not for commercial use. The buyer may not resell products in their original or any modified form. The purchase of products does not include or carry an implied right or license for the buyer to use such products in the provision of services to third parties and a license must be obtained directly from Exiqon A/S for such use.



Table of contents

Product summary	4
Amount	4
Additional required materials	4
Product description	5
Applications	5
Shipping and storage	5
Protocol	6
Resuspension	6
Transfection guidelines	6
Electroporation	8
Related products	9
References	10



Product summary

4

Amount

5 nmol of oligonucleotide, dried down in a screw cap tube.

Additional required materials

Nuclease-free water

Microcentrifuge

DNase-free microcentrifuge tubes or microtiter plate

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 miRCURY LNA™ microRNA Inhibitors 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

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 unopened tube should be stored at -20°C or below. Fluorescence-labeled inhibitors should be protected from light to avoid bleaching. Shelf life is at least 6 months 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-freeze cycles!** Exposure to higher ambient temperatures during shipment does not pose any risk to the stability of the oligonucleotides.



Protocol

6

MICRORNA INHIBITOR | Instruction Manual

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 the screw cap tube 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 cap in step 2.
2. Remove screw cap carefully.
3. Add nuclease-free, sterile water using a pipette with a sterile filter tip to achieve the desired concentration. Stock solutions should not be lower than 10 μM (Adding 100 μl water to 5 nmole microRNA Inhibitor will make a 50 μM solution).
4. Let the tube 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 inhibitor in sister tubes to limit the number of thaw-freeze cycles.
8. Store at -20°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.

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. The effect of transfection 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.



At sufficiently high concentration all oligonucleotides are cytotoxic. The level of toxicity is sequence dependent and the sensitivity of cell lines varies considerably. MicroRNA functional analysis should therefore only be performed under optimized transfection conditions with the minimal required inhibitor concentration. Dose response experiments are often useful for determining the threshold concentration where 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 100nM concentrations. Always 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	6 well
Transfection reagent ^A	0.3 – 1.0 µl	1 – 3 µl	2 – 4 µl	3-36 µl
miRNA inhibitor ^B	5 pmole	25 pmole	50 pmole	150 pmole
Cell density (cells/well) ^C	6000	40000	80000	240.000
Final volume per well	100 µl	500 µl	1000 µl	3000 µl

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

^B The amount shown yields a microRNA Inhibitor concentration of 50 µM.

^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.



Related products

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™ microRNA Inhibitor Library - Human and Mouse

For genome-wide high throughput screening of microRNA function.

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.



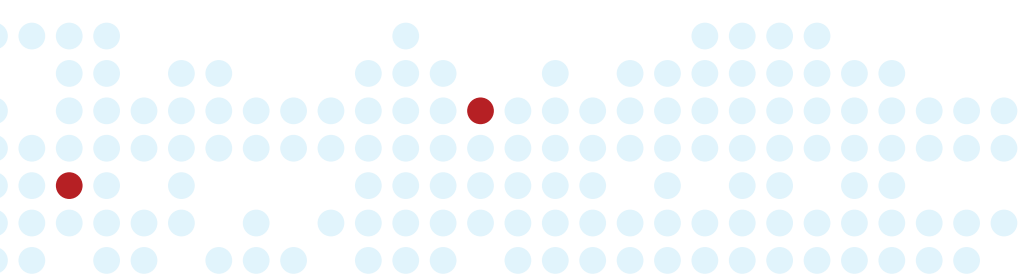
References

10

- 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

EXIQON
Seek Find Verify