Product Catalog
All you need for RNA research
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mRNA function

The role of mRNA is to provide a template for the production of proteins for the cell. The current phase of the ENCODE project catalogues 20,687 protein-coding genes which represents only ~2% of the transcriptome. Various mediators are involved in regulating transcription, via methylation and acetylation of histones, promoter-binding enhancers and repressors, cis and trans-acting factors, modifying the transcript via spliceosomes, polyadenylation and capping enzymes etc. and in targeting mRNA for degradation (i.e. ubiquitination). Any one of these can enhance or suppress the production of the protein and alter phenotypes. Additionally, non-coding RNAs can influence these mediators or directly interact with the mRNA to alter phenotypes.

The long non-coding RNAs - lncRNA

The remaining 98% of the transcriptome, the non-coding RNA population, is quite diverse (Figure 1). Many of these are referred to as long non-coding RNA (lncRNA) with transcripts longer than 200 nts and are thought to encompass more than 10,000 different transcripts in mammals. Some of the lncRNAs work in cis (e.g. XIST for X chromosome activation) and others work in trans [e.g. HOTAIR for chromatin remodeling]. Many regulate gene expression (e.g. Gas5), mRNA splicing (e.g. MALAT-1) and translation (e.g. BACE1-AS) and many more are yet to have their functions defined. What is known is that the expression of lncRNAs varies spatially, temporally, or in response to stimuli. They possess secondary structures which facilitate their interactions with DNA and proteins, and through diverse mechanisms play roles in homoeostasis and disease.

Figure 1. The many forms of RNA.

<table>
<thead>
<tr>
<th>Non-coding RNA</th>
<th>Coding RNA</th>
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</thead>
<tbody>
<tr>
<td>17-25 nt microRNA</td>
<td>incl. rRNA</td>
</tr>
<tr>
<td>24-35 nt piRNA</td>
<td>200-10,000 nt IncRNA</td>
</tr>
<tr>
<td>72-94 nt tRNA</td>
<td>incl. rRNA</td>
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<tr>
<td>86-141 nt rRNA</td>
<td>avg 1,800-2,000 mRNA</td>
</tr>
<tr>
<td>40-150 nt Pre-miRNA</td>
<td>60-200 nt snoRNA</td>
</tr>
<tr>
<td>60-200 nt miRNA</td>
<td>200-100,000 nt</td>
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</table>

With advanced technologies for sequencing and characterizing RNA, the scientific community has taken what was once perceived as the Dark Matter of the Genome and shone a light, revealing many previously unknown players in the biology of an organism. There is a diverse range of species and functions associated with both protein coding and non-coding RNAs. These include the familiar mRNAs, tRNAs and rRNAs but now expand the numbers, and our understanding, of small and long non-coding RNAs.
The short non-coding RNAs - microRNA

MicroRNAs constitute the smallest class (~22 nt) of non-coding RNAs which play key roles in the regulation of gene expression. Their highly tissue-specific expression and distinct temporal expression patterns during embryogenesis suggest that microRNAs play a key role in the differentiation and maintenance of tissue identity. They have been demonstrated to be important for many biological processes and have been linked to many diseases including cancer, heart-disease and neurological disorders. Acting at the post-transcriptional level, these fascinating molecules may fine-tune the expression of as much as 60% of all mammalian protein-encoding genes.

MicroRNAs usually induce gene silencing by binding to target sites found within the 3'UTR of the targeted mRNA. This interaction prevents protein production by suppressing protein synthesis and/or by initiating mRNA degradation. Since most target sites on the mRNA have only partial base complementarity with their corresponding microRNA, individual microRNAs may target as many as 100 different mRNAs. Moreover, individual mRNAs may contain multiple binding sites for different microRNAs, resulting in a complex regulatory network. In addition, many microRNAs are part of families of highly similar sequences.

Exiqon’s tools for mRNA and ncRNA research

Exiqon has pioneered the development of microRNA research and diagnostics tools with leading-edge products and services based on the propriety Locked Nucleic Acid (LNA™) technology. By incorporating LNA™ into our products, we have significantly increased the affinity and specificity of our microRNA mimics, inhibitors, probes, and primers, thereby addressing both challenges described above. Furthermore, with advanced intelligent design, Exiqon’s LNA™-enhanced oligos also benefit from increased stability and potency and are ideal for downregulating mRNA and IncRNA levels as antisense oligonucleotide inhibitors (LNA™ GapmeRs; visit exiqon.com/gapmer) and elucidating RNA interactions (miRCURY LNA™ Target Site Blockers; visit exiqon.com/mirna-target-site-blocker). With LNA™, the diversity of the transcriptome can be further revealed.

RNA as disease biomarkers

When mutation gives rise to aberrant genes, transcripts and proteins, developmental and disease conditions transpire. Expression studies including transgenic and knock-out models, profiling and sequencing, have demonstrated the dysregulation and function of many genes in disease development and progression, yet our ability to prevent, reverse, and treat these are still at the early stages in many cases. As with mRNAs, microRNAs and IncRNAs are presenting themselves as diagnostic and prognostic biomarkers of disease, characterizing those who would benefit from various therapies and those who would not.

ncRNA research challenges

The progression in understanding the roles ncRNAs play has been hampered by the available tools to target these RNAs in a cellular context. Long non-coding RNA are often found within the nucleus, and introducing sequences to enhance or modulate their function, such as siRNA approaches to targeted knock-down, have met with limited success and specificity. The double-stranded siRNA duplex has difficulty crossing the nuclear membrane and the passenger strand (non-targeting sequence) of the duplex can often elicit its own effect, confounding interpretation of results. For microRNA and other small RNA, their small size presents a challenge for DNA-based detection tools to achieve the required specificity and sensitivity.

Interactive microRNA research guide

Let our free online research guide take you through each step of a microRNA experiment, from RNA isolation to functional analysis, learn more at exiqon.com/microRNA-research-guide

Download our new RNA app

Get Xplore™ from the App Store

Discover more about your mRNA and microRNAs of interest with our miRSearch tool at exiqon.com/mirsearch

RNA and microRNA in brief

Discover more about your mRNA and microRNAs of interest with our miRSearch tool at exiqon.com/mirsearch
At a glance

- Excellent sensitivity - significantly increased sensitivity compared to DNA and RNA
- Uniform detection - robust detection of all sequences, regardless of GC-content
- Increased specificity - detection of single nucleotide mismatches
- High stability - superior binding to small RNAs in vivo and in vitro
- Excellent flexibility - can be used for a wide range of samples including biofluids and FFPE

What is LNA™?

Locked Nucleic Acids (LNA™) are a class of high-affinity RNA analogs in which the ribose ring is "locked" in the ideal conformation for Watson-Crick binding (Figure 2). As a result, oligonucleotides containing LNA™ exhibit unprecedented thermal stability when hybridized to a complementary DNA or RNA strand. For each incorporated LNA™ monomer, the melting temperature ($T_m$) of the duplex increases by 2-8 °C (Figure 3). In addition, LNA™ oligonucleotides can be made shorter than traditional DNA or RNA oligonucleotides and still retain a high $T_m$. This is important when the oligonucleotide is used to detect short or highly similar targets.

Since LNA™ oligonucleotides typically consist of a mixture of LNA™ and DNA or RNA, it is possible to optimize the sensitivity and specificity by varying the LNA™ content of the oligonucleotide. As such LNA™ has been proven to be a powerful tool in many molecular biological applications in which standard DNA oligonucleotides or RNA riboprobes do not show sufficient affinity or specificity. Incorporation of LNA™ into oligonucleotides has been shown to improve sensitivity and specificity for hybridization-based technologies including PCR, microarray and in situ hybridization.

![Figure 2. The structure of LNA™. The ribose ring is connected by a methylene bridge (orange) between the 2'-O and 4'-C atoms thus "locking" the ribose ring in the ideal conformation for Watson-Crick binding. When incorporated into a DNA or RNA oligonucleotide, LNA™ makes the pairing with the complementary strand more rapid and increases the stability of the resulting duplex.](image)

![Figure 3. Replace DNA with LNA™ for higher $T_m$. On the left, progressive substitution of DNA nucleotides with LNA™ increases the melting temperature of the oligonucleotide while maintaining the recognition sequence and specificity of the oligonucleotide. On the right, LNA™ substitutions allow shortening of the oligonucleotide while maintaining the same $T_m$.](image)
Tm normalization – robust detection regardless of GC content

The Tm and therefore the affinity of an oligonucleotide duplex can be controlled by varying the LNA™ content. This feature can be used to normalize the Tm across a population of short sequences with varying GC-content. For AT-rich oligonucleotides, which have low Tm, more LNA™ is incorporated into the LNA™ oligonucleotide to raise the Tm of the duplex. This enables the design of LNA™ oligonucleotides with a narrow Tm range. This is beneficial for microarray, PCR and other applications where sensitive and specific binding to many different targets must occur under the same conditions simultaneously. The power of Tm normalization is demonstrated by the comparison of DNA and LNA™ probes for detection of microRNA with varying GC content (Figure 4).

Superior single nucleotide discrimination

Intelligent placement of LNA™ monomers ensures excellent discrimination between closely related sequences. Differences as small as one nucleotide can be detected. The difference in Tm between a perfectly matched and a mismatched target is described as the Δ Tm. Incorporation of LNA™ in oligonucleotides can increase the Δ Tm between perfect match and mismatch binding by up to 8 °C. The increase in Δ Tm enables better discrimination between closely related sequences such as members of microRNA families.

Broad applicability

The affinity-enhancing effects of LNA™ give LNA™ oligonucleotides strand invasion properties making LNA™ excellent for in vivo applications. Incorporation of LNA™ into oligonucleotides further increases resistance to endo- and exonucleases which leads to high in vitro and in vivo stability. Since the physical properties (e.g., water solubility) of these sequences are very similar to those of RNA and DNA, conventional experimental protocols can easily be adjusted to their use.

LNA™ for microRNA research

The small sizes and widely varying GC-content (5-95 %) of microRNAs make them challenging to analyze using traditional methods. The use of DNA or RNA based technologies for microRNA analysis can introduce high uncertainty and low robustness because the melting temperature (Tm) of the oligonucleotide/microRNA duplex will vary greatly depending on the GC content of the sequences. This is especially problematic in applications such as microarray profiling and high throughput experiments where many microRNA targets are analyzed under the same experimental conditions. These challenges in microRNA analysis can be overcome by using LNA™-enhanced oligonucleotides. By simply varying the LNA™ content, oligonucleotides with uniform Tm can be designed, regardless of the GC-content of the microRNA. Exiqon has used the LNA™ technology to Tm-normalize primers, probes and inhibitors to ensure that they all perform well under the same experimental conditions (Figure 6).

Another challenge of studying microRNAs is the high degree of similarity between the sequences. Some microRNA family members differ by a single nucleotide. LNA™ can be used to enhance the discriminatory power of primers and probes to allow excellent discrimination of closely related microRNA sequences.

LNA™ offers significant improvement in sensitivity and specificity and ensures optimal performance for all microRNA targets.

What is LNA™

LNA™ captures microRNA

Figure 4. The power of Tm normalization. The signal from DNA-based capture probes varies with GC content and results in poor detection of many microRNAs, whereas LNA™ probes offer robust detection of all microRNAs. Signal intensity from microarray experiments using LNA™-enhanced (gray) or DNA-based (blue) capture probes. Synthetic microRNAs with varying GC content were added at 100amol each.
LNA™ for other applications

The unique characteristics of LNA™ make it a powerful tool, not only for microRNA research but also for the detection of low abundance, short or highly similar targets in a number of other applications (Figure 5).

LNA™ has been successfully used to overcome the difficulties of studying very short sequences and has greatly improved, and in many cases enabled, specific and sensitive detection of non-coding RNA and other small RNA molecules.

Intelligent and sophisticated design strategies result in highly successful approaches to microRNA mimicry as well as antisense oligonucleotides for target degradation or functional interference (Figure 7).

The unique ability of LNA™ oligonucleotides to discriminate between highly similar sequences has further been exploited in a number of applications targeting longer RNA sequences such as mRNA. In addition, LNA™ has been successfully used for the detection of low abundance nucleic acids and chromosomal DNA.

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**Figure 5. LNA™ applications used in literature.**

<table>
<thead>
<tr>
<th>DNA</th>
<th>ncRNA</th>
<th>mRNA</th>
<th>microRNA</th>
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<tr>
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<td>Real-time/quantitative PCR</td>
<td>Real-time/quantitative PCR</td>
<td>Real-time/quantitative PCR</td>
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<td>Multiplex analysis</td>
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<td>Proteomics of isolated chromatin segments (PIDH)</td>
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<td>Northern Blotting</td>
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<td>Fluorescence activated cell sorting</td>
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<td>Microarray analysis</td>
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<td>In situ hybridization</td>
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<td>Northern Blotting</td>
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<td>Bead-based applications</td>
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<td>Fluorescence activated cell sorting</td>
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<td>Isolation</td>
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<td>In situ hybridization</td>
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<td>Northern Blotting</td>
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<tr>
<td>Bead-based applications</td>
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</table>

**Figure 6. LNA™ microRNA inhibitors have high uniform potency.** The affinity of traditional full length microRNA inhibitors is highly influenced by the GC-content resulting in a Tm span of more than 40°C. In contrast, Exiqon’s inhibitors span just 10°C around an optimal temperature.

**Exiqon is the home of LNA™**

With our proprietary LNA™ technology and more than 10 years experience working with LNA™ applications, Exiqon can provide you with an excellent LNA™ oligonucleotide solution for your research needs - ensuring: optimized Tm, optimal mismatch discrimination and high binding specificity while keeping secondary structure and self-complementarity to a minimum.

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**Figure 7. Intelligent design strategies applying the LNA™ optimally result in powerful applications for RNA silencing and mimicry.**
Introduction

LNA™ Oligonucleotides

Design and order your own custom LNA™-enhanced oligonucleotides directly on our website. Exiqon offers synthesis of custom oligonucleotides with a wide variety of modifications, labels, synthesis scales and purification methods.

At a glance
- LNA™-enhanced oligos for more sensitive and specific RNA and DNA applications
- Design your own LNA™ oligos or have Exiqon’s experts help you
- Apply Exiqon’s LNA™ Tm Prediction tool and LNA™ Oligo Optimizer tool to design the optimal oligo
- Select from a wide variety of modifications, labels, synthesis scales and purification methods

Product Description
LNA™ has been proven to be a powerful tool in many molecular biological applications in which standard DNA oligonucleotides or RNA riboprobes do not show sufficient affinity or specificity. By substituting LNA™ into RNA or DNA oligonucleotides, the LNA™ oligonucleotides can be used to overcome the difficulties of studying very short sequences. This has greatly improved and in many cases enabled specific and sensitive detection of microRNA and other small RNA molecules.

The unique ability of LNA™ oligonucleotides to discriminate between highly similar sequences has been exploited in a number of applications targeting also longer RNA sequences such as mRNA and IncRNA. In addition, LNA™ has also been successfully used to enable detection of low abundance nucleic acids and chromosomal DNA.

Exiqon offers custom synthesized LNA™ oligonucleotides with a variety of synthesis scales and with a wide range of modifications, labels, synthesis scales, purification method options. Table 1 lists a selection of these. For additional options, please visit exiqon.com/custom-lna-oligos

How to design your LNA™ oligonucleotide
Incorporation of LNA™ in a sequence strongly affects the properties of the oligonucleotide and great care must be taken to find the right design for your experimental purpose. By varying the length and LNA™ content of the oligonucleotide you can optimize your design to achieve good mismatch discrimination, and high binding specificity, while avoiding unacceptable secondary structure and self-complementarity. Additional information and a number of useful tools (Figure 10) are found at exiqon.com/oligo-tools

Let Exiqon design your LNA™ oligonucleotide
Exiqon’s in-house LNA™ experts can help you design the best LNA™ oligonucleotide for the application and target of your interest. Your oligonucleotide will be designed for optimal LNA™ content and positioning in order to achieve optimal specificity and minimal secondary structure and self-complementarity.

Figure 8. LNA™ enables detection of SSA4 RNA in Δrip1 fixed yeast cells. Use of a Cy3™-labeled LNA™ probe resulted in improved ISH signal and less background staining compared to a Cy3™-labeled DNA probe (right figure). Thomsen et al. RNA, 2005.
As a standard all oligonucleotides are deprotected and desalted to remove small molecule impurities, quantitated by UV spectrophotometry to provide an accurate measure of yield and finally quality controlled (QC) by mass spectrometry.

Additional purification may be recommended or required depending on potential modifications or the application the oligonucleotide is intended for. This includes PAGE, IE-HPLC, dual HPLC and RNase Free HPLC. Na+ Salt exchange is recommended for oligonucleotides used in applications where the presence of minute amount of toxic salts can cause unwanted side reactions. Specific endotoxin tests are also available.

Please view exiqon.com/oligo-tools or contact us for additional information on our analytical services.

On-line design tools for specific RNA and microRNA applications

Take advantage of the online design tools already available for the following LNA™-enhanced applications at exiqon.com:
• Custom microRNA LNA™ qPCR primer sets
• Custom microRNA qPCR panels
• Batch design and ordering of microRNA LNA™ qPCR assays
• LNA™ Detection Probes for mRNA and IncRNA
• LNA™ qPCR assays for mRNA and IncRNA
• Antisense oligos for IncRNA and mRNA (LNA™ GapmeRs)

Ordering information (Details on page 70)

Custom LNA™ oligonucleotides of your own design are best ordered at exiqon.com/custom-lna-oligos

For assistance in designing your custom LNA™ oligonucleotides, please contact exiqon at Exiqon.com/contact

Figure 9. LNA™ probes are superior to DNA probes for SNP detection. A capture probe targeting each of the two alleles in a SNP were enhanced by LNA™ (nucleotides shown in bold) and hybridized to the target PCR amplicon. LNA™ incorporation increases the Tm of the probes and leads to a larger △Tm thereby improving the the mismatch discrimination ability of the assay. Probe sequences are presented below the bars. Underlined positions indicate the site of the SNP.

Figure 10. Useful LNA™ Oligonucleotide design guidelines and design tools are available at exiqon.com/oligo-tools.

<table>
<thead>
<tr>
<th>LNA™ Oligo Tm Prediction</th>
<th>Predicts the melting temperature of the LNA™ oligonucleotide.</th>
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<tbody>
<tr>
<td>LNA™ Oligo Optimizer</td>
<td>Calculates scores for self-complementarity and secondary structure of the LNA™ oligonucleotide. The scores can be used to predict the temperature at which an oligonucleotide is likely to form undesired secondary structure.</td>
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<tr>
<td>Oligo Dilution Calculator</td>
<td>Calculates how much water or buffer must be added to a oligonucleotide stock solution in order to attain the desired final concentration.</td>
</tr>
<tr>
<td>Oligo Resuspension Calculator</td>
<td>Calculates how much water or buffer is needed to resuspend a lyophilized oligonucleotide in order to attain the desired final concentration.</td>
</tr>
<tr>
<td>Oligo Concentration Converter</td>
<td>Calculates the concentration and total amount of oligonucleotide in your stock solution.</td>
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</table>
Table 1.Custom LNA™ oligonucleotides. Overview of selected options for modified bases, labels and other modifications. For more options, please visit exiqon.com/custom-lna-oligos.

### Modified bases

<table>
<thead>
<tr>
<th>Universal and modified bases</th>
<th>5’end</th>
<th>Internal</th>
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<td>W = A, T</td>
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<tr>
<td>ROX™ NHS Ester</td>
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## RNA Product Overview

### Exiqon NGS Services
- mRNA and whole transcriptome NGS services (page 57)
  - Complete sample-to-answer RNA-seq service
  - Let Exiqon’s service team of experienced RNA scientists and bioinformaticians perform your RNA-seq project

### LNA™ GapmeRs
- LNA™ GapmeRs for knockdown of lncRNA and mRNA (page 20)
  - Highly specific antisense LNA™ GapmeRs with superior potency for inhibition of any lncRNA and mRNA
  - Ideal alternative to siRNA for knockdown of nuclear retained RNAs
  - Active and highly stable in vivo and in vitro

### Expression Profiling
- LNA™ Detection Probes
  - LNA™-enhanced ISH probes (page 18)
    - Sensitive and specific probes for any RNA target of interest
    - Available with a wide variety of modifications

### miRCURY™ RNA Isolation Kits
- Hi-Power RNA Labeling Kits (page 17)
  - Fast, simple and uniform labeling of total RNA for use with microarrays

### miRCURY™ Exosome & RNA Isolation Kits
- Exosome Isolation kits (page 14)
  - High quality recovery of exosomes for RNA profiling and exosome surface markers

### RNA Isolation kits (page 15)
- Set total RNA from a wide range of sources with fast, simple and non-toxic protocols

### RNA isolation services (page 56)
- Have Exiqon isolate your RNA as part of your RNA-seq service project

### LNA™-enhanced detection probes (page 18)
- Ideal for Northern blotting
- Sensitive and specific probes for any RNA target of interest
- Single nucleotide mismatch discrimination for identification of e.g. mutations or splice variants

### LNA™-enhanced ISH probes (page 18)
- Sensitive and specific probes for any RNA target of interest
- Available with a wide variety of modifications

### Exiqon NGS Services
- Exiqon® qPCR system for lncRNA and mRNA
  - New product to be launched Summer 2015. Please visit exiqon.com for more information or for custom designing of LNA™-enhanced primers
"Exiqon has a solution for any step of an RNA research project."
Efficient isolation of exosomes from various biofluids by straightforward protocol in less than 2 hours. Developed for seamless integration with miRCURY™ RNA isolation kits.

**Exosome Isolation Kits**

**At a glance**
- Straightforward protocol for isolation of exosomes in less than 2 hours
- No ultra-centrifugation
- No phenol chloroform required
- High recovery rate of exosomes
- Full compatibility with Exiqon’s miRCURY™ RNA isolation Kits.

**Product coverage**
Two kits are available for exosome isolation:
- **miRCURY™ Exosome Isolation Kit – serum and plasma** for isolation of exosomes from serum and plasma samples
- **miRCURY™ Exosome Isolation Kit - Cells, urine and CSF** for isolation of exosomes from up to 10 mL of sample

Both kits are designed to work optimally with our miRCURY™ RNA Isolation Kits. Please follow Figure 11 for selection of recommended RNA isolation kit. The exosome isolation kits ensures a very high recovery rate of exosomes, and the majority of the membrane encapsulated microparticles ends up in the pelleted fraction (Figure 12).

Furthermore, the miRCURY™ Exosome isolation kits work as a means of concentrating the sample with minimal interference from inhibitors, ensuring high call rates from low-content samples like urine and CSF.

**Figure 11. Exosome and RNA isolation kits selection guide.** Select the optimal combination of miRCURY™ Exosome Isolation kit and miRCURY™ RNA Isolation kit for your samples.

**Figure 12. NanoSight measurements of pelleted exosomes and discarded supernatant demonstrate a very high recovery rate of exosomes from serum using the miRCURY™ Exosome Isolation kit.**

**Ordering information** (Details on page 70)

<table>
<thead>
<tr>
<th>Kit</th>
<th>Product description</th>
<th>Product no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRCURY™ Exosome Isolation Kit - Serum and plasma</td>
<td>All buffers needed for isolation of Exosomes from serum/plasma incl. Thrombin for pretreatment of plasma samples. Reagents for 16 x 1.5 mL sample or 50 x 0.25 mL.</td>
<td>300901</td>
</tr>
<tr>
<td>miRCURY™ Exosome Isolation Kit - Cells, urine and CSF</td>
<td>All buffers needed for isolation of Exosomes from liquid samples other than serum/plasma e.g. Urine or CSF. Reagents for 24 x 5 mL urine isolations or 12 x 10 mL extractions or more than 80 x 1.5 mL.</td>
<td>300902</td>
</tr>
</tbody>
</table>

For updated product information, go to exiqon.com/exosome-isolation-kits
RNA | Isolation

RNA Isolation Kits

Fast, easy and robust RNA isolation from various sample types with superior yield and RNA quality for downstream RNA profiling.

At a glance
• High quality total RNA isolation from a wide range of sources
• Fully compatible with Exiqon’s NGS Services, qPCR and Northern blot products
• Fast, easy and robust protocol for reproducible RNA purifications in just 20 minutes
• Excellent compatibility with RNAlater®
• No toxic organic solvents used

Product coverage
We offer three different RNA purification kits:
• miRCURY™ RNA Isolation Kit – Cell & Plant provides a rapid method for purification of total RNA from cultured animal cells, small tissue samples, blood, yeast, fungi, bacteria and plants
• miRCURY™ RNA Isolation Kit – Tissue is specifically optimized for purification of total RNA from human and animal tissue samples
• miRCURY™ RNA Isolation Kit – FFPE is a fast method for isolating RNA from archived samples using a non-toxic paraffin dissolver

Choose the right isolation kit for your samples using the guide in Figure 14.

Fast, easy and robust RNA isolation
Exiqon’s miRCURY™ RNA Isolation kits offer high quality RNA purification based on spin columns using a separation matrix containing an advanced proprietary resin.

RNA is separated from other cell components, such as proteins, without the use of toxic phenol and chloroform in a fast and easy procedure with high reproducibility between individual isolations. The end result is high-purity total RNA ready for a wide range of downstream applications.

Selected publication

Ordering information (Details on page 70)

<table>
<thead>
<tr>
<th>Kit Product description</th>
<th>Product no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRCURY™ RNA Isolation Kit – Cell &amp; Plant (50)</td>
<td>300110</td>
</tr>
<tr>
<td>50 spin columns, reagents and buffers for total RNA isolation</td>
<td></td>
</tr>
<tr>
<td>miRCURY™ RNA Isolation Kit – Tissue (50)</td>
<td>300111</td>
</tr>
<tr>
<td>50 spin columns, enzyme, reagents and buffers for total RNA isolation</td>
<td></td>
</tr>
<tr>
<td>miRCURY™ RNA Isolation Kit – FFPE (50)</td>
<td>300115</td>
</tr>
<tr>
<td>50 spin columns, enzyme, reagents and buffers including deparaffinization removal for total RNA isolation</td>
<td></td>
</tr>
</tbody>
</table>

For updated product information, go to exiqon.com/rna-isolation

Figure 13. Fast and simple 3-step RNA isolation procedure using miRCURY™ RNA Isolation kits. 1) Lysis and protein precipitation, 2) washing column, 3) Eluting purified RNA.

Step 1: Lysis and protein precipitation. Add ethanol / isopropanol and spin down.

Step 2: Wash the column with the included Wash Solution.

Step 3: Elute your purified total RNA.
Figure 14. Select the right miCURY™ RNA Isolation kit for your samples. Exiqon’s RNA isolation kits are available for many different kinds of samples. Find out which kit is ideal for your samples and what downstream detection method to use.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Recommended kit</th>
<th>RNA fraction</th>
<th>Recommended detection method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultured cells, Plant Tissues</td>
<td>miRCURY™ RNA Isolation Kit - Cell and Plant</td>
<td>Total RNA</td>
<td>Exiqon’s NGS Services, LNA™ qPCR system and LNA™ Detection probes for Northern blotting</td>
</tr>
<tr>
<td>Small human/animal Tissue samples*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole Blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exosomes**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue</td>
<td>miRCURY™ RNA Isolation Kit - Tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFPE***</td>
<td>miRCURY™ RNA Isolation Kit - FFPE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* For brain and adipose tissue use special protocol in appendix C. Extra Lysis Additive (Product No. 300121) required.
** Exosomes derived from cultured cells, urine and CSF (see Figure 11)
*** Yield from FFPE samples vary depending on tissue and input amount, low yield might not be compatible with array. LncRNA/mRNA from FFPE samples is typically fragmented.
Hi-Power Labeling Kits

High-performance RNA labeling kits enabling uniform end labeling of RNAs for consistent and reliable performance in a range of applications.

At a glance
• A simple two-step protocol
• Consistent and reliable results
• Uniform labeling - no post-labeling clean-up necessary
• Compatible with all common microarray scanners, fluorescent microscopes and readers

Product coverage
The miRCURY LNA™ Hi-Power Labeling Kit allows for fast and robust labeling of 3’end labeling of total RNA. It offers truly amazing performance with very high signal intensity compared to competing products. Furthermore, it is very easy to use with uniform labeling of the target RNA molecules.

Kits are available for both single and dual color labeling of total RNA.

Save time and get consistent results
The miRCURY LNA™ Hi-Power Labeling Kits ensure that exactly one fluorescent molecule is attached to each RNA. Use the kits for fast and simple labeling of total RNA. Once labeled, the RNA can be applied to many different downstream applications.

Hi-Power labeling offers very high signal intensity
Exiqon’s miRCURY LNA™ Hi-Power Labeling Kit offers class leading signal-to-noise ratios. This means that RNAs that were previously just below the level of detection, can now be readily detected. More RNA molecules can be detected from the same amount of input RNA. Furthermore, it is possible to work with very small RNA input amounts and still get good data with a high number of detected RNA molecules.

Ordering information (Details on page 70)

For updated product information, go to www.exiqon.com/rna-labeling-kits

For excellent performance, combine with Exiqon’s miRCURY™ RNA Isolation kits for isolation of total RNA from cell, plant, tissue or FFPE material. Learn more at exiqon.com/rna-isolation

Uniform labeling for reliable results
The labeling kits are used for single and dual color uniform 3’end labeling of total RNA samples [See Figure 15]. The dyes used (Hy3™ and Hy5™) are spectrally equivalent to the well-known Cy3 and Cy5 fluorophores, allowing for comparison of the RNA expression patterns.
LNA™ Detection Probes: ISH and Northern Blotting

LNA™-enhanced probes for lncRNA and mRNA in situ hybridization and Northern blotting with superior sensitivity and specificity. Use Exiqon’s design software to design the optimal probe for your target.

At a glance
- Superior sensitivity and specificity compared to DNA probes and riboprobes
- Designed in minutes using proprietary algorithms developed by Exiqon’s LNA™ experts
- No cloning expertise needed
- Excellent tissue penetration
- Available with a wide selection of labels

Product coverage
- Custom LNA™ Detection Probes for lncRNA and mRNA are highly sensitive LNA™-enhanced probes for in situ hybridization and Northern blotting that can be designed specifically to target your lncRNA or mRNA sequence using Exiqon’s sophisticated design tool. They are available with a large selection of 5’ and 3’ labels and in a ready-to-label version for manual labeling using standard end-labeling techniques.

Pre-designed positive and negative control probes are also available.

Sophisticated and fast online design software
Custom LNA™ Detection Probes for Northern blotting and in situ hybridization are designed in minutes for any lncRNA or mRNA target using our advanced online design software. In less than a minute, the software evaluates more than 5,000 probe designs based on more than 20 design criteria. As a result, the process ensures that high-quality probes can be designed for any sequence.

Excellent discrimination between similar RNAs
LNA™ Detection Probes, being short and binding with high specificity, are ideal for discriminating between highly similar RNAs, e.g. closely related isoforms or splice variants. The high affinity of these probes for their target sequences means that stringent hybridization and washing conditions can be used, despite the short lengths of the probes, while both sample input and exposure time can be reduced when used for Northern blotting.

Higher sensitivity with double DIG-labeled probes
For both Northern blotting and in situ hybridization using non-radioactive methods, we recommend double (3’ and 5’) DIG-labeled probes as the signal-to-noise is greatly increased due to a synergistic effect of the labels.

Selected publications
Darnell et al. RNA 2010, 16: 632-7

Ordering information (Details on page 70)

<table>
<thead>
<tr>
<th>Custom LNA™ Detection probes for lncRNA and mRNA</th>
<th>Product no.</th>
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</thead>
<tbody>
<tr>
<td>Custom probe, 250 nmol</td>
<td>300500</td>
</tr>
<tr>
<td>Positive and negative control probes</td>
<td>30051x-xx</td>
</tr>
</tbody>
</table>

Go to Custom LNA™ Detection probe design tool at exiqon.com/mrna-in-situ-hybridization to learn more and order your LNA™ Detection probes.
We got beautiful results repeatedly and could even detect the probes fluorescently.
Antisense GapmeRs

Potent antisense oligonucleotides for highly efficient knockdown of mRNA and lncRNA. RNase H-activating LNA™ GapmeRs designed using advanced algorithms to ensure superior performance and high success rate.

At a glance
- Highly potent single stranded antisense oligonucleotides (ASO) for silencing of lncRNA and mRNA
- Function by RNase H dependent degradation of complementary RNA targets
- Active in vivo and in vitro - enabling the analysis RNA function in a wide range of model systems
- Excellent alternative to siRNA for knockdown of mRNA and lncRNA
- Taken up by cells without transfection reagents
- Designed with sophisticated and empirically developed algorithm for potent and specific knockdown of target RNAs

Product coverage
LNA™ GapmeRs can be designed for any RNA target > 200 nucleotides and are available in four different categories depending on application:

- LNA™ GapmeR in vitro Standard Cost effective for initial screening of multiple designs using standard cell-lines
- LNA™ GapmeR in vitro Premium HPLC- purified GapmeRs with guaranteed purity suitable for most cell assays, also available with 5’ or 3’ fluorescent labels
- LNA™ GapmeR in vivo Ready High quality, animal-grade GapmeRs recommended for any projects that have in vivo testing as the ultimate goal. Also recommended for hard-to-transfect cell lines such as B-cells, primary cell lines, cells in suspension etc
- Custom LNA™ GapmeR – in vivo Large Scale The same high quality and purity as the in vivo Ready GapmeRs available with custom large scale yields.

Efficient silencing and fewer off-target effects
LNA™ GapmeRs are ASOs 14-16 nucleotides in length. They contain a central stretch (‘gap’) of DNA monomers flanked by blocks of LNA™ modified nucleotides (Figure 16). LNA™ in the flanks confers nuclease resistance to the oligo while at the same time increasing target affinity regardless of GC content. The central DNA “gap” activates RNase H cleavage of the target RNA upon binding (Figure 17). LNA™ GapmeRs have fully phosphorothioated backbones which ensure exceptional resistance to enzymatic degradation.

RNase H enables extremely efficient knockdown of both nuclear retained RNAs (e.g. lncRNAs) and RNAs destined for the cytoplasm. Being single stranded, LNA™ GapmeRs allow strand-specific knockdown of RNAs and minimize off-target effects due to the lack of a passenger strand. Furthermore, LNA™ GapmeRs act independently of the RNA induced silencing complex (RISC), so there are no issues with saturation of RISC.

Figure 16. Unique short single-stranded ASO design. LNA™ GapmeRs contain a DNA part flanked by LNA™. The LNA™ parts increase the affinity for the target and confers nuclease resistance. RNase H is activated by the DNA part of the ASO.

Validated positive and negative controls are also available.

Design your LNA™ GapmeRs at exiqon.com/gapmers
You can also submit your RNA sequence and let our tech support handle the design for you: exiqon.com/contact
Sophisticated and fast online design software tool
LNA™ GapmeRs are designed using an empirically derived design tool that incorporates our more than 20 years of experience with LNA™ design. For each RNA target the tool evaluates thousands of possible GapmeR designs against >30 design parameters and identify the GapmeR most likely to give potent and specific target knockdown.

Potent knockdown of mRNA
The efficacy of mRNA knockdown with LNA™ GapmeRs rivals that of siRNA [Figure 18]. LNA™ GapmeRs are therefore an excellent alternative for researchers looking for a method that works independently of RISC and has no microRNA-like off-target effects.

The application of choice for knockdown of lncRNA
Loss of function analysis of lncRNA is particularly challenging for several reasons. Many lncRNAs are involved in transcriptional regulation by attracting chromatin modifying enzymes to certain DNA targets. Confined to the nuclear compartment these lncRNAs are inefficiently targeted by siRNA. In contrast, nuclear retained RNAs are particularly sensitive to LNA™ GapmeRs exactly because they share the nuclear compartment with RNaseH, the endonuclease responsible for LNA™ GapmeR activity (Figures 17, 19 and 20).

In addition lncRNAs often derive from transcriptionally complex loci with overlapping sense and antisense transcripts. Strand specific knockdown is therefore of crucial importance which is guaranteed with GapmeRs because they are single stranded.

No transfection reagent needed
Due to their small size, exceptional potency and stability, LNA™ GapmeRs are taken up efficiently by cells directly from the culture medium. With many cell lines potent knockdown of target RNA is therefore achievable with unassisted delivery [Figure 19], avoiding the confounding cytotoxic effects normally associated with transfection reagents.

Study RNA function in live animal models
Excellent pharmacokinetic and pharmacodynamic properties of LNA™ GapmeRs have been demonstrated in many different tissues and organs. LNA™ antisense oligonucleotides are well tolerated and show low toxicity in vivo. In addition, short, high affinity LNA™ GapmeRs are active at lower concentrations compared to other antisense oligonucleotides. The incorporation of LNA™ increases the serum stability of the ASO.
LNA™ GapmeRs have also been shown to have high potential to penetrate the cell membrane barrier and successfully interact with intracellular and even nuclear retained targets. Effective and long lasting knockdown of mRNA and lncRNA can be achieved in a broad range of tissues with LNA™ GapmeRs administered in live animal models (Figure 20).

In addition, formulation (e.g. liposomes or cationic complexes) is not required for efficient delivery in vivo, making the workflow easier.

**Ordering information** (Details on page 70)

<table>
<thead>
<tr>
<th>LNA™ GapmeR</th>
<th>Product no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>in vitro Standard, 5 nmol</td>
<td>300600</td>
</tr>
<tr>
<td>in vitro Premium, ready to label, 5 nmol</td>
<td>300601-00</td>
</tr>
<tr>
<td>in vitro Premium, 5' fluorescein label, 5 nmol</td>
<td>300601-04</td>
</tr>
<tr>
<td>in vitro Premium, 3' fluorescein label, 5 nmol</td>
<td>300601-08</td>
</tr>
<tr>
<td>in vivo Ready, ready to label, 5 nmol</td>
<td>300602-00</td>
</tr>
<tr>
<td>in vivo Ready, 5' fluorescein label, 5 nmol</td>
<td>300602-04</td>
</tr>
<tr>
<td>in vivo Ready, 3' fluorescein label, 5 nmol</td>
<td>300602-08</td>
</tr>
<tr>
<td>in vivo Ready, ready to label, 20 nmol</td>
<td>300603-00</td>
</tr>
<tr>
<td>in vivo Ready, 5' fluorescein label, 20 nmol</td>
<td>300603-04</td>
</tr>
<tr>
<td>in vivo Ready, 3' fluorescein label, 20 nmol</td>
<td>300603-08</td>
</tr>
</tbody>
</table>

Selected publications
Xing et al. Cell 2014, 159: 1110-1125

**Figure 19.** LNA™ GapmeRs are taken up excellently by unassisted delivery (gymnosis). LNA™ GapmeRs can be delivered to cell-lines by adding them directly to the culture medium without transfection reagents - so called gymnosis. This can be useful with hard-to-transfect cell lines and to avoid experimental artifacts introduced by transfection reagents.

**Figure 20.** Efficient and long lasting knockdown in vivo with LNA™ GapmeRs. LNA™ GapmeR for knockdown of Malat1 in mice was injected subcutaneously over a period of 4 weeks. Samples from a broad range of tissues from the mice were collected up to 5 weeks after last LNA™ GapmeR administration, where the knockdown effect was still highly efficient in all tissues.
Antisense GapmeRs offer efficient knockdown of mRNA and lncRNA.

Design a GapmeR for your favorite RNA at exiqon.com/gapmers
**Product Overview**

**MicroRNA**

**Isolation**

- **miRCURY™ Exosome & RNA Isolation Kits**
  - **Exosome Isolation Kits (page 26)**
    - High quality recovery of exosomes for microRNA profiling
  - **RNA Isolation Kits (page 28)**
    - Get total RNA from a wide range of sources with fast, simple and non-toxic protocols

**Exiqon NGS Services**

- **microRNA and Small RNA NGS Services (page 57)**
  - Complete sample-to-answer NGS service
  - Let Exiqon’s service team of experienced RNA scientists and bioinformaticians perform your microRNA or small sequencing project

**miRCURY LNA™ Universal RT microRNA PCR**

- **Universal RT microRNA PCR (page 33)**
  - Complete qPCR platform optimized for microRNA quantitation including all reagents needed
  - Superior sensitivity and specificity for accurate microRNA profiling from various types of samples incl. biofluids
  - Flexible assay format: individual primer sets, miRNome panels, focus panels and fully customizable panels

**microRNA and Small RNA NGS Services**

- **Hi-Power Labeling Kits (page 30)**
  - Fast, simple and uniform labeling of total RNA for use with microarrays

**miRCURY LNA™ microRNA Array**

- **microRNA microarray – hsa, rno, mmu (page 31)**
  - Extremely sensitive and specific microarray for robust and reliable global microRNA expression profiling

**Hi-Power Labeling Kits**

- **microRNA Microarray Profiling Services (page 61)**
  - Complete sample-to-answer microRNA profiling service performed by Exiqon’s microRNA experts

**miRCURY LNA™ microRNA Detection Probes**

- **microRNA Detection Probes for in situ hybridization (page 40)**
  - Sensitive and specific ISH probes for all microRNAs

**miRCURY LNA™ microRNA ISH Optimization Kits, FFPE (page 44)**

- **Kits and a one-day protocol for optimization of microRNA ISH from many sample sources**

**miRCURY LNA™ microRNA Inhibitors**

- **microRNA Inhibitors and Power Inhibitors (page 46)**
  - Highly potent inhibitors for specific and long-lasting knockdown of microRNA

**miRNA LNA™ microRNA Inhibitor**

- **In vivo LNA™ microRNA inhibitors (page 49)**
  - Superior inhibition of microRNA in animal models

**miRCURY LNA™ microRNA Target Site Blocker**

- **microRNA Target Site Blockers (page 52)**
  - High-affinity microRNA target site blockers (TSBs) for the study of single microRNA target sites – in vivo and in vitro

- **Ideal for determining which pathway is involved in the observed effects of microRNA inhibition**

**miRCURY LNA™ microRNA Mimic**

- **microRNA Mimics (page 50)**
  - Unique triple RNA strand mimics design for specific microRNA mimicry without off-target microRNA activity

- **Biotinylated mimics for pull-down studies**
"Exiqon’s technical support is very efficient and I believe they strive to give the best possible service to their customers."
Exosome Isolation Kits

Efficient isolation of exosomes from various biofluids by straightforward protocol in less than 2 hours. Developed for seamless integration with miRCURY™ RNA isolation kits.

At a glance
- Straightforward protocol for isolation of exosomes in less than 2 hours
- No ultra-centrifugation
- No phenol chloroform required
- High recovery rate of exosomes
- Full compatibility with Exiqon’s miRCURY™ RNA isolation Kits and miRCURY LNA™ Universal RT microRNA PCR

Product coverage
Two kits are available for exosome isolation:
- miRCURY™ Exosome Isolation Kit – Serum and plasma
- miRCURY™ Exosome Isolation Kit - Cells, urine and CSF

Both kits are designed to work optimally with our miRCURY™ RNA Isolation Kits. Please follow Figure 21 for selection of compatible RNA isolation kit. The exosome isolation kits ensures a very high recovery rate of exosomes, and the majority of the membrane encapsulated microparticles ends up in the pelleted fraction (Figure 22).

Furthermore, the miRCURY™ Exosome isolation kits work as a means of concentrating the sample with minimal interference from inhibitors, ensuring high call rates from low-content samples like urine and CSF.

Figure 21. Exosome and RNA isolation kits selection guide. Select the optimal combination of miRCURY™ Exosome isolation kit and miRCURY™ RNA Isolation kit for your samples.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Recommended exosome extraction kit</th>
<th>Recommended Exosome Isolation Kit</th>
<th>Recommended Detection method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultured cells, urine, CSF or other biofluids</td>
<td>miRCURY™ Exosome Isolation Kit – Cells, urine and CSF</td>
<td>miRCURY™ RNA Isolation Kit – Cell and Plant</td>
<td>miRCURY LNA™ Universal RT microRNA PCR</td>
</tr>
<tr>
<td>Serum or plasma</td>
<td>miRCURY™ Exosome Isolation Kit – Serum and plasma</td>
<td>miRCURY™ RNA Isolation Kit – Biofluids</td>
<td></td>
</tr>
</tbody>
</table>
Why study exosomes?

Exosomes are cell derived membranous particles ranging in size from 20 to 120 nm, approximately the same size as viruses but considerably smaller than microvesicles (Figure 23). Exosomes are excreted from cells into the surrounding media and can be found in many if not all body fluids. Their proposed role as intercellular hormone-like messengers together with their stability as carrier of proteins and RNA make them ideal as biomarkers for a variety of diseases and biological processes.

Exosomes are secreted by most cell types and are formed by the fusion of multivesicular bodies with the plasma membrane. They are believed to be involved in a number of functions, including:

- **Immune regulation** (e.g. tumor derived exosomes may help the tumor to evade the immune response)
- **Blood coagulation**
- **Cell migration**
- **Cell differentiation**
- **Cell-to-cell communication**

Microvesicles that are larger than exosomes (up to 1 μm) are typically formed by blebbing of the plasma membrane, whereas exosomes are released by exocytosis from multivesicular bodies of the endosome.

**Figure 23. The structure of an exosome.** Exosomes are membrane-encapsulated particles typically ranging from 20 to 120 nm in size and contain multiple macromolecules: Proteins, mRNA and microRNA and recently they have also been reported to contain DNA. A number of surface proteins are found exclusively in exosomes.

---

Superior RNA isolation from Exosomes

RNA Isolation Kits

Fast, easy and robust RNA isolation from various sample types with superior yield and RNA quality for downstream microRNA profiling.

At a glance
- High quality total RNA isolation from a wide range of sources
- Fully compatible with Exiqon’s PCR, Array, NGS Services and Northern blot products
- Fast, easy and robust protocol for reproducible RNA purifications in just 20 minutes
- Excellent compatibility with RNAlater®
- No toxic organic solvents

Product coverage
We offer four different RNA purification kits:
- **miRCURY™ RNA Isolation Kit – Biofluids** purifies low abundance small RNA (<1000 bp) from samples such as serum, plasma, urine and CSF
- **miRCURY™ RNA Isolation Kit – Cell & Plant** provides a rapid method for purification of total RNA from cultured animal cells, small tissue samples, blood, yeast, fungi, bacteria and plants
- **miRCURY™ RNA Isolation Kit - Tissue** is specifically optimized for purification of total RNA from human or animal tissue samples
- **miRCURY™ RNA Isolation Kit - FFPE** is a fast method for isolating RNA from archived samples using a non-toxic paraffin dissolver

Choose the right isolation kit for your samples using the guide in Figure 25.

Fast, easy and robust RNA isolation
Exiqon’s miRCURY™ RNA Isolation kits offer high quality RNA purification based on spin columns using a separation matrix containing an advanced proprietary resin.

RNA is separated from other cell components, such as proteins, without the use of toxic phenol and chloroform in a fast and easy procedure with high reproducibility between individual isolations.

The end result is high-purity RNA ready for a wide range of downstream applications.

Selected publication

Figure 24. Fast and simple 3-step RNA isolation procedure using miRCURY™ RNA Isolation kits. 1) Lysis and protein precipitation, 2) washing column, 3) Eluting purified total RNA.

Ordering information (Details on page 70)

<table>
<thead>
<tr>
<th>Kit</th>
<th>Product description</th>
<th>Product no.</th>
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</thead>
<tbody>
<tr>
<td>miRCURY™ RNA Isolation Kit – Cell &amp; Plant (50)</td>
<td>50 spin columns, reagents and buffers for total RNA isolation</td>
<td>300110</td>
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<tr>
<td>miRCURY™ RNA Isolation Kit – Tissue (50)</td>
<td>50 spin columns, enzymes, reagents and buffers for total RNA isolation</td>
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<tr>
<td>miRCURY™ RNA Isolation Kit – Biofluids (50)</td>
<td>50 spin columns, reagents and buffers for miRNA isolation</td>
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<td>miRCURY™ RNA Isolation Kit – Tissue (10)</td>
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<tr>
<td>miRCURY™ RNA Isolation Kit – FFPE (50)</td>
<td>50 spin columns, enzymes, reagents and buffers including deparaffinization removal for total RNA isolation</td>
<td>300115</td>
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</tbody>
</table>

For updated product information, go to [exiqon.com/rna-isolation](http://exiqon.com/rna-isolation)
Figure 25. Choose the right miCURY™ RNA Isolation kit for your samples. Exiqon RNA isolation kits are available for many different kinds of samples. Find out which kit is ideal for your samples and what downstream detection method to use.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Recommended kit</th>
<th>RNA fraction</th>
<th>Recommended detection method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum / Plasma, Urine</td>
<td>miCURY™ RNA Isolation Kit - Biofluids</td>
<td>Small RNA</td>
<td>miCURY LNA™ Universal RT microRNA PCR</td>
</tr>
<tr>
<td>CSF, Other biofluids</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Exosomes from serum/plasma</td>
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<tr>
<td>Cultured cells, Plant Tissues</td>
<td>miCURY™ RNA Isolation Kit - Cell and Plant</td>
<td>Total RNA</td>
<td>miCURY LNA™ Universal RT microRNA PCR or miCURY LNA™ microRNA Array or Exiqon Services (NGS, qPCR or Array)</td>
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<tr>
<td>Small human/animal tissue sample*</td>
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<td></td>
<td></td>
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<tr>
<td>Exosomes***</td>
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<td></td>
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<tr>
<td>Whole Blood</td>
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<td></td>
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<tr>
<td>Tissue</td>
<td>miCURY™ RNA Isolation Kit - Tissue</td>
<td>Total RNA</td>
<td>miCURY LNA™ Universal RT microRNA PCR or miCURY LNA™ microRNA Array or Exiqon Services (NGS, qPCR or Array)</td>
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<tr>
<td>FFPE***</td>
<td>miCURY™ RNA Isolation Kit - FFPE</td>
<td>Total RNA</td>
<td>miCURY LNA™ Universal RT microRNA PCR or miCURY LNA™ microRNA Array or Exiqon Services (NGS, qPCR or Array)</td>
</tr>
</tbody>
</table>

* For brain and adipose tissue use special protocol in appendix C. Extra Lysis Additive (Product No. 300121) required.
** Exosomes derived from cultured cells, urine, CSF [see Figure 21].
*** Yield from FFPE samples vary depending on tissue and input amount, low yield might not be compatible with array. LncRNA/mRNA from FFPE samples is typically fragmented.

Best-in-class RNA isolation
In a study comparing Exiqon’s miCURY™ RNA Isolation Kit for Biofluids to competing commercial kits, it was found that Exiqon’s kit offers the best combination of performance and ease-of-use. Reference: McAlexander et al. Front. Genet. 2013, 4: 83
Hi-Power Labeling Kits

High-performance RNA labeling kits for use with microRNA microarrays. Uniform labeling of microRNAs for consistent and reliable single or dual color experiments.

At a glance
- A simple two-step protocol requiring no small RNA enrichment
- Consistent and reliable results
- Uniform labeling - no post-labeling clean-up necessary
- Compatible with all common microarray scanners
- Optimized for use with Exiqon’s miRCURY LNA™ microRNA microarrays

Product coverage
The miRCURY LNA™ microRNA Hi-Power Labeling Kit allows for fast and robust labeling of 3’end labeling of total RNA. It offers truly amazing performance with very high signal intensity compared to competing products. Furthermore, it is very easy to use for uniform labeling of the microRNAs.

Kits are available for both single and dual color labeling of total RNA.

Save time and get consistent results
The miRCURY LNA™ microRNA Hi-Power Labeling Kits are the perfect complement to Exiqon’s highly sensitive and specific LNA™ microarrays. Use the kits for fast and simple labeling of total RNA. Once labeled, the RNA can be applied directly to the microarray without subsequent microRNA enrichments or other time-consuming sample handling steps.

Uniform labeling for reliable results
The labeling kits are used for single and dual color uniform 3’end labeling of total RNA samples [Figure 26]. The dyes used (Hy3™ and Hy5™) are spectrally equivalent to the well-known Cy3 and Cy5 fluorophores, allowing for comparison of microRNA expression patterns.

Figure 26 Uniform labeling. The miRCURY LNA™ microRNA Hi-Power Labeling Kits incorporate only one fluorescent label per microRNA. This results in uniform labeling independent of the sequence at the 3’-end of the microRNA.

Hi-Power labeling offers very high signal intensity
Exiqon’s miRCURY LNA™ microRNA Hi-Power Labeling Kit offers class leading signal-to-noise ratios. This means that microRNAs that were previously just below the level of detection, can now be readily detected. More microRNAs can be detected from the same amount of input RNA. Furthermore, it is possible to work with very small RNA input amounts and still get good data with a high number of detected microRNAs.

Selected publications
Zaravinos et al. PLOS one, 2014, 9: e91646
Gorini et al. PLOS one, 2013, 8: e82565
Bach et al. J Photochem Photobiol B. 2013, 120: 74-81

Ordering information (Details on page 70)

For excellent performance, combine with Exiqon’s miRCURY™ RNA Isolation kits for isolation of total RNA including microRNA from cell, plant, tissue or FFPE material and biofluids including serum/plasma, urine and CSF. Learn more at exiqon.com/rna-isolation

For updated product information, go to exiqon.com/rna-labeling-kits
**microRNA | Expression Profiling**

**Microarrays**

Sensitive and specific microRNA microarrays – ideal for global microRNA expression profiling. LNA™-enhanced and $T_m$-optimized capture probes give uniform detection of all microRNAs. Exiqon offers a streamlined workflow from RNA labeling to data analysis.

**At a glance**
- $T_m$-optimized microarray for robust detection of ALL microRNAs, regardless of GC-content
- Validated LNA™-enhanced capture probes for increased sensitivity and specificity
- Excellent sensitivity - microRNA profiling starting from 30ng total RNA

**Product coverage**
- miRCURY LNA™ microRNA Array, 7th gen - hsa, mmu & rno The 7th generation of our array covers all human, mouse and rat microRNAs annotated in miRBase 19.0, as well as all viral microRNAs related to these species.

**Advantages of LNA™ capture probes**
As a unique feature of Exiqon’s microRNA array, all capture probes are LNA™-enhanced. LNA™ probes have two important advantages over traditional DNA probes (Figure 27):

1. **High affinity** - The addition of LNA™ to the capture probes results in high melting temperatures ($T_m$) of the probe-target duplex, thus increasing the specificity and sensitivity of the array.

2. **Uniform affinity** - Unlike DNA capture probes, $T_m$-normalized LNA™ probes bind to their target sequences with equal affinity regardless of the GC-content of the microRNA. This can be achieved by varying the positions and amount of LNA™ in each probe.

As a consequence, all probes will perform optimally under the same high-stringency hybridization conditions.

**Unmatched sensitivity**
In combination with the miRCURY LNA™ microRNA Hi-Power Labeling Kit, the performance in sensitivity is unmatched (Figure 28). More than half of the LNA™ capture probes on the array have a detection limit of <0.5 amol.

**High specificity with single nucleotide discrimination**
miRCURY LNA™ microRNA Arrays are highly specific for their microRNA targets. The combination of $T_m$-normalized LNA™ capture probes and hybridization conditions optimized for high stringency binding, dramatically increases the specificity of the capture probes. As a result, Exiqon arrays provide superior discrimination between closely related microRNA family members.

**Experimentally validated capture probes**
All capture probes on the miRCURY LNA™ microRNA Arrays have been experimentally validated using synthetic microRNAs. This ensures high confidence in the experimental outcome and a high success rate in validation experiments.

---

**Figure 27. LNA™-enhanced capture probes ensure robust detection of all microRNAs.** With DNA capture probes, half of the microRNAs were either undetected or poorly detected. Signal strength (log$_2$ signal/100amol target) from 660 synthetic microRNAs hybridized to Exiqon’s microarray and Supplier A’s DNA-based array are compared.
Spike-in miRNA Kit v2 for data quality improvement
The 7th gen microRNA Array includes a kit with 52 synthetic spike-in microRNAs that can be used as controls for the labeling reaction and hybridization, scanner settings, data normalization, array replicates and technical variability.

A robust system with high reproducibility
The miRCURY LNA™ microRNA Arrays feature very high reproducibility due to a stringent manufacturing process that ensures high quality uniform spots. This results in very low coefficient of variation (CV) values for the four replicate spots as well as excellent correlation between individual array slides. This makes the array ideal for single as well as dual color array experiments.

Data analysis software for Exiqon arrays
In collaboration with BioDiscovery, Exiqon offers ImaGene® 9 and Nexus Expression™ 3. Analyze your data with this leading-edge software specifically adapted for use with Exiqon’s microarray platform.

Validate your results with Exiqon’s LNA™ qPCR system
Our qPCR system offers the best available combination of performance and ease-of-use on the microRNA qPCR market and is the ideal solution for validating your microarray results. Identical positive controls on both platforms allows for robust cross-platform comparison of results.

Selected publications
Esguerra et al. PLoS One 2011, 6: e18613

Ordering information
Go to exiqon.com/array for more publications and updated product information.
Exiqon’s microRNA qPCR system combines the speed of a Universal RT reaction with the sensitivity and specificity of LNA™-enhanced PCR primers. Complete your microRNA profiling in just 3 hours without the need to pre-amplify. Choose between individual assays, miRNome Panels, Custom Pick-&-Mix Panels and Focus Panels.

**At a glance**
- Exceptional sensitivity – reliable quantification of individual microRNA from as little as 1pg total RNA
- Accurate profiling of hundreds of microRNA in 96- and 384-well plates using just 20ng total RNA
- Superior specificity – LNA™-enhanced primers enable specific quantification of microRNA differing by a single nucleotide
- Fast and easy – Universal RT protocol completed in 3 hours
- Choose from 20,000 assays covering all organisms in miRBase
- Complete data analysis solution with Exiqon GenEx

**LNA™ PCR amplification** – Both PCR amplification primers (forward and reverse) are microRNA-specific and optimized with LNA™. This results in exceptional sensitivity, extremely low background and highly specific assays that allow discrimination between closely related microRNA sequences.

**Product coverage**
Exiqon offers a solution for the entire microRNA qPCR workflow - from cDNA synthesis to data analysis and for microRNA expression profiling, validation or quantitation of multiple as well as individual microRNAs.

1. **cDNA synthesis kit:** Use our Universal cDNA Synthesis Kit for fast, easy and reliable first strand synthesis. The same single cDNA synthesis reaction can be used as template in the PCR amplification, regardless of whether you are using PCR plates or individual assays.

2. **PCR panels and assays for microRNA amplification:** Exiqon offers a wide range of PCR panels and assays. Our ready-to-use PCR panels include miRNome panels, Focus panels and custom-designed Pick-&-Mix panels (Figure 29). Alternatively, you can choose from over 20,000 pre-designed individual primer sets or design your own assays using our intuitive and powerful online design tool.
Use the ExiLENT SYBR® Green Master Mix to get the most out of your samples. This high-performance PCR master mix kit was specifically designed for Exiqon’s qPCR system.

3. Analysis software. Exiqon GenEx is a powerful, yet easy-to-use software suite with all the tools you need for qPCR data analysis.

**Accurate microRNA quantification using just 1pg total RNA**

Use of LNA™-enhanced Tm-normalized primers means that the PCR amplification is extremely sensitive and therefore allows accurate and reliable quantification of individual microRNAs from as little as 1 pg of total RNA input in the first-strand cDNA synthesis reaction (Figure 32).

Pre-amplification of the cDNA is not required. MicroRNAs can be profiled in 384-well plates using just 20ng total RNA. This is important when working with samples that contain very little total RNA, such as FFPE sections, LCM, serum/plasma and other biofluids.

The incorporation of LNA™ in the PCR amplification primers (forward and reverse) facilitates the design of assays that can distinguish between microRNA sequences that differ by a single nucleotide (Table 2). In addition, the assays can discriminate between mature and precursor microRNAs.

**Fast, easy and reproducible**

The easy-to-follow protocol takes only 3 hours to complete. The Universal RT reaction simplifies the reaction set up and saves both time and effort. Furthermore, the number of pipetting steps is reduced and technical variation is minimized.

As a result, it is possible to achieve extremely high reproducibility from day-to-day and site-to-site.

**Biomarker discovery**

Exiqon’s qPCR system is the ideal solution for biomarker discovery. miRNome panels give a quick overview of which microRNAs are present in a given sample collection. Focus panels or Pick-&-Mix panels allow cost effective screening of larger numbers of samples by including only those microRNAs that are relevant for the specific study (Figure 33).
In a new large study published in Nature Methods, Pieter Mestdagh et al. compare the performance of commercially available microRNA profiling platforms in key areas. Exiqon is the only platform to be a top performer in all areas, combining both high sensitivity and specificity.

To find out more information, please go to: www.exiqon.com/mirqc
Individual assays targeting 20,000 different microRNA sequences are available for delivery in either tubes or plates with 200 reactions/tube or well. Of these 20,000 assays, the 1,400 most popular have been thoroughly wet lab validated.

Ready-to-use PCR panels
All panels are delivered in a ready-to-use format with 10 μl reaction volume per well. Just add cDNA and Exiqon’s ExiLENT SYBR® Green master mix to the plates and run the real-time PCR. The whole process takes only 3 hours.

Exiqon’s panels include several reference genes and controls and are compatible with most real-time PCR instruments. For a complete list of Focus and miRNome panels, please see page 38.

miRNome Panels
These panels contain pre-aliquoted PCR primer sets in 384-well PCR plates for human, mouse and rat microRNAs. MicroRNA profiling from two PCR plates using just 40 ng total RNA - no pre-amplification necessary.

- 752 human and 752 rodent microRNA assays available
- Recommended for projects involving a large number of microRNAs
- Includes reference genes, inter-plate calibrator and control primer sets

Pick-&-Mix panels
Design your own panels from our wide selection of primer sets and six plate layouts. Design the plates the way you want them using our online plate configurator tool. Layouts of any miRNome or Focus panels can be customized in the configurator. These panels are ideal for investigating or validating signatures and subsets of microRNA on a medium to large number of samples.

- Fully customizable to favored plate type, layout, PCR instrument and batch size
- Choose primer sets from Exiqon’s vast collection of thoroughly validated miRCURY LNA™ Universal RT microRNA PCR assays or use custom designed primer sets

Table 2. Excellent discrimination between closely related microRNA family members. Examples of single nucleotide discrimination in the miR-181 family.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sequence</th>
<th>Template</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-181a-5p</td>
<td>AACAUUCAACCGUGUCGGUGAGU</td>
<td>100.0%</td>
</tr>
<tr>
<td>miR-181b-5p</td>
<td>AACAUUCAACCGUGUCGGUGAGU</td>
<td>100.0%</td>
</tr>
<tr>
<td>miR-181c-5p</td>
<td>AACAUUCAACCGUGUCGGUGAGU</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Figure 32. Accurate quantitation from 1 pg total RNA starting material. Data from the amplification of 6 microRNAs in serial dilutions of human AM6000 total reference RNA are shown. All miRNA assays exhibit linear read-out with correlation coefficients R(2) > 0.99.

Let Exiqon perform your experiments
Nobody knows our PCR system as well as we do. Let Exiqon services perform your microRNA qPCR experiments. Learn more on page 64.

Get expert advice on your microRNA profiling project: exiqon.com/contact

Individual custom assays
Design LNA™-enhanced qPCR primer sets for any microRNA or small RNA using our easy-to-use online batch design tool.

- Optimal PCR primers are designed using Exiqon’s online design tool based on advanced in-house algorithms
- Primer designs are species specific, taking target organism sequence composition into account

RNA Quality control
Perform quality control of your RNA samples using the RNA Spike-in kit and carefully selected RNA QC panel available in ready-to-use PCR plates. Control primer sets for the RNA spike-ins are also available as individual assays and on the PCR panels.

- Control RNA yield, cDNA synthesis and PCR efficiency
- Unique hemolysis indicator on QC panel
Reference genes and normalization

Twelve reference genes targeting endogenous small non-coding RNAs that are constitutively expressed in a variety of tissues are available. However, we recommend using stably expressed microRNAs for normalization.

All ready-to-use panels contain appropriate reference gene candidates. It is always important to ensure that putative reference genes are expressed at a constant level in all samples before using them for normalization.

Exiqon GenEx data analysis software

Exiqon offers a specifically adapted version of the comprehensive yet easy-to-use qPCR analysis software GenEx with all the tools needed for qPCR data analysis:

- Intuitive interface for easy generation of templates compatible with most real-time PCR instruments and rapid import of data directly into GenEx
- Includes pre-processing of qPCR data, fast and easy data import with Exiqon import wizard, easy selection of reference genes, straightforward normalization and easily implemented statistical analysis
- Publication-ready plots and graphs
- Perpetual or time limited license and free support including detailed manual and online tutorials
- Download a 14 day free trial and use our step-by-step guide to get started with your data analysis

GenEx offers user-friendly step-by-step guides to data preprocessing. You are guided through interplate calibration, normalization and calculation of relative values without the need of advanced bioinformatics skills. In addition, sophisticated statistical analysis tools are included in the software.

For more information and to download a free trial, please see exiqon.com/mirna-pcr-analysis

Selected publications

Jensen et al. BMC Genomics 2011, 12: 435
Jorde et al. BMC Research Notes 2012, 5: 245

For more publications and updated product information, please visit: exiqon.com/mirna-pcr
### Ordering Information (Details on page 70)

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Product description</th>
<th>Product no.</th>
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<tbody>
<tr>
<td>Universal cDNA Synthesis Kit II</td>
<td>Polyadenylation and cDNA synthesis kit (8 to 64 rxns)</td>
<td>203301</td>
</tr>
<tr>
<td>ExiLENT SYBR® Green master mix, 2.5ml</td>
<td>250 rxns of 20μl or 500 rxns of 10μl</td>
<td>203403</td>
</tr>
<tr>
<td>ExiLENT SYBR® Green master mix, 20ml</td>
<td>2000 rxns of 20μl or 4000 rxns of 10μl</td>
<td>203421</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Panel</th>
<th>Organism (number of assays in panel)</th>
<th>Format</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>miRNome</td>
<td></td>
<td>384</td>
<td>Human or rodent miRNome; panels available in one or two 384 well plates that covers the most microRNAs in miRbase 20.</td>
</tr>
<tr>
<td>Serum plasma</td>
<td></td>
<td>Ws/384</td>
<td>Each panel contains LNA™ primers targeting those microRNAs that are predominantly expressed in serum and plasma samples.</td>
</tr>
<tr>
<td>Toxicology</td>
<td></td>
<td>Ws/384</td>
<td>The panels contain LNA™ primers for all relevant toxicity-related microRNAs. Ideal for microRNA biomarker detection in cell cultures such as serum/plasma and urine.</td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td>Ws/384</td>
<td>PCR panels contain LNA™ primers for microRNAs related to cancer. Both cancer specific assays and general cell cycle related assays are included.</td>
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<tr>
<td>Urine Exosomes</td>
<td></td>
<td>Ws/384</td>
<td>The panel is available through Exiqon's flexible Pick- &amp; Mix. It targets microRNAs that are typically found in exosomes from urine samples.</td>
</tr>
<tr>
<td>CSF Exosomes</td>
<td></td>
<td>Ws/384</td>
<td>The panel is available through Exiqon's flexible Pick- &amp; Mix. It targets microRNAs that are typically found in exosomes from CSF samples.</td>
</tr>
<tr>
<td>Stem cell</td>
<td></td>
<td>Ws/384</td>
<td>Exiqon's Pluripotent Stem Cell Focus microRNA PCR Panel targets microRNAs important for either hESC or iPSC stem cell research.</td>
</tr>
<tr>
<td>QC</td>
<td></td>
<td>Ws/384</td>
<td>PCR panels to check the quality of your isolated RNA, look for outlier samples, identify the presence of nucleases and PCR inhibitors and look for signs of contamination.</td>
</tr>
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Individual Assays

<table>
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<th>Individual Assays</th>
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<tr>
<td>xxx-miR-xxx, LNA™ PCR primer set, UniRT</td>
<td>microRNA primer set, 200 rxns in tube or plate</td>
<td>204000-206997</td>
</tr>
<tr>
<td>Reference gene Assay Primer, UniRT</td>
<td>Reference gene primer set, 200 rxns in tube or plate</td>
<td>203901-203912</td>
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<tr>
<td>Pick- &amp; Mix LNA™ microRNA PCR panel</td>
<td>8 PCR plates with custom selection of LNA™ microRNA primer sets</td>
<td>203891-203897</td>
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<tr>
<td>96 well, Ready-to-Use plates</td>
<td>8 PCR plates with custom selection of LNA™ microRNA primer sets</td>
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RNA Spike-In

<table>
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<tr>
<th>RNA Spike-in kit, UniRT</th>
<th>LNA™ microRNA PCR, Set of two vials with synthetic RNA spike-in templates for qPCR control (UniSp2, UniSp4, UniSp5)</th>
<th>203203</th>
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<tbody>
<tr>
<td>UniSp2, LNA™ control primer set, UniRT</td>
<td>LNA™ microRNA PCR, spike-in control primer set, 200 rxns</td>
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<tr>
<td>UniSp3, LNA™ control primer set, UniRT</td>
<td>LNA™ microRNA PCR, spike-in control primer set, 200 rxns</td>
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<tr>
<td>col-miR-39-3p, LNA™ control primer set, UniRT</td>
<td>LNA™ microRNA PCR, spike-in control primer set, 200 rxns</td>
<td>203952</td>
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<tr>
<td>UniSp4, LNA™ control primer set, UniRT</td>
<td>LNA™ microRNA PCR, spike-in control primer set, 200 rxns</td>
<td>203953</td>
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Exiqon GenEx Software

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<tr>
<th>Exiqon GenEx Software</th>
<th>Product description</th>
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</tr>
</thead>
<tbody>
<tr>
<td>GenEx 6 Pro</td>
<td>Exiqon GenEx, qPCR analysis software, available as industrial or academic licenses</td>
<td>See website</td>
</tr>
<tr>
<td>GenEx 6 Enterprise</td>
<td>Exiqon GenEx, qPCR analysis software, industrial license available as industrial or academic licenses</td>
<td>See website</td>
</tr>
</tbody>
</table>

Each panel product is available in different types of plates for specific real-time PCR instrument compatibility: Roche LightCycler 480 (Plate R), ABI 7900HT, ABI Vii7, ABI Quantstudio w. 384 well block, Bio-Rad CFX384 (Plate M). Compatible with 96 well PCR systems: Roche LightCycler 480 (Plate R), ABI 7900 series FAST, ABI StepOnePlus, ABI Vii7 FAST, ABI Quantstudio w. 96 well FAST block (Plate AF), ABI7000 series standard, ABI Vii7, ABI Quantstudio w. 96 well standard, Bio-Rad Cycler IQ, iQ5 and MyIQ, Eppendorf Mastercycler replex, Stratagene Mx4000 (Plate M), Stratagene Mx3000P (Plate ST), Bio-Rad CFX96, please enquire.

*Number of assays for this organism is based on profiling from the human panel version.
Exiqon knows about microRNA profiling in blood serum and plasma

We have taken advantage of Exiqon’s pioneering clinical diagnostic work on microRNA expression profiling in serum and plasma when designing our new Serum/Plasma Focus microRNA PCR Panel. It has been thoroughly validated for use with clinical samples. Furthermore, the panel is well-suited for a clinical workflow as it is automatable and fully compatible with standard FDA-approved qPCR equipment. All 179 microRNA assays on our focus panel have been carefully selected based on our vast number of in-house analyses of microRNA expression in blood serum and plasma samples as well as on peer-reviewed published papers available.

Over 1 million in-house and collaborative data points from samples collected from healthy as well as diseased individuals have been used in the selection of relevant microRNAs for the panel. This includes microRNA expression data from different disease stages from various types of cancer, neurological disorders, allergies, diabetes, inflammation etc.

For more information, visit the Biofluids Reading Room at exiqon.com/biofluids
microRNA | Localization

Detection Probes: ISH and Northern Blotting

Extremely sensitive and specific LNA™-enhanced probes targeting any microRNA or small RNA in a wide range of sample sources. Superior for use in Northern and in situ hybridization studies.

At a glance
- Unmatched sensitivity and specificity
- Probes for all known microRNAs as well as custom sequences
- Fully developed protocols including Exiqon’s proprietary One-day ISH protocol
- Northern blots ready in just a few hours with microRNA detection from as little as 2.5 μg total RNA
- Available unlabeled, in a wide selection of pre-labeled versions, or with custom labels
- Compatible with radioactive and non-radioactive methods

Product coverage
There are two kinds of microRNA detection probe products for ISH and Northern blotting available:
- Pre-designed miRCURY LNA™ microRNA Detection Probes are available for all invertebrate, vertebrate and plant microRNAs annotated in miRBase.
- Custom miRCURY LNA™ microRNA Detection Probes are available for any microRNA or small RNA, including precursor microRNAs. Let our experts design the optimal probe for you.

Positive and negative control probes are also available. We offer Detection Probes in a “ready-to-label” format. These probes can be conveniently labeled using standard end-labeling techniques e.g. enzymatic or radioactive labeling. Alternatively, detection Probes may be ordered pre-labeled with a range of different labels available.

Sensitive and specific detection
miRCURY LNA™ microRNA Detection Probes for Northern blotting offer very high binding affinity and discrimination, resulting in highly specific and sensitive microRNA detection from 10 times less sample than when using traditional DNA probes (Figure 34). Moreover, the exposure time is reduced to just a few hours. The high specificity of the probes means that they can be used to discriminate between single nucleotide differences (Figure 35).

Figure 34. LNA™ probes are superior to DNA probes. A thaliana total RNA was hybridized with 32P-labeled DNA and LNA™ probes for miR-171. From Válóczi et al. 2004, Nucleic Acids Res. e175; reprinted with permission from Oxford University Press.
Higher sensitivity with double DIG-labeled probes
For researchers who wish to perform Northern blotting using non-radioactive methods, we recommend double [3’ and 5’] DIG-labeled probes. These probes offer excellent sensitivity [see Kim et al. 2010].

Selected publications
Kim et al. Nucleic Acids Res. 2010, 1-7

Sensitive microRNA detection
miRCURY LNA™ microRNA Detection Probes for in situ hybridization bind to their targets with high affinity, resulting in very specific and sensitive detection of microRNAs in whole mounts, single cells and sections from frozen or formalin-fixed paraffin-embedded (FFPE) tissues (including archived samples). For FFPE samples, we recommend using the probes in conjunction with one of our miRCURY LNA™ microRNA ISH Optimization Kits [page 44].

The miRCURY LNA™ microRNA Detection Probes for in situ hybridization have been used with great success in a variety of samples [Figures 34-37]. This is evident from the large number of peer-reviewed publications based on results obtained using these probes in various cells and tissues. Our detection probes help researchers to accurately address “when” and “where” a particular microRNA is expressed.

Double DIG labels for higher sensitivity
Double (5’ and 3’) DIG-labeled probes offer substantially higher sensitivity than single labeled probes (Figure 37). A cooperative effect of the two DIG labels results in greatly increased signal to noise ratio (up to 10-fold higher) which means that even low abundance microRNAs can be reliably detected. We recommend this labeling option for optimal results.

Figure 35. LNA™ probes readily discriminate between single nucleotide differences. The specificity was assessed using 32P-labeled probes, with and without mismatches (MM), targeting miR-171 in A. thaliana flowers [1] and leaves [2]. From Válóczi et al. 2004, Nucleic Acids Res. e175; reprinted with permission from Oxford University Press.

Figure 36. MicroRNA detection in zebrafish. Detection of miR-122a (top), miR-206 (middle) and miR-124a (bottom) using LNA™ probes in whole mount zebrafish embryos. Image kindly provided by Dr. Ronald Plasterk, Hubrecht Laboratory, The Netherlands.
**Selected publications**


**Figure 37. Double DIG labeling is more sensitive than single DIG labeling.**

hsa-miR-21 detection in FFPE tissue sections using an LNA™ probe with a double DIG (5’ and 3’) label at 40nM (A) or a single 3’ DIG label at 80nM (B).

**Ordering information** [Details on page 70]

<table>
<thead>
<tr>
<th>miRCURY LNA™ microRNA Detection Probe</th>
<th>Product no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5’ and 3’ DIG labeled, 250 pmol</td>
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<tr>
<td>Ready-to-label*, 250 pmol</td>
<td>xxxxx-00</td>
</tr>
<tr>
<td>5’ DIG labeled, 250 pmol</td>
<td>xxxxx-01</td>
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<tr>
<td>5’-biotin labeled, 250 pmol</td>
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<td>5’-fluorescein labeled, 250 pmol</td>
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<td>Sense miR-199, Negative Control, 250 pmol</td>
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<tr>
<td>Scramble-miR, Negative Control, 250 pmol</td>
<td>99004-xx</td>
</tr>
</tbody>
</table>

*Other modifications available at: [exiqon.com/oligonucleotide-modifications](http://exiqon.com/oligonucleotide-modifications)*

*“Ready-to-label” means that the miRCURY LNA™ microRNA Detection Probe can be enzymatically labeled with the detection moiety of choice. For example DIG, radiolabel, biotin or fluorophores.*
LNA™ technology is superior to any other for the detection of small RNA species.
microRNA in situ hybridization kit for FFPE samples. Optimize the procedure for your samples with the included DIG-labeled LNA™ probes.

**At a glance**
- The shortcut to successful microRNA ISH – few experimental steps leaving minimal of optimization
- Fast and easy – one-day microRNA ISH protocol
- Superior sensitivity and specificity – essential reagents and double DIG-labeled LNA™ probes for optimal ISH analysis
- Very robust – can be used for both high throughput and individual microRNA localization studies
- Highly flexible – no advanced instruments needed
- Validated in a wide range of tissues – ideal for use with clinical and experimental FFPE samples

**The easiest way to get started with microRNA ISH**
A miRCURY LNA™ microRNA ISH Optimization Kit (FFPE) is the ideal option for getting started with or optimizing microRNA in situ hybridization (ISH) experiments on formalin-fixed paraffin embedded (FFPE) tissue samples.

Based on the highly popular and highly sensitive double (5’ and 3’) DIG-labeled miRCURY LNA™ microRNA Detection Probes, the kits provide the sensitivity and specificity needed to perform successful microRNA ISH analysis (Figure 38). All the kits come with reagents, including a non-toxic, formamide-free ISH buffer, specifically adapted for use with LNA™ probes in FFPE tissue sections.

Use the included probes to optimize the procedure for your samples. Then use double DIG-labeled miRCURY LNA™ microRNA Detection Probes to detect your microRNAs of interest (page 41).

The accompanying instruction manual carefully explains each step of the ISH experiment and provides tips and recommendations for a successful experiment. Furthermore, it includes a thoroughly validated one-day protocol for fast and trouble-free ISH analysis.

**Flexible and robust**
The kits can be used for a large number of applications including cellular and sub-cellular microRNA localization studies and determination of spatial microRNA expression.

Exiqon’s scientists have developed a very fast protocol which eliminates several of the steps normally associated with ISH, such as pre-hybridization, post-fixation and acetylation, thus making the protocol very robust and easy to optimize. Furthermore, the procedure is completely formamide-free and non-radioactive, which minimizes the exposure to harmful chemicals. Taken together, the flexibility of the kits makes them ideal for use in both clinical and research laboratories and for use in both automated and manual set-ups.

**A solution for every sample**
Seven different miRCURY LNA™ microRNA ISH Optimization Kits are available. Each kit comes with positive and negative control probes, hybridization buffer and Proteinase K. A unique tissue-specific miRCURY LNA™ microRNA Detection Probe is included in each kit (Table 3). These positive control probes have been validated in a variety of tissues and cell types and are used as positive control probes during the initial set-up and optimization procedure (Figures 39-41).
Product content

- Unique microRNA LNA™ probe (double DIG-labeled, kit specific)
- Scrambled LNA™ probe (double DIG-labeled, negative control)
- U6 LNA™ probe [5’ DIG-labeled, positive control]
- Hybridization buffer (2x, formamide-free)
- Proteinase K (12 mg, lyophilized)

The unique microRNA LNA™ probes have been validated in a variety of tissues and are therefore ideal positive controls for optimizing ISH experimental settings (Figures 39-41).

Selecting the appropriate miRCURY LNA™ microRNA ISH Optimization Kit.

Table 3. The tissues and cell types in which each of the kits has been validated.

<table>
<thead>
<tr>
<th>Cell entity</th>
<th>Kit 1</th>
<th>Kit 2</th>
<th>Kit 3</th>
<th>Kit 4</th>
<th>Kit 5</th>
<th>Kit 6</th>
<th>Kit 7</th>
<th>Kit 8</th>
<th>Kit 9</th>
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<tbody>
<tr>
<td>Brain</td>
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<td></td>
<td></td>
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</tr>
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<td>✓</td>
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<td></td>
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<tr>
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<td></td>
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<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

Selected publications

Jørgensen et al. Methods 2010, 52: 375-81

Ordering information (Details on page 70)

microRNA ISH Optimization Kit
- Kit 1 (miR-1) Includes controls and buffer 90001
- Kit 2 (miR-21) Includes controls and buffer 90002
- Kit 3 (miR-122) Includes controls and buffer 90003
- Kit 4 (miR-124) Includes controls and buffer 90004
- Kit 5 (miR-126) Includes controls and buffer 90005
- Kit 7 (miR-145) Includes controls and buffer 90007
- Kit 8 (miR-205) Includes controls and buffer 90008
- Kit 9 (miR-223) Includes controls and buffer 90009

Other reagents
- microRNA ISH Buffer 25ml (1000 slides) 90000
- microRNA ISH Buffer and Controls kit 90010

Did you know?
- microRNA ISH kits can also be used for fresh frozen samples. Please visit: exiqon.com/ish for more information

For updated product information, please visit: exiqon.com/mirna-ish-kit
At a glance
- $T_m$ normalized inhibitors with unmatched potency against all microRNA regardless of GC content
- Power inhibitors so potent that they work by unassisted delivery without the need for transfection reagents
- Superior specificity and biological stability for long lasting antisense activity
- 1 nmol, 5 nmol and 15 nmol quantities
- Fluorescent labels available for convenient monitoring of transfection efficiency
- Libraries for high throughput screening
- Specially designed family inhibitors

Potent microRNA inhibitors and Power inhibitors
Exiqon’s miRCURY LNA™ microRNA Inhibitors are ideal for use as specific suppressors of microRNA activity. Use them to determine the role of microRNAs in cellular processes and pathological pathways or for identification and validation of microRNA targets.

All microRNA inhibitors were developed using an advanced design algorithm that identifies the optimal combination of length, sequence and LNA™ positioning. In addition we have exploited the high affinity properties of LNA™ chemistry to create $T_m$ normalized inhibitors. This ensures that our inhibitors offer a high uniform potency towards all microRNAs regardless of their GC content (Figure 42) combined with excellent specificity and biological stability.

An added benefit of the inhibitor design is that LNA™ bases are distributed throughout the entire length, which ensures that LNA™ inhibitor/RNA duplexes are not recognized as substrates for RNase H. As a consequence, there will be minimal off-target effects on unrelated longer RNAs that share the same target sequence.

Product coverage
We offer pre-designed inhibitors according to their annotation in miRBase, as well as custom designed inhibitors.
- miRCURY LNA™ microRNA inhibitors Efficient inhibitors with normal phosphodiester backbone and subnanomolar potency for in vitro transfection experiments (Figure 43)
- miRCURY LNA™ microRNA Power inhibitors come with a fully phosphorothioate (PS) modified backbone, which dramatically improves their stability against enzymatic degradation. As seen in Figure 44, the
efficacy of these inhibitors is significantly higher than our regular inhibitors. Their increased stability and potency allows their direct addition to the cell culture medium without the need for transfection reagents (Figure 45). Power inhibitors are therefore especially useful for difficult applications, i.e. hard-to-transfect cells, highly expressed microRNA targets, long duration experiments and when normal transfection procedures have unacceptable phenotypic consequences
• miRCURY LNA™ microRNA family inhibitors are designed to simultaneously silence all members of a microRNA family. Family inhibitors are available for more than 40 microRNA families conserved in human and mouse. Available as Family Inhibitors and as Power Family Inhibitors with PS backbones
• Custom miRCURY LNA™ microRNA inhibitors If your choice of microRNA inhibitor is not available among the pre-designed products, Exiqon will design it for you. We also provide inhibitors with a range of chemical modifications and different types of purification
• miRCURY LNA™ microRNA inhibitor libraries (see below)

**Figure 43. Excellent subnanomolar potency of miRCURY LNA™ microRNA inhibitors.** Cells were transfected with a plasmid containing a Renilla luciferase gene and a microRNA target sequence upstream of a Firefly luciferase reporter gene. Firefly luciferase expression is suppressed by endogenous microRNAs in the cell. The cells were then transfected with microRNA inhibitors and negative controls. MicroRNA inhibitors sequester the endogenous microRNA and prevent it from suppressing Firefly luciferase, resulting in increased levels of Firefly luciferase. Reporter gene expression was measured and ratios of Firefly and Renilla luciferase activity calculated and normalized to values obtained with a Firefly Luciferase reporter with no miR target sequence (pLuc). LNA™-enhanced microRNA inhibitors display subnanomolar potency.

**Figure 44. Enhanced potency of miRCURY LNA™ microRNA Power Inhibitors.** Power inhibitors offer even greater performance than regular microRNA inhibitors. The experiment was performed as described in Figure 43.

**Figure 45. Potent microRNA inhibition easily obtained by adding miRCURY LNA™ microRNA Power inhibitors by gymnosis.** Efficient microRNA inhibition can be achieved by adding high concentrations of Power inhibitor directly to the culture medium without use of transfection reagent (gymnosis). The results also show that regular inhibitors with an unmodified normal phosphodiester backbone are ineffective with gymnotic delivery. The experiment was performed as described in Figure 43.

**MicroRNA inhibitor libraries**

The miRCURY LNA™ microRNA Inhibitor Libraries enable convenient high-throughput screening of mouse and human microRNA function. The libraries are based on our renowned Tₘ-normalized miRCURY LNA™ microRNA Inhibitors with phosphodiester backbone.
The inhibitor libraries offer coverage of key microRNAs listed in miRBase v. 20. A number of microRNAs have been excluded for which there is either no or very limited direct experimental evidence. This significantly reduces the cost of screening and time wasted on potentially false positive results with very little impact on the “true” coverage of the screen.

**Inhibitor coverage of the libraries:**
- **Human library:** 1,972 inhibitors of human microRNAs
- **Mouse library:** 1,624 inhibitors of mouse microRNAs

**Plate layout**
The inhibitor libraries are provided in 96-well plates. The plates are all organized with empty outer rows and columns. This facilitates easy pipetting into 96-well culture plates in a setup that avoids edge effects due to evaporation of culture medium (Figure 46).

**Selected publications**
- Polesskaya et al. PLoS One 2013, 8:e71927

**Ordering information**
(Details on page 70)

<table>
<thead>
<tr>
<th>Product no.</th>
<th>miRCURY LNA™ microRNA inhibitors*</th>
<th>miRCURY LNA™ microRNA Power Inhibitors*</th>
<th>miRCURY LNA™ microRNA family inhibitors*</th>
<th>miRCURY LNA™ microRNA Power family inhibitors*</th>
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<tr>
<td>4100001-4104908-000</td>
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<td>miRCURY LNA™ microRNA Power Inhibitors*</td>
<td>miRCURY LNA™ microRNA family inhibitors*</td>
</tr>
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<tr>
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<td>miRCURY LNA™ microRNA Power family inhibitors*</td>
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<tr>
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<td>miRCURY LNA™ microRNA inhibitor libraries</td>
<td>miRCURY LNA™ microRNA Power family inhibitors*</td>
<td>miRCURY LNA™ microRNA family inhibitors*</td>
</tr>
<tr>
<td>4100001-4104908-108</td>
<td>15 nmol</td>
<td>miRCURY LNA™ microRNA inhibitor libraries</td>
<td>miRCURY LNA™ microRNA Power family inhibitors*</td>
<td>miRCURY LNA™ microRNA family inhibitors*</td>
</tr>
<tr>
<td>4100001-4104908-109</td>
<td>15 nmol</td>
<td>miRCURY LNA™ microRNA inhibitor libraries</td>
<td>miRCURY LNA™ microRNA Power family inhibitors*</td>
<td>miRCURY LNA™ microRNA family inhibitors*</td>
</tr>
</tbody>
</table>

*All inhibitors are available with fluorescent labels. Negative controls for regular and Power inhibitors are also available. Custom miRCURY LNA™ microRNA Inhibitors and Power Inhibitors are available for microRNAs or other short ncRNA sequences not in miRBase. For more information please visit: exiqon.com/mirna-inhibitors

Exiqon offers complete set of tools for microRNA functional analysis:
- **miRCURY LNA™ microRNA Inhibitors**
  For loss of function analysis
- **miRCURY LNA™ microRNA Mimics**
  For gain of function analysis
- **miRCURY LNA™ microRNA Target site blockers**
  For the study of microRNA function in molecular detail, by analyzing the significance of specific microRNA/mRNA interactions

Comprehensive plate layout files can be downloaded at exiqon.com/mirna-inhibitor-library

The inhibitors are positioned in the plates according to the amount of supporting scientific data. This 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.
**At a glance**
- Potent inhibition of microRNAs in broad range of tissues
- Enables the discovery of surprising microRNA functions in live animals
- Custom designed and highly purified
- Superior serum stability and nuclease resistance

**Well-documented inhibition of microRNAs in vivo**
Effective microRNA inhibition has been achieved in multiple organs and tissues by systemic and local administration of custom designed in vivo LNA™ microRNA inhibitors (see Table 4). As a result, surprising discoveries about microRNA function have been made that could not have been achieved by cell culture experiments. Successful phase 2 human trials with an LNA™ miR-122 inhibitor for treatment of HCV infections is a testimony to the unique drug-like properties of these short antisense molecules.

**Product description**
The in vivo LNA™ microRNA Inhibitors are highly purified custom designed microRNA inhibitors optimized for in vivo use. We exploit LNA™ technology by designing short (14-16mer) inhibitors with fully modified phosphorothioate (PS) backbone in order to optimize the pharmacokinetic and pharmacodynamic properties and to minimize toxicity. As an added benefit, the in vivo LNA™ microRNA Inhibitors are easily taken-up without the need for cholesterol-conjugation.

The high affinity of the LNA™-enhanced in vivo microRNA inhibitors makes them highly effective at physiological temperatures and when used in low concentrations, thereby minimizing potential secondary effects not related to the antisense activity of the microRNA inhibitor. In addition, LNA™ incorporation enhances serum and nuclease stability.

The in vivo inhibitors are available with fluorescein and other custom modifications, in amounts ranging from 5 mg to kg scales. If needed, they can be delivered in the quantity and quality required for preclinical toxicity studies.

**Selected publications with miRCURY LNA™ in vivo microRNA inhibitors**
- Boon et al. Nature 2013, 495: 107-10
- Sene et al. Cell Metab. 2013, 17:549-61
- Son et al. Nature Communications 2013, 4:3000
- Seeger et al. Obesity 2014, 22:2352-60

**Ordering information**
Please contact us at exiqon.com/contact

---

**Table 4. In vivo inhibition of microRNA in a variety of tissues using in vivo LNA™ microRNA inhibitors.**

<table>
<thead>
<tr>
<th>microRNA</th>
<th>Tissue</th>
<th>Organism</th>
<th>Process/Disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-802</td>
<td>Liver</td>
<td>Mouse</td>
<td>Obesity induced impaired insulin signaling</td>
<td>Kornfeld et al. Nature 2013</td>
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<tr>
<td>miR-142-3p</td>
<td>Spleen dendritic cells</td>
<td>Mouse</td>
<td>Endotoxemia</td>
<td>Sun et al. Blood 2011</td>
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<td>miR-212</td>
<td>Brain</td>
<td>Rat</td>
<td>Cocaine addiction</td>
<td>Hollander et al. Nature 2010</td>
</tr>
<tr>
<td>miR-34a</td>
<td>Heart</td>
<td>Mouse</td>
<td>Cardiac aging</td>
<td>Boon et al. Nature 2013</td>
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<tr>
<td>miR-21-5p</td>
<td>Bone marrow, hematopoietic stem cells</td>
<td>Mouse</td>
<td>Myelodysplastic syndromes</td>
<td>Bhagat et al. Blood 2013</td>
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<td>miR-199a, miR-1908</td>
<td>Human melanoma</td>
<td>Mouse</td>
<td>Melanoma metastasis</td>
<td>Parcheva et al. Cell 2012</td>
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<td>miR-712</td>
<td>Endothelium</td>
<td>Mouse</td>
<td>Atherosclerosis</td>
<td>Son et al. Nature Commun 2013</td>
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<tr>
<td>miR-21-5p</td>
<td>White adipose tissue - heart</td>
<td>Mouse</td>
<td>Obesity</td>
<td>Seeger et al. Obesity 2014</td>
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<tr>
<td>miR-33</td>
<td>Choroidal macrophages of the eye</td>
<td>Mouse</td>
<td>Age related macular degeneration</td>
<td>Sene et al. Cell Metabolism 2013</td>
</tr>
</tbody>
</table>
Sophisticated high-quality mimics designed to simulate naturally occurring mature microRNAs in functional analysis studies. A unique LNA™-enhanced triple RNA strand design ensures excellent specificity with no off-target effects from the passenger strand.

<table>
<thead>
<tr>
<th>At a glance</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Third generation highly potent mature microRNA mimics with unique triple RNA strand design</td>
</tr>
<tr>
<td>• No off-target microRNA activity from the segmented LNA™-enhanced passenger strand</td>
</tr>
<tr>
<td>• No chemical modification of the microRNA (guide) strand</td>
</tr>
<tr>
<td>• microRNA strand sequence according to the annotation in miRBase</td>
</tr>
<tr>
<td>• Available in 5 and 20 nmol quantities</td>
</tr>
<tr>
<td>• Comprehensive product offering - fluorescently labeled, biotinylated and in vivo mimics are also available</td>
</tr>
</tbody>
</table>

In contrast, the passenger strand is divided into two LNA™-enhanced RNA strands. This ensures that only the microRNA strand is loaded into the RNA induced silencing complex (RISC) with no resulting microRNA activity from the two complementary passenger strands (Figure 50). Such off-target effects can be a problem using traditional microRNA mimics.

**Product coverage**

miRCURY LNA™ microRNA Mimics have been pre-designed for most human, mouse and rat microRNAs listed in miRBase. Since many microRNAs are phylogenetically conserved, our microRNA mimics cover a large proportion of vertebrate and invertebrate microRNAs.

Fluorescently and biotin labeled mimics as well as negative control mimics are also available.

LNA™ microRNA Mimics are offered in 5 nmol and 20 nmol quantities and are delivered desalted and dried-down. Once dissolved, they are ready for transfection or electroporation using standard techniques. Mimics for in vivo use are available at mg scales.

**A unique triple-RNA strand design**

miRCURY LNA™ microRNA Mimics have a unique and novel innovative design (Figure 47). They are based on three RNA strands rather than the two RNA strands that characterize traditional microRNA mimics. The microRNA (guide) strand is an unmodified RNA strand with a sequence corresponding exactly to the annotation in miRBase.

---

Figure 47. The unique triple RNA strand design ensures completely specific microRNA mimicry. Only the microRNA strand is incorporated by RISC. The two passenger strands are too short to act as microRNAs and are rapidly degraded after displacement from the microRNA strand. Off-target effects from the passenger strands are therefore minimal with miRCURY LNA™ microRNA Mimics.
Applications
MicroRNA mimics serve to simulate the natural functions of endogenous microRNAs and are primarily used in gain-of-function studies by assessing the biological consequences of increasing microRNA activity. The effect of increasing the cellular content of a microRNA (using microRNA mimics) 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 of putative microRNA targets.

MicroRNA mimics are also frequently used for validating microRNA targets in combination with microRNA inhibitors and target site blockers (Figure 52).

Excellent potency
The microRNA strand of miRCURY LNA™ microRNA Mimics is unmodified RNA in order to achieve as accurate microRNA mimicry as possible. The sequence and LNA™ spiking pattern of the two complementary passenger strands have been carefully designed to optimize efficient incorporation of the microRNA (guide) strand into RISC.

Biotinylated mimics for pull-down experiments
Biotinylated LNA™ microRNA mimics are highly effective tools for identification of microRNA targets in RNA pull-down experiments [Figure 49]. Recent advances with this experimental approach have revealed that non-canonical microRNA – mRNA interactions (ignored by target prediction tools) are frequent and lead to target repression.

Ordering information [Details on page 78]

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<td>Negative controls (4, 5, cel-miR-39-3p, 20 nmol)</td>
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<table>
<thead>
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<tr>
<td>FAM-labeled, 5 nmol</td>
<td>479995-011</td>
</tr>
<tr>
<td>FAM-labeled, 20 nmol</td>
<td>479995-014</td>
</tr>
<tr>
<td>Premium, Biotinylated, 5 nmol, HPLC purified</td>
<td>479997-671</td>
</tr>
<tr>
<td>Premium, Biotinylated, 20 nmol, HPLC purified</td>
<td>479997-674</td>
</tr>
<tr>
<td>Premium, FAM-labeled Ready, 5 nmol, HPLC purified</td>
<td>479997-611</td>
</tr>
<tr>
<td>Premium, FAM-labeled Ready, 20 nmol, HPLC purified</td>
<td>479997-614</td>
</tr>
</tbody>
</table>

Learn more about microRNA target identification by RNA pull-down studies in the tech note on use of biotinylated miRCURY LNA™ microRNA Mimics at [exiqon.com/mirna-mimics-pulldown](exiqon.com/mirna-mimics-pulldown)

Figure 48. Highly efficient miRCURY LNA™ microRNA Mimics display sub-nanomolar potency. Using a Firefly luciferase reporter assay, HeLa cells were transfected with different concentrations of LNA™ microRNA mimic or a cel-miR-39-3p negative control mimic. The results illustrate that LNA™ microRNA Mimics display sub-nanomolar potency under optimal transfection conditions.

Figure 49. Effective microRNA target identification with biotinylated miRCURY LNA™ mimics.

Figure 50. Perfect microRNA strand specific activity with miRCURY LNA™ microRNA Mimics. Cells harboring hsa-miR-16-3p (below) and hsa-miR-16-5p (not displayed) luciferase reporter plasmids respectively were transfected with hsa-miR-16-3p and hsa-miR-16-5p mimics and a negative mimic control. The results demonstrate that suppression of luciferase activity is only achieved with the miRCURY LNA™ microRNA Mimic corresponding to the reporter assay. Visit [exiqon.com/mirna-mimics](exiqon.com/mirna-mimics) for the full study.
microRNA Target Site Blockers

Use target site blockers to study microRNA function in molecular detail.

At a glance
• Target site blockers enable detailed study of which microRNA/mRNA interactions are important for microRNA function
• A target site blocker (TSB) stimulates translation of a specific mRNA by masking a microRNA binding site
• A target site blocker hybridizes with a microRNA binding site on a specific mRNA (or non-coding RNA) preventing the microRNA from interacting with the site without inhibiting the microRNA itself
• Sophisticated and innovative custom design
• Unmatched high efficiency in vivo and in vitro

Target site blockers (TSB)

microRNAs are typically involved in regulation of large number of genes. Therefore, the phenotype observed upon changes in microRNA activity is a combined effect of several targets being deregulated, although a few of these targets will often contribute more significantly than others. Identification of such targets is important to understanding microRNA function. It allows researchers to study the effects of a microRNA on a single target.

The contribution of an individual target to the obtained phenotype can be investigated with a target site blocker (TSB) – an antisense oligonucleotide that specifically prevents interaction of a microRNA with one of its RNA targets. The TSB is designed to mask the microRNA target site in the RNA target of interest and will not affect the activity of the microRNA per se. As a result, the TSB will achieve specific derepression of a single intended target only, enabling simple phenotypic interpretation (Figure 52).

The TSB mode of action is illustrated in Figure 51. TSBs can be used effectively in combination with microRNA inhibitors and mimics see Figure 52.

Figure 51. A target site blocker (TSB) stimulates translation of a specific mRNA by masking the microRNA binding site. The TSB will compete effectively with microRNA/RISC for the microRNA target site. In addition, LNA™ distribution throughout the LNA™/DNA mixmer ensures that the antisense oligonucleotide does not catalyze RNaseH dependent degradation of the mRNA. As a result the TSB will cause increased production of the protein encoded by the targeted mRNA by preventing microRNA mediated translational attenuation.

Target site blockers are custom designed. Send us your RNA sequence with an indication of the microRNA binding site to exiqon.com/contact. We will design your TSB.
Product coverage
miRCURY LNA™ microRNA Target Site Blockers are available in several different purity grades depending on application. They can be use for in vitro experiments as well as for in vivo models.

The TSB’s are custom designed to match your specific needs. Once you send us your RNA sequence with an indication of the microRNA binding site, our in-house RNA experts will design the most optimal TSB for you.

miRCURY LNA™ microRNA Target Site Blockers are LNA™-enhanced and are available with a phosphorothioate modified backbone for maximum potency.

Selected publications
Dajas-Bailador et al., Nat Neurosci 2012, 15: 697-69
Cardenas et al., PLoS Genet 2013, 9: e1003291
Viart et al., Eur Respir J 2015, 45: 116-28
Ortega et al., Leukemia 2014, 29: 968-976

Figure S2. Unravel microRNA function with LNA™ Target Site Blockers (TSB). Combined use of TSB and microRNA inhibitors and mimics. (A) An interesting phenotype is observed with a microRNA inhibitor. microRNA inhibition leads to increased translation of multiple mRNAs. Question: Which upregulated genes are responsible for the phenotype? This question can be answered by testing a TSB for one mRNA suspect at a time. TSBs that phenocopy the microRNA inhibitor identify important microRNA targets. (B) An interesting phenotype is observed with a microRNA mimic. The increased microRNA activity suppresses translation of multiple mRNAs. Question: Which downregulated genes are responsible for the phenotype. This question can be answered by cotransfecting TSBs of mRNA suspects with the microRNA mimic. TSBs that rescue the phenotype identify important microRNA targets.

Ordering information (Details on page 70)

<table>
<thead>
<tr>
<th>Product no.</th>
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<tbody>
<tr>
<td>480001-00</td>
</tr>
<tr>
<td>480003-00</td>
</tr>
<tr>
<td>480004-00</td>
</tr>
</tbody>
</table>

**“Ready-to-label” means that the miRCURY LNA™ microRNA Target Site Blocker can be enzymatically labeled with the detection moiety of choice. For example DIG, radiolabel, biotin or fluorophores.**

All Target Site Blockers (except 480001-00) have a phosphorothioate (PS) backbone modification.
Exiqon Services

Introduction – RNA Services

Send Exiqon your samples and let us perform your RNA experiments. We offer high-quality RNA isolation, and profiling using Next Generation Sequencing (NGS), microarray and qPCR. All experiments are performed by a team of seasoned RNA experts in state-of-the-art laboratories using the best available technologies.

At a glance

- Comprehensive all-inclusive RNA analysis services ranging from genome-wide screening by to single target validation
- Complete sample-to-answer service tailored to your research needs and budget
- Industry-leading service reports and support
- Close consultation throughout your project with experienced RNA scientists
- State-of-the-art laboratories with optimized processes and protocols
- More than 10 years of experience with RNA research and product development for RNA analysis
- SOPs, LIMS, and rigorous QC procedures

A comprehensive and flexible RNA analysis service

Exiqon offers a suite of RNA profiling and analysis services ranging from exosome and RNA isolation to expression profiling by NGS, microarray and qPCR. Combine your NGS or microarray analysis with validation by qPCR. Figure 53 displays an overview of Exiqon’s service products.

Take advantage of our comprehensive sample-to-answer service that covers every step in the process from experimental design to a biologically meaningful interpretation of your results. We offer everything from an initial consultation with our highly experienced RNA scientists to define the experimental design, to industry-leading data analysis and delivery of a comprehensive final report with publication-grade figures.

We are committed to providing personalized, accurate and responsive guidance from helping you to tailor the exact level of service and study you need, to making sure you get on the fast track to actionable results.

All experiments are carried out in our state-of-the-art laboratories using the latest technologies. Our high-throughput procedures enable fast turn-around times and allow capacity for large projects.

Benefit from a well-established and highly experienced RNA service provider

As the first commercial microRNA service provider, Exiqon Services have profiled over 30,000 samples and delivered high quality services to more than 1,800 customers in pharma and the food industry, biotech and academia since 2006.

Figure 53. Overview of Exiqon Services.
Our team takes pride in ensuring that you get the best possible RNA analysis. We know how important it is to make unbiased conclusions on results, which is why we focus on quality control and reproducibility in all steps of the project; from the initial control of the RNA samples to the data analysis and report.

Exiqon’s highly skilled and seasoned team of researchers has extensive experience with RNA profiling and data analysis. We use Exiqon’s commercially available products in our services. We know the products better than anyone and take great advantage from the knowledge we have gained from in-house research and development of the products.

**Experts in analyzing microRNA from biofluids**

Exiqon Services have years of experience analyzing microRNA in challenging clinical samples such as biofluids. As part of our own diagnostics development program, Exiqon has pioneered the field of microRNA biomarker development in serum/plasma. This knowledge has been applied in establishing optimized protocols and special QC procedures for biofluids in our microRNA NGS and qPCR services.

**The QA and security standards you expect**

Exiqon has implemented a QA Program incorporating important components of ISO 17025 and GLP ensuring all work is performed in accordance with high standards. Our Quality Management System includes SOPs covering all activities.

IT Security is very important to Exiqon, all security standards are derived from ISO 17799/DS484 and BS 7799.

Exiqon offers a complete sample-to-answer RNA service.
Exiqon Services

RNA Isolation

Send us your biological samples and we will prepare high quality total RNA suitable for profiling by Next Generation Sequencing, microarray and qPCR. We can handle a broad range of sample types and have protocols optimized for samples with minute RNA content.

At a glance
- All processes are carried out by RNA experts
- Standard Operating Procedure (SOP) protocols for optimal quality and efficiency
- Optimized protocol for samples with minute RNA content such as biofluids and FFPE samples
- Exosome isolation also available
- High quality RNA extraction suitable for Exiqon’s NGS, Array and PCR Services
- Consultation with RNA experts throughout the process

Consultation and sample submission
Exiqon can perform the initial RNA isolation for your NGS, microarray and qPCR service projects. We can isolate high quality RNA suitable for profiling from many different sample sources. In addition, we offer isolation of exosomes from various types of biofluids combined with the downstream RNA isolation.

Details on how to submit your samples will be tailored according to your sample type and requirements. Exiqon provides recommendations on the best way to ship your samples.

Sample handling and total RNA Isolation
All RNA Isolation Service projects are carried out in our state-of-the-art service facilities. Exiqon’s miRCURY™ RNA Isolation and miRCURY™ Exosome Isolation kits are used for the isolation services (for product details, please refer to relevant product pages in the catalogue). RNA isolation is performed following optimized certified Standard Operating Procedure (SOP) protocols to ensure optimal quality and efficiency. Quality control on the extracted total RNA is performed using procedures tailored to the particular sample type.

RNA isolation from a broad range of sample types
We offer RNA isolation on the following types of samples:
- Serum/plasma, urine, CSF and other biofluid samples
- Clinical tissue and FFPE samples
- LCM samples
- Cell lines
- Whole blood (e.g. PAXgene blood RNA tubes)
- Exosomes (we also perform exosome isolation)
- Other sample types upon request

Learn more!
For more information and ordering please visit exiqon.com/rna-isolation-services

Experts in profiling for microRNA biomarkers in serum and plasma
From years of in-house microRNA biomarker diagnostics development, Exiqon Services have vast experience with profiling of circulating microRNA in serum and plasma samples.

We pioneered the microRNA biomarker development in serum/plasma.
Exiqon Services

Next Generation Sequencing

Send us your samples and let our Next Generation Sequencing (NGS) experts perform your RNA-seq analysis in state-of-the-art automated laboratories with rigorous quality control and fast turn-around times. Exiqon offers a complete sample-to-answer solution from sample isolation to data interpretation and validation.

At a glance
- Complete sample-to-answer solution
- Thorough QC of RNA samples and libraries
- Consultation with RNA and NGS experts throughout the process
- Flexible capacity using Illumina MiSeq, NextSeq and HiSeq instruments
- Fast turn-around times - delivery of the final report within 4 - 6 weeks
- Rigorous quality control in all steps of the workflow
- Combine with downstream validation using the market-leading LNA™-enhanced qPCR platform

Exiqon’s scientists can also assist you in how to validate your NGS results.

A complete RNA sequencing service
Exiqon Services offers RNA Sequencing services using the Illumina technology:
- Whole Transcriptome Sequencing
- mRNA Sequencing
- microRNA Sequencing (including microRNA discovery)
- microRNA in biofluids Sequencing (includes RNA isolation with Exiqon’s optimized serum/plasma protocols)
- small RNA Sequencing
- Custom Sequencing

Figure 54 shows the workflow for the RNA NGS Service. Details on each step of the process are described below. For more information and updates, please visit exiqon.com/NGS-services

Exiqon’s NGS services include every step from initial consultation, RNA isolation, RNA QC, library preparation, to sequencing and full data analysis. All analyses are performed by NGS expert scientists who will ensure that you get the best service throughout the project. Data and results from your sequencing project will be delivered in an easy-to-read report with publication-grade illustrations including an Excel file with all data needed for publication. Complete raw data can be provided on an encrypted hard disk.

1. Consultation
When you engage in an NGS Service project with Exiqon, you are assured direct communication with the scientists performing your experiments throughout the duration of the project. Each project begins with a free consultation with an

Figure 54. Workflow for Exiqon’s RNA Sequencing Services.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Consultation and experimental design</td>
<td>Tailored experimental design to suit your research needs and budget</td>
</tr>
<tr>
<td>2. RNA sample submission or RNA Isolation</td>
<td>Total RNA is recommended for RNA Sequencing. At Exiqon we also offer to isolate your RNA for sequencing</td>
</tr>
<tr>
<td>3. RNA sample quality control (QC)</td>
<td>Assessment of purity and integrity of total RNA samples (NanoDrop or Qubit and Bioanalyzer)</td>
</tr>
<tr>
<td>4. Library preparation</td>
<td>Library generation, quality control and quantification (Bioanalyzer and qPCR) Size selection of the library (for microRNA and small RNA)</td>
</tr>
<tr>
<td>5. Sequencing</td>
<td>Sequencing using the Illumina technology (MiSeq, NextSeq or HiSeq)</td>
</tr>
<tr>
<td>6. Data analysis and interpretation</td>
<td>Filtering, mapping, normalization and differential expression analysis GO Enrichment analysis and Biological Interpretation</td>
</tr>
<tr>
<td>7. Report and final consultation</td>
<td>An extensive report is accompanied by follow-up scientific discussion</td>
</tr>
</tbody>
</table>
RNA and NGS expert. Together, we design an experimental setup that best satisfies your research needs and budget. Next, you complete a detailed online sample submission form ensuring that all experimental details and subsequent analysis are clearly defined and understood by both parties.

2. RNA sample submission
High quality samples are important for accurate sequencing. At the initial consultation, we offer recommendations on suitable extraction and clean-up methods. Alternatively, you can take advantage of our expertise and submit your samples to our RNA isolation service (see page 56).

The amount of total RNA recommended varies depending on the sequencing service you choose, and the type of samples you have. We also accept enriched RNA or purified mRNA as well as biofluid samples. More information can be found in Exiqon’s NGS Guidelines document available for download here: exiqon.com/ngs-services

3. RNA sample quality control (QC)
Our experts will determine the integrity and quantity of each sample using Bioanalyzer and NanoDrop™ or Qubit instruments. Possible contaminations are likewise assessed for each sample. You will receive a report with the results of these analyses prior to the sequencing.

Perform microRNA-Seq in biofluids with the experts
Exiqon pioneered microRNA biomarker discovery in serum/plasma. Take advantage of our years of experience in handling and profiling serum/plasma samples when having your biofluids samples sequenced.

Read tips on working with serum/plasma samples at exiqon.com/biofluids

4. Library preparation
Following quality control, we generate libraries according to protocols optimized for each type of sequencing service.

For whole transcriptome Sequencing, ribosomal RNA depletion is first performed using biotin-streptavidin based bead with target-specific depletion oligonucleotides. Specialized depletion protocols are available for whole blood and plant samples.

For mRNA Sequencing, Poly-A RNA selection is first performed using an oligo-dT magnetic bead system.
Following library preparation, a quality control assessment of each library is performed.

The insert rate of the desired RNA type is evaluated using a Bioanalyzer DNA high sensitivity chip. Libraries for microRNA and small RNA Sequencing are then size fractionated using the appropriate size range (which may also be customized), to ensure only the relevant RNA fraction is sequenced. qPCR based quantification of each library is performed, and samples are normalized and pooled in equimolar ratios. After pooling of sample libraries, qPCR based quantification is performed on the library pool to ensure optimal concentration for cluster generation on the flowcell.

5. Sequencing

Exiqon Services offers five types of sequencing using the Illumina platform:

<table>
<thead>
<tr>
<th>RNA-seq Service</th>
<th>Type of sequencing offered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Transcriptome</td>
<td>Single or paired end reads</td>
</tr>
<tr>
<td>mRNA</td>
<td>Single or paired end reads</td>
</tr>
<tr>
<td>microRNA including microRNA discovery</td>
<td>50 bp single end processing</td>
</tr>
<tr>
<td>microRNA biofluids</td>
<td>Single end</td>
</tr>
<tr>
<td>small RNA</td>
<td>200 bp single end processing</td>
</tr>
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During your consultation with Exiqon, we will discuss the specific aims of your project and make recommendations regarding the optimal number of reads per sample, read length and single or paired end reads. All of these factors can be tailored to your project.

6. Data Analysis and Interpretation

The bioinformatics is an integrated part of our NGS platform (Figure 56) and our scientists have a strong background in both the experimental and analytical aspects of Next Generation Sequencing.

Our data analysis includes data QC and mapping, characterization of the read populations, normalization, differential expression analysis, and comparison of sample groups (unsupervised and supervised clustering via two dimensional heat maps, PCA plots, and pairwise comparison and visualization by T-tests, ANOVAs, and volcano plots).

microRNA sequencing projects also include prediction of novel microRNAs, as well microRNA target identification using Exiqon’s miRSearch database (a rich source of curated information related to microRNAs and their targets).

Figure 56. Data analysis and Interpretation is central to Exiqon’s RNA Sequencing Service.
Gene Ontology (GO) enrichment analysis is performed to determine which GO terms are overrepresented in the differentially expressed transcripts or identified microRNA target genes. In connection with large service projects we also offer extensive customized bioinformatics service.

7. Report and final consultation
The final report is delivered as link to our secure webserver and will contain:
- An easy-to-read data report as pdf containing a description of the project, assessments of sample and data quality and an overview of the results of the data analysis with publication-grade illustrations.
- Extensive Excel files with all the major findings and statistical analysis which is sufficient for publication, or performing your own analysis of the data if needed.

The complete encrypted raw data can be provided on an encrypted hard disk or USB3-stick.

Exiqon always offers a free consultation with one of our NGS experts to discuss the data, answer any questions you may have, and discuss the next steps for the project including qPCR validation or functional analysis.

**Sequencing FFPE samples**
When sequencing FFPE samples there is a risk that a significant portion of the reads can result from degraded RNA species (rRNA/mRNA/tRNA).

Contact Exiqon to discuss your project and our NGS experts will be happy to advise on the best approach for sequencing your FFPE samples.

**The NGS report consists of:**
An easy-to-read data report as pdf
- QA/QC of sample and data
- Mapping and Yields
- PCA plots and Heat maps based on unsupervised clustering
- Results of statistical analysis of customer defined group comparisons
- Volcano plots
- Identification of novel transcripts
- GO Enrichment Analysis
- Summary of results
- A materials and methods section ready to use for publication purposes
- Project summary Excel report
- Mapped reads and counts
- Filtered high quality data
- Normalized data for each individual sample and gene
- Statistical analysis according to supplied groupings: T-test or ANOVA including multiple testing corrections

View a sample NGS report
Report examples are available for download at exiqon.com/ngs-services

Did you know?
Exiqon’s Whole transcriptome or mRNA sequencing Service includes identification of:
- Splice junctions
- Alternative splicing and splice variants
- Novel transcripts
- Antisense transcripts
- Gene fusions
Exiqon Services

Microarray Profiling

Exiqon offers a comprehensive microRNA profiling service based on our miRCURY LNA™ Arrays with unrivaled accuracy and sensitivity. Let our microarray experts perform your analysis in state-of-the-art automated laboratories with rigorous quality control, fast turn-around times and advanced data analysis, with experimental design tailored to your research needs and budget.

At a glance
- Covers everything from initial consultation to the final report including all raw data and detailed data analysis
- Consultation with microRNA and array experts throughout the process
- Profiling from as little as 200 ng total RNA
- Fast turn-around times - delivery of the final report within 2-4 weeks of receiving your sample
- Rigorous quality control in all steps of the analysis
- Normalization using either Lowess (dual-color experiments) or Quantile (single-color experiments)

A complete microRNA Array profiling service
Our microRNA Array service includes every step from initial consultation, sample labeling and hybridization to full data analysis. All analyses are performed by microRNA expert scientists ensuring the best service throughout the project. Data and results from your microRNA profiling project will be delivered in an easy-to-read report with publication-grade illustrations including an Excel file with all the raw data.

Figure 57 shows the workflow for the Array Service. Details on each step of the process are described below. For more information and updates, please visit www.exiqon.com/microRNA-array-services.

1. Consultation
When you engage in an Array Service project with Exiqon, you are assured direct communication with the scientists performing your experiments throughout the duration of the project. Each project begins with a free consultation with a microRNA expression profiling expert. Together, we design an experimental setup that best satisfies your research needs and budget.

2. RNA sample submission
High quality samples are important for accurate microRNA profiling. At the initial consultation, we offer recommendations on suitable extraction and clean-up methods. Alternatively, you can take advantage of our expertise and submit your samples to our RNA isolation service (see page 56).

Due to the sensitivity of our microarrays, we can perform analysis with low RNA input. We normally use between 250 - 750ng total RNA depending on the experimental design.
3. RNA sample quality control (QC)
After receiving your RNA samples, our experts will determine the integrity and quantity of each sample using Bioanalyzer and NanoDrop™ instruments. Possible contaminations are likewise assessed for each sample. A report with summary of the results will be forwarded to you prior to the array profiling.

4. Labeling, hybridization and scanning
Following quality control, your RNA samples will be labeled using our miRCURY LNA™ Hi-Power Labeling Kit for efficient and uniform labeling. Next, the labeled samples are hybridized to a miRCURY LNA™ microRNA Array. All hybridization and washing steps are fully automated to ensure high reproducibility.

microRNA expression profiling from FFPE samples
A unique feature of our Array Services is our ability to provide high quality microRNA expression profiling from FFPE samples (Figure 57).

FFPE samples are generally characterized by poorly preserved RNA. Due to the selective nature of LNA™ capture probes and the high-stringency hybridization conditions, the miRCURY LNA™ Array platform is uniquely robust towards interference from small mRNA and rRNA degradation products typically present in FFPE samples.

Figure 58. Good correlation between expression profiles from FFPE and fresh frozen samples. MicroRNA log2 intensity scatterplots of FFPE and fresh frozen samples from the same tissue using Exiqon’s microarrays. The excellent correlation between the two expression profiles suggests that microRNAs are well conserved in FFPE samples and can be accurately detected using the miRCURY LNA™ Arrays.

Let our RNA array experts perform your analysis in state-of-the-art automated laboratories with rigorous quality control and fast turn-around times tailored to your research needs and budget.
5. Data Analysis

After scanning the arrays, we perform a technical quality assessment of the data based on the results from spike-in controls, flagging of spots, background intensity levels and signal intensity distribution.

Following normalization, the microarray data is assessed in multiple ways, including principle component analysis (PCA) and heat-maps with unsupervised clustering. In the biological quality assessment, we check if samples group according to biology and look for any signs of experimental bias in the data set.

Finally, statistical analysis of customer defined group comparisons is performed. In connection with large service projects we also offer extensive customized bioinformatics analysis.

6. Report and final consultation

An easy-to-read summary report is provided containing a description of the project, assessments of sample and data quality and an overview of the results of the data analysis with publication-grade illustrations. Extensive Excel files with all raw data is also included. The final report is delivered as a link to our secure webserver.

Scientific follow-up is always offered to answer any questions to your final report and to discuss how to proceed with the obtained results.

The microRNA Array Profiling report consists of:

- An easy-to-read summary report
- QC of sample and data
- Plots showing the effect of normalization
- Volcano plots
- Heat maps and PCA plots based on unsupervised clustering
- Results of statistical analysis of customer defined group comparisons.
- Summary of results

Materials and methods section

- Ready to use for publication purposes

Project summary Excel report

- Normalized data for each individual sample
- Expression matrices with statistical analysis according to customer specifications: T-test or ANOVA including multiple testing corrections (Benjamini-Hochberg).
- Indication of microRNAs that pass the statistical restriction
- MA plots

Great flexibility in experimental design

Our spotted microarrays allow both dual and single color experiments due to highly controlled production and strict QC-criteria. This provides great flexibility in the choice of experimental design and method of normalization.

Dual color with common reference for optimal intraslide normalization (global LOWESS), minimizing effect of sample/slide variation in labeling and hybridization efficiency.

Single color with interslide normalization (Quantile) enabling comparison of data from independent array experiments accumulating over time.

Learn more!

For more information and ordering, please visit exiqon.com/microRNA-array-profiling-services

View a sample microRNA Array Service report

Report examples are available for download at exiqon.com/microRNA-array-profiling-services
At a glance
- Based on our highly specific and sensitive miRCURY LNA™ Universal RT microRNA PCR system
- Covers everything from an initial consultation to the final report, including all raw data and detailed data analysis
- Consultation with microRNA and PCR experts throughout the process
- Rigorous quality control in all steps
- System flexibility and sensitivity ensures cost-efficient experimental setup
- Experiments performed by expert scientists in state-of-the-art laboratories
- Fast turn-around times - delivery of the final report within 4-6 weeks of receiving your samples

2. RNA samples
High quality samples are important for accurate microRNA quantification. At the initial consultation, we offer recommendations on suitable RNA extraction and clean-up methods. Alternatively, you can take advantage of our expertise and submit your samples to our RNA Isolation Service. Due to the sensitivity of our miRCURY LNA™ microRNA PCR system, we can perform analysis with minute RNA input. We normally use 40ng total RNA for full miRNome profiling and RNA corresponding to just 16μl plasma (purified from 250 ul plasma) for profiling with our Serum/Plasma Focus microRNA PCR Panels. Indeed, the LNA™ PCR system is well suited for the analysis of samples with low RNA content, including biofluids and exosomes.

A complete profiling service
Exiqon’s microRNA PCR experts will ensure that you get the best service throughout the projects, from the initial consultation and tailored experimental setup to the data analysis and delivery of a comprehensive yet easy-to-read final report with publication-grade illustrations. Figure 59 shows the standard workflow for an Exiqon PCR service project.

1. Consultation and experimental design
When you engage in qPCR Service project with Exiqon, you are assured direct communication with the scientists performing your experiments throughout the duration of the project. Each project begins with a free consultation with a microRNA qPCR expression profiling expert.

Together, we design an experimental setup that best satisfies your research needs and budget. Following this you will complete a detailed online sample submission form ensuring that all experimental details and subsequent analyses are clearly defined and understood by both parties.
3. RNA sample quality control (QC)

After receiving your RNA samples our specialists can assess the integrity, quantity and purity of each sample using Bioanalyzer and NanoDrop™ instruments. In addition, we offer qPCR-based QC tests to assess the performance of samples prior to RNA qPCR profiling. Samples are tested by PCR QC for the amplification of selected endogenous microRNAs and synthetic RNA spike-ins. Amplification levels are tested to be within known ranges and compared between samples to identify potential outliers. Two tests are offered: basic and extended PCR QC (see info box below).

All PCR reactions are performed using our miRCURY LNA™ Universal RT PCR products. The Universal RT reaction enables reverse transcription of all RNA species in the sample simultaneously in one tube. The flexibility of the LNA™ PCR platform gives superior experimental flexibility. For microRNA analysis, a range of PCR panels are available: full miRNome Panels, Focus Serum/Plasma, Cancer, Toxicology, Stem cells and Exosome Panels and custom Pick-&-Mix Panels (see page 36). Custom Pick-&-Mix Panels may include LNA™ PCR assays for any RNA species including novel RNAs.

**qPCR-based quality control of samples:**

**RNA PCR QC – Basic:**
- Quantification of five pre-selected endogenous microRNAs and a three synthetic RNA spike-ins
- Useful to monitor RNA extraction efficiency, presence of inhibitors, hemolysis and identify any potential outlier samples that should be omitted or replaced in the study

**RNA PCR QC – Extended:**
- Quantification of five pre-selected endogenous microRNAs and a three synthetic RNA spike-ins
- Three different RNA input amounts are tested
- Useful for determination of optimal RNA input
- Useful to monitor RNA extraction efficiency, presence of inhibitors, hemolysis and identify any potential of outlier samples that should be omitted or replaced in the study

We recommend that basic PCR QC is performed on all samples where standard RNA quality control is not applicable (e.g. low RNA content samples, such as serum/plasma RNA, or samples where the RNA quality/quantity is expected to be a challenge).

Extended PCR QC is recommended for samples that may contain PCR inhibitors and for sample types we have little experience with, or where there is a strong potential for qPCR inhibition.

The flexibility of Exiqon’s qPCR system allows us to identify and focus on the microRNA that carry information.
4. RNA qPCR

Our state-of-the-art laboratories use high throughput robotic pipetting stations that ensure superior reproducibility. Risk of template contamination is minimized by performing cDNA synthesis and PCR reactions in separate locations. All projects include negative control samples to determine background levels. Our procedures are in accordance with MIQE guidelines.

5. PCR quality assessment

Due to their small size, microRNA are extremely challenging PCR targets. We have therefore developed a unique automated QC system that allows for careful analysis of the quality of each individual PCR reaction prior to data analysis. Melting curves are inspected, amplification efficiencies are calculated and quantification cycle (Cq) values are compared to background levels in the negative control samples. Based on these analyses, reactions are flagged and removed from the data set if they show:

- several melting points or have melting points that are not within assay specifications
- amplifications with efficiencies outside our accepted range
- amplifications with Cq values within a threshold range of background signal

6. Normalization and data analysis

Before data analysis, we make sure your data are normalized to correct for potential overall differences between samples. The method of normalization is optimized for each individual project using sophisticated software packages. In case of biological replicates, average ΔCq values are calculated and ΔΔCq values are determined based on the biological grouping of samples.

Data analysis appropriate for the experiment at hand is performed, e.g., Principle Component Analysis (PCA) and heat maps based on unsupervised clustering. We check if samples group according to biology and look for any signs of experimental bias in the data set. Finally, statistical analysis of customer defined group comparisons is performed.

A wide range of statistical tests are available:

- T-test
- ANOVA
- ANCOVA
- Chi2 test for presence (for identification of microRNAs only detectable in one group)
- Power analysis (to determine the number of samples required in a validation study)
- Normality tests
- Wilcoxon tests

Cost-efficient serum/plasma microRNA profiling

The sensitivity and specificity of our microRNA PCR system make it ideally suited for the detection of microRNAs in blood serum and plasma.

Using our new Focus microRNA Panels (see page 36), we can profile serum/plasma samples at a fraction of the cost of a full genome profiling and still deliver the same high quality data on all the relevant microRNAs.
Exiqon’s qPCR analysis pipeline is integrated with Exiqon’s database XploreRNA™ (a rich source of curated information related to microRNAs and their targets, expression and disease association). Exiqon’s app for transcription analysis can be downloaded from Google Play and App Store free of charge. In connection with large service projects we also offer an extensive customized bioinformatics analysis.

7. Report and final consultation
An easy-to-read summary report is provided containing a description of the project, assessments of sample and data quality and an overview of the results of the data analysis with publication-grade illustrations. An extensive Excel file with all raw data is also included. The final report is delivered as a link to our secure webserver.

Scientific follow-up is always offered to answer any questions to your final report and to discuss how to proceed with the obtained results.

The qPCR profiling report consists of:
An easy-to-read summary report
• Quality assessment of sample and data
• Heat maps and PCA plots based on unsupervised clustering
• Results of statistical analysis of customer defined group comparisons
• Summary of results
• If relevant, data is compared to Exiqon’s in-house microRNA expression database

Materials and methods section
Project Summary Excel Report
• All raw data and normalized Cq values
• Statistical analysis and heatmaps according to customer specifications
• Ready to use for publication purposes

View a sample microRNA PCR Service report
Report examples are available for download at exiqon.com/microRNA-PCR-services

Learn more!
For more information and ordering please visit exiqon.com/microRNA-PCR-services
Exiqon has extensive knowledge of microRNA profiling in a wide range of samples and has been involved in numerous biomarker discovery projects.

We are currently working on an advanced and promising program on early detection of colorectal cancer (CRC) by microRNA analysis of patient blood plasma using our LNA™ microRNA qPCR platform (Table 5).

In addition to our extensive knowledge of biomarkers, we are experts on our own qPCR system. We are therefore uniquely positioned to assist you in all phases of biomarker discovery projects – from experimental design guidance to advanced bioinformatics. An overview of the general biomarker discovery project setup is presented in Figure 60.

Together with you and based on your requirements and budget we will design the best experiments and subsequent data analyses for your project.

Table 5. Experimental details of our colorectal cancer (CRC) biomarker study.

<table>
<thead>
<tr>
<th>Experimental stage*</th>
<th>Study size</th>
<th>MicroRNAs studied</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNAome screening</td>
<td>50 controls</td>
<td>730 microRNAs screened</td>
</tr>
<tr>
<td></td>
<td>50 CRC patients</td>
<td></td>
</tr>
<tr>
<td>Identify candidate microRNAs</td>
<td>76 controls</td>
<td>378 custom defined microRNAs screened</td>
</tr>
<tr>
<td></td>
<td>151 CRC patients</td>
<td></td>
</tr>
<tr>
<td>Validation of microRNA signatures</td>
<td>1000 patients</td>
<td>Defined microRNA signature</td>
</tr>
</tbody>
</table>

* See Figure 60

Figure 60. Biomarker discovery workflow using Exiqon’s LNA™ microRNA PCR system. Pilot miRNAome-wide screenings on experimental groups with a limited number of individuals are performed with our miRNAome PCR panels. Subsequently, biomarker candidate discovery screens on more individuals can be performed with a subset of microRNA PCR assays using our Serum/Plasma or Cancer Focus microRNA PCR panels or custom Pick-&-Mix panels. Final biomarker validation can be performed on large groups of individuals with a small set of microRNA assays in custom Pick-&-Mix panels.

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**Exiqon Services**

**Biomarker Discovery**

Exiqon’s scientists have expertise in biomarker discovery through several in-house projects, as well as experience in handling challenging clinical samples. They are available to assist with all phases of your project – from guidance on experimental design to advanced bioinformatics.
Our uniquely flexible PCR panel formats allow us to conduct cost effective biomarker screening projects by focusing only on the microRNAs with biomarker potential.
General Information

How to order

Below you will find information on how to place an order with Exiqon. In countries where Exiqon is represented by a local distributor, orders will have to be placed with the distributor. A list of distributors is shown on page 72 and 73.

1. Ordering options

You may place an order in one of the following ways. Information needed by Exiqon to handle your order is described below this section.

Order online:
Most products can be ordered directly online at Exiqon’s webpage. Go to exiqon.com, click on “Products” in the main menu and find your product(s) of interest in the product list. Click on the product line and follow the directions for online purchasing. Immediately after check out, you will receive an order confirmation by email.

If you already know the product number for the products you wish to order, use our Express Order option on the front page (exiqon.com)

Order by Email:
Place an order by contacting us at exiqon.com/contact

Order by Fax:
A signed order can be faxed to:
North America: +1 781 376 4152
Rest of the World: +45 4566 1888

Order by Phone:
To place an order by phone, call:
North America: +1 781 376 4150
Toll free (US & Canada): +1 888 miRCURY
Rest of the World: +45 4565 0929

Information needed:
When placing an order, please provide the following information:
• Product information
• Name (contact person), phone number, email address for order confirmation
• Billing Address
• Shipping address (including contact person)
• Purchase order (PO #) if applicable

• Institute TAX/VAT ID number for orders purchased and shipped within Europe
• Credit card (Visa, Mastercard, American Express) payment is possible upon receipt of invoice, where instructions for payment will be given

NOTE: For software orders, it is necessary to provide the end-user email address as product activation codes (serial number and download specifications) are provided by email ONLY. If an end-user email is not provided, the activation codes will be sent to the purchaser.

2. Finding a product number

We recommend visiting our website for the latest information on products and product numbers. New products and product updates may have been launched after print of this catalogue.

For microRNA-specific products such as detection probes, inhibitors and primer sets, the unique product number is only available on Exiqon’s website. Please go to the relevant product page on exiqon.com and find the product number by searching for the microRNA of interest.

Custom LNA™ Oligonucleotides of your own design are best ordered at exiqon.com/custom-lna-oligos. If you need assistance in designing your custom LNA™ Oligonucleotides, please contact Exiqon at exiqon.com/contact.

3. Shipping details

All products except software are shipped with FedEx. Shipping time depends on the product. Once your order has been processed, you will receive an order confirmation email stating an expected shipping date. Items in stock will be shipped by FedEx within 1-3 business days and custom-made products are normally shipped within 10-12 business days. Details are found at exiqon.com under product details for the specific product of interest.

For software orders, the activation code is sent by email within 2-5 days of purchase. Please then allow time to activate the programs. For GenEx, a 30-day fully functional trial version can be used in the meantime.
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