Unlock the Secrets of microRNA

miRCURY LNA™ Products for microRNA Research

2012

www.exiqon.com
MicroRNAs are small wonders. To study them, life science researchers need extremely accurate and sensitive tools for detection, profiling, localization and functional analysis.

Exiqon’s miRCURY™ product line offers unmatched performance in microRNA research. Based on our proprietary Locked Nucleic Acid-technology, the miRCURY™ tools give you reliable and specific results from strikingly small amounts of sample.

We offer a solution for every research need. From sample preparation to functional analysis, you can take advantage of our tools and expertise. We can even run the entire experiment for you with our customized, microRNA services. We’ll enable your microRNA discovery.

Enabling your groundbreaking microRNA discovery
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What is microRNA?

MicroRNAs constitute a class of small (~22 nt), non-coding RNAs that play key roles in the regulation of gene expression. 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 are important for many biological processes and have been linked to many diseases including cancer, heart-disease and neurological disorders.

The function of microRNAs
MicroRNAs have been shown to be involved in a wide range of biological processes such as cell cycle control, apoptosis and several developmental and physiological processes. These include stem cell differentiation, hematopoiesis, hypoxia, cardiac and skeletal muscle development, neurogenesis, insulin secretion, cholesterol metabolism, aging, immune responses and viral replication. In addition, 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.

MicroRNA and gene expression
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 highly similar microRNA families.

Figure 1. MicroRNA biogenesis. MicroRNA genes are transcribed by RNA polymerase II as large primary transcripts (pri-microRNA) that are processed by a protein complex containing the RNase III enzyme Drosha, to form an approximately 70 nucleotide long precursor microRNA (pre-microRNA). This precursor is subsequently transported to the cytoplasm where it is processed by a second RNase III enzyme, DICER, to form a mature microRNA of approximately 22 nucleotides. The mature microRNA is then incorporated into a ribonuclear particle to form the RNA-induced silencing complex, RISC, which mediates gene silencing. MicroRNAs are sometimes encoded by multiple loci, some of which are organized in tandemly co-transcribed clusters.
**MicroRNA as disease biomarkers**
In addition to their important roles in healthy individuals, microRNAs have also been implicated in a number of diseases including a broad range of cancers, heart disease and neurological diseases. Consequently, microRNAs are intensely studied as candidates for diagnostic and prognostic biomarkers and predictors of drug response.

**MicroRNA research**
MicroRNAs were first reported in mammalian systems in 2001. In the latest release of miRBase [v.17], more than 16000 microRNAs have been annotated, highlighting the rapid growth of this field of research. However, the functions of most of these microRNAs still remain to be discovered.

The challenges of studying microRNAs are two-fold. First, microRNAs are very short (~22 nt). This means that traditional DNA-based methods are not sensitive enough to detect these sequences with any reliability. Second, closely related microRNA family members differ by as little as one nucleotide, emphasizing the need for high specificity and the ability to discriminate between single nucleotide mismatches.

**Exiqon’s microRNA tools**
Exiqon has pioneered the development of microRNA research and diagnostics tools with leading-edge products and services based on the proprietary Locked Nucleic Acid (LNA™) technology. By incorporating LNA™ into our products, we have significantly increased the affinity and specificity of our probes, inhibitors and primers for their microRNA targets, thereby addressing both challenges described above.

All of Exiqon’s miRCURY LNA™ products are based on LNA™ technology.

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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 www.exiqon.com/microRNA-research-guide
What is LNA™?

An LNA™ oligonucleotide offers substantially increased affinity for its complementary strand, compared to traditional DNA or RNA oligonucleotides. This results in unprecedented sensitivity and specificity and makes LNA™ oligonucleotides ideal for the detection of small or highly similar DNA or RNA targets.

At a glance

- Excellent sensitivity - significantly increased sensitivity compared to DNA and RNA
- Uniform detection - robust detection of all microRNAs, 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. Incorporation of LNA™ into oligonucleotides has been shown to improve sensitivity and specificity for hybridization-based technologies including PCR, microarray and in situ hybridization.

LNA™-enhanced oligonucleotides can be designed to have a similar affinity towards all types of sequences regardless of the GC-content.

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.

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 probe. On the right, LNA™ substitutions allow shortening of the probe while maintaining the same \( T_m \).

### Same length, higher \( T_m \)

<table>
<thead>
<tr>
<th>DNA</th>
<th>23-mer</th>
<th>tcgatcgattagctagctagcga</th>
<th>( T_m: 60^\circ C )</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA/LNA™</td>
<td>23-mer</td>
<td>tcgatcgattagctagctacgta</td>
<td>( T_m: 64^\circ C )</td>
</tr>
<tr>
<td>DNA/LNA™</td>
<td>23-mer</td>
<td>tcgatcgattagctacgtacgta</td>
<td>( T_m: 78^\circ C )</td>
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### Shorter length, similar \( T_m \)

<table>
<thead>
<tr>
<th>DNA</th>
<th>23-mer</th>
<th>tcgatcgattagctagctagcga</th>
<th>( T_m: 60^\circ C )</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA/LNA™</td>
<td>16-mer</td>
<td>tcgatcgattagctacgtacgta</td>
<td>( T_m: 60^\circ C )</td>
</tr>
<tr>
<td>DNA/LNA™</td>
<td>8-mer</td>
<td>tcgatcgattagctacgtacgta</td>
<td>( T_m: 61^\circ C )</td>
</tr>
</tbody>
</table>

DNA: atcg  LNA: ATCG
T<sub>m</sub> normalization – robust detection regardless of GC content

The T<sub>m</sub> and therefore the affinity of an oligonucleotide duplex can be controlled by varying the LNA™ content. This feature can be used to normalize the T<sub>m</sub> across a population of short sequences with varying GC-content. For AT-rich oligonucleotides, which have low T<sub>m</sub>, more LNA™ is incorporated into the LNA™ oligonucleotide to raise the T<sub>m</sub> of the duplex. This enables the design of LNA™ oligonucleotides with a narrow T<sub>m</sub> range. This is beneficial for microarray, PCR and other applications where sensitive and specific binding to many different targets must occur under the same experimental conditions.

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 T<sub>m</sub> between a perfectly matched and a mismatched target is described as the delta T<sub>m</sub>. Incorporation of LNA™ in oligonucleotides can increase the delta T<sub>m</sub> between perfect match and mismatch binding by up to 8 °C. The increase in delta T<sub>m</sub> 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 [T<sub>m</sub>] 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 specific T<sub>m</sub> can be designed, regardless of the GC-content of the microRNA. Exiqon has used the LNA™ technology to T<sub>m</sub>-normalize primers, probes and inhibitors to ensure that they all perform well under the same experimental conditions (Figure 5).

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.

`Figure 4. The power of T<sub>m</sub> 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 (blue) or DNA based (gray) capture probes. MicroRNA targets with varying GC content were added at 100amol each.`

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

See how LNA™ works...

Watch the LNA™ movie at www.exiqon.com/e-talk
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 6).

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.

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.

Exiqon is the home of LNA™

Exiqon’s scientists have the expertise to design LNA™-enhanced oligonucleotides with high melting temperatures (Tm), optimal mismatch discrimination and high binding specificity while keeping secondary structure and self-complementarity to a minimum.

With our proprietary LNA™ technology and more than 10 years of experience in working with LNA™ applications, we can provide you with an excellent solution for your research needs.

Figure 5. 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 over 10°C around an optimal temperature.

Figure 6. Proven LNA™ applications. LNA™ is a powerful tool in many applications where standard DNA or RNA oligonucleotides do not have sufficient affinity or specificity. The figure shows an overview of some of the LNA™ applications that have been used for the study of RNA and DNA.

DNA

- Real-time /quantitative PCR
- SNP detection/allele specific PCR
- Methylation analysis
- Bead-based applications
- Chromosomal FISH
- Comparative genome hybridization
- Proteomics of isolated chromatin segments [PIChi]
- Antigen inhibition
- Mutagenesis

mRNA

- Real-time /quantitative PCR
- Microarray analysis
- In situ hybridization
- Northern blotting
- Bead-based applications
- Fluorescence activated cell sorting
- Inhibition of RNA function
- RNA modification [frame shifting/exon skipping

miRNA

- Real-time /quantitative PCR
- Microarray analysis
- In situ hybridization
- Northern blotting
- Bead-based applications
- Inhibition of RNA function

Bead-based applications

- Hybridization based approaches
- In vivo based approaches

Technical and scientific literature on LNA™ is available on www.exiqon.com/lna-technology
You can order your own custom LNA™ oligonucleotides directly on our website...

Your choice
Exiqon offers synthesis of custom oligonucleotides with a wide variety of modifications, labels, synthesis scales and purification methods.

We also offer design guides and online LNA™ tools to aid in the design process. Use our LNA™ Tₘ Prediction tool and LNA™ Oligo Optimizer tool to design the right oligo for your application.

When ordering an LNA™ oligonucleotide from Exiqon you can either design the sequence and LNA™ spiking pattern yourself or let Exiqon’s experts help you with the design.

Find out more...
Visit our website at www.exiqon.com/order-lna-oligos
1. Product Overview
# Product Overview

Overview of Exiqon’s miRCURY™ products for microRNA research.

## RNA Isolation

<table>
<thead>
<tr>
<th>miRCURY™ RNA Isolation Kits [page 12]</th>
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<tbody>
<tr>
<td>• Get total RNA from a wide range of sources</td>
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<tr>
<td>• Ideal for microRNA research</td>
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## Microarray

<table>
<thead>
<tr>
<th>miRCURY LNA™ microRNA Hi-Power Labeling Kits [page 14]</th>
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<tr>
<td>• Fast and simple labeling of total RNA</td>
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<tr>
<td>• Ideal for use with Exiqon’s microRNA arrays</td>
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<tr>
<th>miRCURY LNA™ microRNA Array, 7th gen - hsa, mmu &amp; rno [page 16]</th>
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<tr>
<td>• Our sensitive and specific human, mouse and rat microRNA microarray</td>
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<tr>
<th>Spike-in microRNA Kits [page 18]</th>
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<tr>
<td>• Improve the quality of your array data with these synthetic microRNAs</td>
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<tr>
<th>miRCURY LNA™ microRNA Array Analysis Software [page 18]</th>
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<tr>
<td>• Analyze your microarray data using these specifically adapted versions of ImaGene® 9 and Nexus Expression™ 3</td>
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## qPCR

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<tr>
<th>Universal cDNA Synthesis and SYBR® Green Master Mix kits [page 22]</th>
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<tr>
<td>• Optimized reagents for use with the miRCURY LNA™ Universal RT microRNA PCR system</td>
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<tr>
<th>miRCURY LNA™ Universal RT microRNA PCR Primer Sets [page 21]</th>
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<tr>
<td>• Individual microRNA PCR primer sets for quantification of microRNAs</td>
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<tr>
<td>• Design custom LNA™ primers for any small RNA online</td>
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<tr>
<th>mirNome PCR panels [page 21]</th>
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<tr>
<td>• Pre-aliquoted microRNA PCR primer sets in 384-well PCR plates</td>
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<tr>
<th>microRNA Pick-&amp;-Mix PCR panels [page 21]</th>
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<tr>
<td>• Design your own 96 and 384 well PCR plates</td>
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<tr>
<th>Focus microRNA PCR panels [page 21]</th>
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<tbody>
<tr>
<td>• 96 and 384 well PCR plates for microRNA profiling in serum/plasma, cancer, stem cells or toxicology</td>
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<tr>
<th>Exiqon GenEx software [page 23]</th>
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<tr>
<td>• Analyze qPCR data with powerful and easy to use software</td>
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## Northern blot

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<tr>
<th>miRCURY LNA™ microRNA Detection Probes [page 26]</th>
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<tr>
<td>• Get improved specificity and sensitivity with these pre-designed or custom LNA™ Northern blot probes</td>
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## In situ hybridization

<table>
<thead>
<tr>
<th>miRCURY LNA™ microRNA Detection Probes [page 28]</th>
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<tr>
<td>• Sensitive and specific probes for all microRNAs. Available with a wide variety of modifications</td>
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<tr>
<th>miRCURY LNA™ microRNA ISH Optimization Kits (FFPE) [page 30]</th>
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<tr>
<td>• Kits for optimizing microRNA ISH from many sample sources</td>
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<tr>
<td>• First optimize your experiment, then use an LNA™ probe for your microRNA of interest</td>
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## Inhibition

<table>
<thead>
<tr>
<th>miRCURY LNA™ microRNA Inhibitors [page 32]</th>
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<tbody>
<tr>
<td>• Pre-designed and custom inhibitors for specific suppression of all microRNAs in miRBase and more</td>
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<tr>
<th>miRCURY LNA™ microRNA Power Inhibitors [page 32]</th>
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<tr>
<td>• Our most potent inhibitors</td>
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<tr>
<td>• Synthesized with PS backbones</td>
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<tr>
<th>miRCURY LNA™ microRNA Inhibitor Libraries [page 34]</th>
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<tbody>
<tr>
<td>• Libraries available for human and mouse</td>
</tr>
<tr>
<td>• Ideal for high throughput microRNA inhibition</td>
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<table>
<thead>
<tr>
<th>miRCURY LNA™ microRNA Family Inhibitors [page 32]</th>
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<tbody>
<tr>
<td>• Inhibitors of all microRNAs within a family</td>
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<tr>
<td>• Available for more than 40 microRNA families</td>
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miRCURY™
RNA Isolation Kits

Kits for extraction of total RNA from a wide range of sample sources. Get high quality RNA in just 20 minutes using column-based protocols. RNA isolated using these kits is ideal for downstream microRNA experiments.

At a glance
• High quality total RNA isolation from a wide range of sources
• Fully compatible with Exiqon’s Array, PCR and Northern blot products
• Fast, easy and robust protocol for reproducible RNA purifications in just 20 minutes
• Excellent compatibility with RNAlater®
• Phenol-free procedure

Product coverage
We offer two total RNA purification kits:
• The miRCURY™ RNA Isolation Kit – Cell & Plant provides a rapid method for purification of total RNA from cultured animal cells, small tissue samples, blood, bacteria, yeast, fungi and plants.
• The miRCURY™ RNA Isolation Kit – Tissue is an excellent method for purification of total RNA from animal tissue samples.

Both kits contain spin columns and reagents for 50 total RNA purifications. Since these kits are used for the isolation of total RNA, ranging from large messenger and ribosomal RNAs down to microRNAs and other small RNAs, the purified total RNA can be used for a large number of downstream applications.

Purification technology
The miRCURY™ RNA Isolation Kits are based on spin column chromatography using a proprietary resin as separation matrix. Total RNA is separated from other cell components, such as proteins, without the use of toxic phenol or chloroform in an easy 20-50 min (depending on the sample source) protocol.

The miRCURY™ RNA Isolation Kits include optimized protocols for a large number of sample types. These protocols all consist of four simple steps (Figure 7).

High quality phenol-free total RNA isolation
The quality of the RNA isolated from our miRCURY™ RNA Isolation Kits is at least as high as that obtained using phenol/chloroform-based approaches, as determined by OD measurements and analysis using Agilent Bioanalyzers (Figure 8). However, column-based purification techniques offer distinct advantages over phenol or Trizol-based techniques in that high quality results are achieved very easily and with higher reproducibility. Furthermore, downstream applications such as qPCR and microarray analysis are very sensitive to phenol carry-over, a problem that is completely avoided by using Exiqon’s column-based kits.

Figure 7. Workflow for total RNA purification using miRCURY™ RNA Isolation Kits.
Total RNA is isolated in 20-50 min using easy-to-use spin column-based protocols.

Figure 8. Exiqon’s isolation kits produce high-quality RNA without the use of toxic phenol. Total RNA was purified from 10^6 HEK293 cells using kits from Exiqon and two leading rivals. Both competitors use phenol-based protocols.
Easy RNA extraction from difficult tissues

The miRCURY™ RNA Isolation Kit – Tissue provides rapid protocols for RNA purification from all types of animal tissue. For better removal of proteins from difficult fiber-rich tissue, such as brain, the kit has been enhanced with Proteinase K. The miRCURY™ RNA Isolation Kits yield high quality RNA even from such difficult tissues (Figure 9).

Excellent compatibility with RNAlater®

When using the kits on samples stored in RNAlater®, the purified RNA is of the same high quality as RNA from fresh frozen tissue as determined by microarray and qPCR analysis (Figures 10 & 11). This is important for researchers who store their samples before RNA extraction.

Ideal for a number of downstream applications

The purified total RNA is of the highest quality and can be used in a large number of downstream applications, including quantitative real-time PCR, microarray analysis, Northern blotting, RNase protection and primer extension assays. In addition, these kits are ideal for use with Exiqon’s miRCURY LNA™ Universal RT microRNA PCR, Array and Northern blot products (Figures 10 & 11).

Selected publications

Eldh et al. Mol. Immunol 2012
Mohan et al. PLOS One 2012

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

Ordering information

<table>
<thead>
<tr>
<th>miRCURY™ RNA Isolation Kit</th>
<th>Product description</th>
<th>Product no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell and Plant</td>
<td>50 spin columns, reagents and buffers for total RNA isolation</td>
<td>300110</td>
</tr>
<tr>
<td>Tissue</td>
<td>50 spin columns, reagents and buffers for total RNA isolation</td>
<td>300111</td>
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</table>

Figure 9. High quality total RNA from difficult fatty or fiber-rich tissues. Total RNA isolation from 5 mouse tissues using the miRCURY™ RNA Isolation Kit.

Figure 10. Excellent correlation between RNA from fresh frozen tissue and tissue stored in RNAlater®. Total RNA was isolated from fresh frozen (FF) mouse tissue or tissue stored in RNAlater® (RL) using Exiqon’s RNA isolation kit and those of two leading rivals. Next, the purified total RNA was analyzed using miRCURY LNA™ microRNA Arrays. There is excellent correlation between the RNA expression from FF and RL samples isolated using Exiqon’s kit, as seen from the correlation coefficients of the log ratios [R²].

Figure 11. Similar PCR results from fresh frozen and RNAlater® samples. Total RNA was purified from fresh frozen (FF) mouse tissues and tissues stored in RNAlater® (RL) using Exiqon’s miRCURY™ RNA Isolation Kit. Mouse miR-21 expression levels were then measured using Exiqon’s microRNA PCR system and the average cycle number values of four 10 fold dilutions from 3 experiments were compared. The results are very similar for the FF and RL samples, which indicates that Exiqon’s kit offers excellent performance for tissue stored in RNAlater®.
**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. Hi-Power kits offer double signal intensity compared to previous versions.

---

**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

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**Product coverage**

Two different labeling kits are available:

- **The miRCURY LNA™ microRNA Hi-Power Labeling Kit** is our new and improved labeling kit. It offers truly amazing performance with close to double signal intensity compared to our standard labeling kit. This means that the amount of sample can be reduced by almost half without sacrificing the quality of the microarray data.

- **The miRCURY LNA™ microRNA Power Labeling Kit** is our well-known RNA labeling kit. It combines ease-of-use with high performance and uniform labeling.

Both kits are available for single or dual color labeling of total RNA, which make the kits very flexible.

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**Save time and get consistent results**

The miRCURY LNA™ microRNA 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 (Figure 12).

---

**Uniform labeling for reliable results**

The labeling kits are used for single and dual color uniform labeling of total RNA samples (Figure 13). The dyes used (Hy3™ and Hy5™) are spectrally equivalent to the well-known Cy3 and Cy5 fluorophores, allowing for comparison of microRNA expression patterns.

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**Hi-Power labeling offers close to double signal intensity**

Exiqon’s miRCURY LNA™ Hi-Power microRNA Labeling Kit has been substantially improved and offers almost double signal-to-noise ratios compared to our standard labeling kit (Figure 14). 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, the same number of microRNAs can be detected with half the amount of RNA when using the Hi-Power kit (Figure 15).

Although there is a dramatic difference in the performance of the labeling kits, the labeling patterns of the kits are very similar (Figure 16). This means that microarray data obtained using the Power labeling kit can be reliably reproduced using the miRCURY LNA™ Hi-Power microRNA Labeling Kit.

---

**Figure 12. Hi-Power Labeling Kits Workflow.**

- **miRCURY LNA™ microRNA Hi-Power Labeling Kits**
  - **CIP treatment:** 5’ dephosphorylation of the RNA to prevent self-circularization
  - **Labeling reaction:** 3’ labeling of the RNA with Hy3™ or Hy5™ fluorescent dyes

- **miRCURY LNA™ microRNA Arrays**
  - Apply labeled samples to microarray
  - Hybridize over night
  - Wash and dry slides

- **miRCURY LNA™ microRNA Array Analysis Software**
  - Image acquisition and data analysis
Figure 13. Uniform labeling independent of the sequence of the microRNA. One fluorescent label is incorporated per microRNA regardless of the sequence at the 3’end of the molecule. This results in uniform labeling for all microRNAs in the sample.

Figure 14. Hi-Power labeling results in double signal intensity. Signal-to-noise ratios are almost doubled when using the miRCURY LNA™ Hi-Power Labeling kit compared to the regular miRCURY LNA™ Power labeling kit.

Figure 15. Detect the same number of microRNAs using half the RNA input. Signal intensities were compared between the Hi-Power and Power labeling kits. The experiments were conducted on Exiqon microRNA Arrays.

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

Ordering information (Details on page 48).

<table>
<thead>
<tr>
<th>miRCURY LNA™ microRNA Labeling Kits</th>
<th>Product description</th>
<th>Product no.</th>
</tr>
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<tr>
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<td>208034</td>
<td></td>
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<tr>
<td>Hy5™ Fluorescent labeling of microRNAs from total RNA samples. 24 rxns</td>
<td>208033</td>
<td></td>
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<tr>
<td>Hy3™/Hy5™ Fluorescent labeling of microRNAs from total RNA samples. 2x12 rxns</td>
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<table>
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<tr>
<td>Hy5™ Fluorescent labeling of microRNAs from total RNA samples. 24 rxns</td>
<td>208030-A</td>
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<td>Hy3™/Hy5™ Fluorescent labeling of microRNAs from total RNA samples. 2x12 rxns</td>
<td>208032-A</td>
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</table>
miRCURY LNA™ microRNA Arrays

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

- Truly global microRNA profiling – 3100 capture probes cover all human, mouse and rat microRNAs in miRBase V18.0
- \( T_m \)-optimized 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
- Efficient discrimination of closely related microRNA family members
- Leading-edge data analysis software customized to Exiqon arrays available

Product coverage

- miRCURY LNA™ microRNA Array, 7th gen - hsa, mmu & rno
  The 6th generation of our array contains more than 3100 capture probes, covering all human, mouse and rat microRNAs annotated in miRBase 18.0, as well as all viral microRNAs related to these species. In addition, this array contains capture probes for miRPlus™ human microRNAs. These are proprietary microRNAs not found in miRBase.

Exiqon strives to continuously update the contents of the array. Go to www.exiqon.com/array for the latest product news.

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 (Figures 17 & 18):

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 new miRCURY LNA™ microRNA Hi-Power Labeling Kit, Exiqon’s array has dramatically improved sensitivity (Figure 19). More than half of the LNA™ capture probes on the array have a detection limit of \( \leq 0.5 \) amol.

Exiqon’s microRNA arrays can produce reliable results from as little as 30ng of total RNA (Figure 20). Because of the high specificity of the platform, the sample size can be scaled up without compromising the quality of the data. This is especially important when studying microRNAs expressed at low levels.

Figure 17. LNA™-enhanced capture probes ensure robust detection of microRNAs.

With DNA-based capture probes, half of 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.
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 [Table 1].

Experimentally validated capture probes
All capture probes of the miRCURY LNA™ microRNA Arrays have been experimentally validated using synthetic microRNAs. Probes not performing according to our strict quality standards are replaced and never make it onto the final product.

Broad dynamic range
miRCURY LNA™ microRNA Arrays offer superior dynamic range over more than 5 orders of magnitude, ensuring that microRNAs with high and low expression levels will be detected well within the linear detection range [Figure 21].

Table 1. Superior discrimination between microRNA family members. There is very little cross-hybridization between let-7 family members. The experiments were performed with synthetic let-7 spike-in microRNA (300 amol) in a background of tRNA.

<table>
<thead>
<tr>
<th>let-7a</th>
<th>let-7b</th>
<th>let-7c</th>
<th>let-7d</th>
<th>let-7e</th>
<th>let-7f</th>
<th>let-7g</th>
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Figure 18. LNA™ capture probes have high uniform $T_m$. LNA™ probes have substantially higher $T_m$ than DNA probes. In addition, they can be $T_m$-normalized which means that all capture probes perform well under the same high stringency conditions.

Figure 19. The most sensitive array available. Due to optimally designed $T_m$ normalized capture probes and extremely efficient labeling, the Exiqon array detects a significantly higher percentage of microRNAs than competitor arrays.

Figure 20. Reliable microRNA expression profiles with as little as 30 ng total RNA. Four different microarray experiments with varying amounts of input RNA from oesophageal cancer (T) and normal adjacent (N) tissue were compared. A very high correlation is obtained when plotting the results from the experiment using 1000ng input RNA against those using 300, 100 and 30 ng.

Array movie tutorial
Watch Exiqon’s movie on manual hybridization of miRCURY LNA™ microRNA Arrays. It provides valuable guidance on how to get started with your microarray experiment. Go to www.exiqon.com/e-talk
Spike-in miRNA Kit v2 for data quality improvement

The 6th gen microRNA array includes a kit with 52 synthetic spike-in microRNAs that can be detected on the array by specifically designed capture probes. When the spike-in microRNAs are added to the labeling reactions before array hybridization, the signals from the spike-in capture probes can be used as controls for the labeling reaction and hybridization, scanner settings, data normalization, array replicates and technical variability.

In addition, the array contains capture probes for an additional 10 spike-in microRNAs, which can be used for further calibration or control of the profiling experiment.

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 of 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.

Ideal for both single and dual-color experiments

The production of our latest, 7th generation, microRNA array has been further optimized to support single-color experiments. When used together with the miRCURY LNA™ Hi-Power Labeling Kit median CV values of ≤ 10% can be routinely obtained. In our hands, we obtain average CV values of 2-4 % even between different batches of arrays. This allows researchers to use the array for reliable single color experiments saving valuable sample.

Data analysis software for Exiqon arrays

In collaboration with BioDiscovery, Exiqon offers ImaGene® 9 and Nexus Expression™ 3 for rapid and easy analysis of miRCURY LNA™ microRNA Array data [Figure 22]. Analyze your data with this leading-edge software specifically adapted for use with Exiqon’s microarray platform.

ImaGene® is a very powerful tool for microarray image analysis. Through an intuitive interface, it lets the user extract signal intensities from the scanned array and flag [poor] spots either automatically or manually. ImaGene® can also be used for easy visualization of microarray data in scatter or M-A plots.

Nexus Expression™ is a very feature-packed but easy-to-use program for the analysis of microarray experiments. Using a simple workflow, raw data from ImaGene® is background-subtracted and normalized, after which differentially expressed microRNAs can be identified. An intuitive interface guides you through each step of the analysis process. Read more about the software at www.exiqon.com/mirna-array-software.

Exiqon’s LNA™-based arrays are superior for microRNA detection.

Ina K. Dahlsveen, Ph.D., Product Manager
Validate your results with Exiqon’s 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. The combination of a Universal RT reaction and LNA™-enhanced PCR primers results in unmatched sensitivity and specificity. Ready-to-use microRNA PCR panels enable fast and easy microRNA expression profiling (Figure 23). Identical positive controls on both platforms allows for robust cross-platform comparison of results.

Selected publications
Ralfkiaer et al. Blood 2011

For more publications and updated product information, go to www.exiqon.com/array

Did you know?
Exiqon can perform your microRNA profiling and data analysis for you. Read more on page 34.

Figure 23. Excellent correlation between microarray and qPCR results. The results you get with our array system can be validated using our qPCR system – Get results you can trust. The array data was normalized (quantile normalization) and microRNAs with log2 ratios > or < 0.5 were included in the study. The qPCR data was normalized to reference genes. Only microRNAs that were detected (Cp < 36 for all replicates) were included. A total of 26 microRNAs were included in the study.

For more publications and updated product information, go to www.exiqon.com/array

<table>
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<tr>
<th>miRCURY LNA™ microRNA Array, 7th gen, hsa/mmu/rno</th>
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<td>208501 (6 slides)</td>
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<td>Hybridization buffer</td>
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<td>Salt buffer</td>
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<td>Detergent solution</td>
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<th>Product no.</th>
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<td>Microarray Analysis Software</td>
<td>208220</td>
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<td>ImaGene®/Nexus™ - 30 day license/24 slides</td>
<td>Microarray Analysis Software</td>
<td>208221</td>
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</table>
miRCURY LNA™ Universal RT microRNA PCR

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.

A unique system for microRNA profiling

The miRCURY LNA™ Universal RT microRNA PCR system (Figure 24) offers the best available combination of performance and ease-of-use as it unites two important features (Figures 25 & 26):

• **Universal RT** – One first-strand cDNA synthesis reaction (or RT reaction) can be used as template for multiple microRNA real-time PCR assays. This saves precious sample, reduces technical variation and saves time and effort in the laboratory.

• **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 solutions for both microRNA expression profiling and for quantification of individual microRNAs (Figure 24). Our products are optimized to give you a flexible and easy to use solution for all stages of your PCR microRNA expression profiling study.

Use our optimized cDNA synthesis kit for fast, easy and reliable first strand synthesis. PCR reactions can be carried out in one of our Ready-to-use PCR panels: miRNome panels, serum/plasma or cancer focus panels or custom-designed Pick-&-Mix plates.

Alternatively, use our pre-designed individual PCR primers or design your own custom primers. Our PCR primer sets have been designed for optimal performance when used with Exiqon’s SYBR® Green master mix. Use of other master mixes may affect the quality of the results.

In addition, to help you get the most of you microRNA PCR experiment, we offer a customized and powerful software solution, Exiqon GenEx, which provides an easy-to-use platform with all the tools you need for the qPCR data analysis.

Figure 24. Overview of the miRCURY LNA™ Universal RT microRNA PCR system.
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 SYBR® Green master mix to the plates and run the real-time PCR. The whole process takes only 3 hours (Figure 26). Exiqon’s panels include several reference genes and controls and are compatible with most PCR instruments.

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 40ng total RNA – no pre-amplification necessary.

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

Focus panels

Panels contain validated assays for the relevant research area or application. The Focus miRNA assays are based on Exiqon’s collection of data from thousands of clinical samples. Selection of panels currently available:

- Serum/plasma Focus panels allow for accurate and sensitive profiling of microRNAs present in serum and plasma using just 20μl serum/plasma [see info box on page 24]
- Cancer Focus panels include PCR assays for all relevant oncogenes and/or tumor suppressor microRNAs
- Stem Cell Focus panels contain all microRNAs relevant for embryonic and induced pluripotent stem cell research
- Toxicology Focus panels are available for both human, rat, dog and monkey, covering all relevant toxicity-related microRNAs
- RNA QC panels contain sets of 12 specially selected PCR assays for quality control of RNA samples

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. These panels are ideal for investigating or validating microRNA signatures and subsets 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
- Choose pre-designed Focus panels and customize to your needs
- Available in 96 and 384 well plate format

Individual assays

Pre-designed assays

Validated qPCR primer sets for quantification of human, mouse and rat microRNAs and 12 endogenous reference genes are available

- Choose from 1207 fully validated and optimized LNA™-enhanced PCR primer sets
- microRNA quantification from just 1pg total RNA

Custom assays

Design primer sets for your own custom microRNA or small RNA using our easy-to-use online design tool

- Design LNA™-enhanced qPCR primer sets for any microRNA
- Optimal PCR primers are designed using Exiqon’s online design tools using advanced in-house design algorithms

Figure 26. Overview of the miRCURY LNA™ Universal RT PCR workflow. The PCR primer sets have been designed for optimal performance when used with Exiqon’s SYBR® Green master mix. Use of other master mixes may affect the quality of the results. Ready-to-use (miRNome, Focus and Pick- & -Mix) panels can be replaced by individual PCR primers in this workflow.
Reagents
Optimized reagents for use with Exiqon’s PCR panels and individual primer sets.

cDNA synthesis kit
First-strand cDNA synthesis
• Fast, simple and reliable first strand synthesis

SYBR® Green master mix
The kit contains high-performance reagents for quantitative real-time PCR amplification.
• Optimized for use with Exiqon’s qPCR system. Use of other kits will affect the quality of the results

Analysis software
Exiqon GenEx
Easy-to-use software solution with all the tools needed for qPCR data analysis.
• 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 license and free support including detailed manual and online tutorials
• Download a 30 day free trial and use our step-by-step guide to get started with your data analysis

Accurate microRNA quantification using just 1pg total RNA
Use of LNA™-enhanced T_m-normalized primers means that the PCR amplification is extremely sensitive which allows for accurate and reliable quantification of individual microRNAs from as little as 1 pg of total RNA input in the first-strand cDNA synthesis reaction (Figures 27 & 28). No pre-amplification of the cDNA is 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 (Figures 29 & 30).

Use less sample with universal RT
Exiqon’s qPCR system uses a single universal RT reaction, which means that the same first-strand cDNA pool can be used as template in all microRNA PCR amplification assays. This saves precious sample, reduces technical variation, and minimizes the amount of pipetting compared to systems that are based on a microRNA specific RT reaction.

Unmatched specificity
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.

All miRCURY LNA™ Universal RT microRNA PCR primer sets have been optimized and strictly validated for specific amplification of the target and for minimal background signal.

Fast, easy and reproducible
Save time and effort in the laboratory with the 3 hour easy-to-follow protocol. By using the same RT reaction as template in all subsequent PCR reactions, the procedure is greatly simplified compared to systems that require microRNA-specific first-strand synthesis. 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.

Reference and control primer sets
There are twelve reference primer sets available for reliable data normalization. These primers target endogenous small non-coding RNAs that are constitutively expressed in a wide variety of tissues (see www.exiqon.com/mirna-PCR for more details).

As an alternative to these genes, microRNAs can be used as reference genes. However, it is important to ensure that the microRNAs are expressed at constant levels in all samples before using them as reference genes.

All ready-to-use panels contain 6 reference primer sets as well as one control primer set and an inter-plate calibrator in triplicate. The control primer set targets a synthetic spike-in RNA that is included in the miRCURY LNA™ Universal cDNA synthesis kit.

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 36.
Customized data analysis software

Exiqon offers a specifically adapted version of the comprehensive qPCR analysis software GenEx. A new user interface allows generation of templates for use with different real-time PCR instruments and rapid import of data directly into GenEx.

GenEx offers user-friendly step-by-step guides to data pre-processing. 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 www.exiqon.com/mirna-pcr-analysis

Selected publications

Kazenwadel et al. Blood 2010
Jensen et al. BMC Genomics 2011
Jorde et al. BMC Research Notes 2012

Working with serum or plasma samples?

Table 2. Excellent discrimination between closely related microRNA family members.

Each PCR assay was tested against synthetic RNA from the 8 family members of the let-7 family. All assays were found to be very specific for the microRNAs for which they were designed.

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<thead>
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<th>let-7a</th>
<th>let-7b</th>
<th>let-7c</th>
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Figure 28. 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 microRNA assays exhibit linear read-out with correlation coefficients R(2) > 0.99.

Figure 29. Expression profiling of 742 microRNAs using 40 ng total RNA from tumor and normal FFPE sections. Real-time PCR was performed using triplicate RT reactions per sample on human miRNome microRNA panels I and II. Data from 424 microRNAs with Cq values <37 is included. Normalized expression is shown as fold changes in tumor compared to normal. Out of 424 microRNAs expressed, 144 were > 2-fold down-regulated in the tumor (blue dots) and 26 were > 2-fold up-regulated in the tumor (red dots).

Figure 30. microRNA profiling in blood serum and plasma. Serum/Plasma Focus microRNA PCR Panels were used to profile 175 microRNAs commonly found in serum/plasma. MicroRNAs for sample quality control in orange and interesting microRNAs in red.
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 175 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 the limited number of peer-reviewed published papers available (Figure 30).

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.
Ordering information [Details on page 48].

<table>
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<th>Reagents</th>
<th>Product description</th>
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<tr>
<td>Universal cDNA Synthesis Kit</td>
<td>Polyadenylation and cDNA synthesis kit (16 to 32 runs)</td>
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<td>SYBR® Green master mix, Universal RT, 2.5ml</td>
<td>250 rxns of 20μl or 500 rxns of 10μl</td>
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<td>250 rxns of 20μl or 500 rxns of 10μl</td>
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<td>xxx-miRxxxx, LNA™ PCR primer set, UniRT</td>
<td>microRNA primer set, 200 runs</td>
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<tr>
<td>Reference gene PCR primer set, UniRT</td>
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<th>PriRNome panels</th>
<th>Product description</th>
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<tr>
<td>microRNA Ready-To-Use PCR, Human panel I+II</td>
<td>4x plate I and 4x plate II in 38-well PCR plates, 74L human microRNAs and 6 reference genes*</td>
<td>203607-203608</td>
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<tr>
<td>microRNA Ready-To-Use PCR, Human panel I</td>
<td>4x plate I in 38-well PCR plates, 375 human microRNAs and 6 reference genes*</td>
<td>203409-203410</td>
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<tr>
<td>microRNA Ready-To-Use PCR, Mouse &amp; Rat panel I+II</td>
<td>4x plate I and 4x plate II in 38-well PCR plates, 74L mouse and rat microRNAs and 6 reference genes*</td>
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<tr>
<td>microRNA Ready-To-Use PCR, Mouse &amp; Rat panel I</td>
<td>4x plate I in 38-well PCR plates, 376 mouse and rat microRNAs and 6 reference genes*</td>
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<table>
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<tr>
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<th>Product no.</th>
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<tr>
<td>96 well, Ready-to-use plates</td>
<td>8 PCR plates with custom selection of microRNA primer sets*</td>
<td>203801</td>
</tr>
<tr>
<td>384 well, Ready-to-use plates</td>
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<table>
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<th>Focus microRNA PCR Panel</th>
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<tr>
<td>Serum/Plasma Focus, 96 well Ready-to-use plates</td>
<td>4 Panels in 8 PCR plates. Each panel contains LNA™ primers for 168 human serum/plasma microRNAs and 7 reference microRNAs*</td>
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<tr>
<td>Serum/Plasma Focus, 384 well Ready-to-use plates</td>
<td>8 Panels in 2 PCR plates. Each panel contains LNA™ primers for 168 human serum/plasma microRNAs and 7 reference microRNAs*</td>
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<tr>
<td>Cancer Focus, 96 well Ready-to-use plates</td>
<td>4 Panels in 4 PCR plates. Each panel contains LNA™ primers for 83 human cancer microRNAs and 6 reference microRNAs*</td>
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<tr>
<td>Cancer Focus, 384 well Ready-to-use plates</td>
<td>8 Panels supplied in two PCR plates. Each panel contains LNA™ primers for 83 human cancer microRNAs and 6 reference microRNAs*</td>
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| microRNA QC PCR Panel, 96 well Ready-to-use plates | 16 Ready-To-Use microRNA QC PCR Panels, supplied in two 96-well plates. Each panel comprises 6 LNA™ microRNA primer sets and 5 reference genes and UniSp3 for evaluation of microRNA quality*. | 203844-203847|
| microRNA QC PCR Panel, 384 well Ready-to-use plates | 32 Ready-To-Use microRNA QC PCR Panels, supplied in one 384-well plate. Each panel comprises 6 LNA™ microRNA primer sets and 5 reference genes and UniSp3 for evaluation of microRNA quality*. | 203848-203849|

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<th>RNA Spike-In</th>
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<tr>
<td>RNA Spike-in kit, UniRT</td>
<td>miRCURY LNA™ Universal RT microRNA PCR, Set of two vials with synthetic RNA spike-in templates for qPCR control (UniSp2; UniSp3, UniSp5 RNA Spike-in template mix and cel-miR-39-3p RNA Spike-in template)</td>
<td>203203</td>
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| UniSp2, LNA™ control primer set, UniRT | miRCURY LNA™ Universal RT microRNA PCR, spike-in control primer set, 200 rxns | 203950      |
| UniSp5, LNA™ control primer set, UniRT | miRCURY LNA™ Universal RT microRNA PCR, spike-in control primer set, 200 rxns | 203951      |
| cel-miR-39-3p, LNA™ control primer set, UniRT | miRCURY LNA™ Universal RT microRNA PCR, spike-in control primer set, 200 rxns | 203952      |
| UniSp4, LNA™ control primer set, UniRT | miRCURY LNA™ Universal RT microRNA PCR, spike-in control primer set, 200 rxns | 203953      |

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<th>Exiqon GenEx Software</th>
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<tr>
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</tr>
<tr>
<td>Pre Academic</td>
<td>Exiqon GenEx, qPCR analysis software, academic license</td>
<td>207006</td>
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<tr>
<td>Enterprise Industrial</td>
<td>Exiqon GenEx, qPCR analysis software, industrial license</td>
<td>207007</td>
</tr>
<tr>
<td>Enterprise Academic</td>
<td>Exiqon GenEx, qPCR analysis software, academic license</td>
<td>207008</td>
</tr>
<tr>
<td>Import wizard</td>
<td>Exiqon qPCR plate import wizard addition to GenEx, for current GenEx customers</td>
<td>207009</td>
</tr>
<tr>
<td>Pre, Portable USB license key</td>
<td>Multiple-user license upgrade to the Pro version</td>
<td>207011</td>
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<tr>
<td>Enterprise, Portable USB license key</td>
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</table>

* Each panel product is available in different types of plates for specific real-time PCR instrument compatibility. Plate types for all major brands of real-time PCR instruments are covered including ABI 7000 series, FAST series, StepOnePlus, 7900HT and Viia 7, Roche (LightCycler 480), BioRad (Cycler, iQ series, and CFX96), Eppendorf (Mastercycler ep Realplex), and Stratagene (Mx4000 and Mx3000 series). The product number relates to the specific plate type. For details, please visit www.exiqon.com/mirna-pcr.
miRCURY LNA™ microRNA Detection Probes for Northern blotting

Extremely sensitive and specific LNA™-enhanced Northern blot probes targeting any microRNA or small RNA.

**Product coverage**

There are two kinds of products available for Northern blotting:
- Pre-designed miRCURY LNA™ microRNA Detection Probes are available for most 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.

**Sensitive and specific detection**

miRCURY LNA™ microRNA 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 31). 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 32).

**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 (Figure 33).

Detection Probes are also available in "ready-to-label" [using enzymatic labeling kits] format. These probes can be conveniently labeled using standard end-labeling techniques.

**Selected publications**


**Ordering information** (Details on page 48).

<table>
<thead>
<tr>
<th>miRCURY LNA™ microRNA Detection Probe</th>
<th>Product no.</th>
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<tbody>
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<td>5’-DIG Labeled, 250 pmol</td>
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<tr>
<td>5’-biotin Labeled, 250 pmol</td>
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<tr>
<td>5’-fluorescein Labeled, 250 pmol</td>
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<tr>
<td>3’-DIG Labeled, 250 pmol</td>
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<tr>
<td>3’-amino Labeled, 250 pmol</td>
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</tr>
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<td>U6 Positive Control, 250 pmol</td>
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<tr>
<td>Sense miR-159, Negative Control, 250 pmol</td>
<td>99003-xx</td>
</tr>
<tr>
<td>Scramble-miR, Negative Control, 250 pmol</td>
<td>99004-xx</td>
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</table>

Other modifications are also available. Learn more at www.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.
Figure 31. 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.

Figure 32. 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 33. DIG-labeled LNA™ probes outperform DNA probes. Total RNA (3 or 6 μg) was hybridized with DNA and LNA™ detection probes targeting miR-21. Exposure time was 1 minute. From Kim et al. 2010, reprinted with permission from Oxford University Press.
miRCURY LNA™ microRNA

Detection Probes for
in situ hybridization

LNA™-enhanced microRNA in situ hybridization probes with a wide selection of labels. Sensitive and specific detection of microRNAs from a wide range of sample sources.

At a glance
• LNA™-enhanced probes with a wide selection of labels
• Probes available for all known microRNAs as well as custom sequences
• Unmatched sensitivity and specificity
• Fully developed protocols available

Product coverage
There are two kinds of products available for microRNA in situ hybridization:
• 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 other microRNA or small RNA, including precursor microRNAs. Our experts will design the optimal probe for you.

In addition, we offer positive and negative control probes.

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 30).

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. 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 (Figure 38). We recommend this labeling option for optimal results.

Selected publications

Visit our microRNA ISH gallery
Nearly 1000 images are on display at: www.exiqon.com/gallery-of-in-situ-hybridization-images

For more publications and updated product information, please visit www.exiqon.com/ish

Figure 34. Detection of a brain-specific microRNA. LNA™ probes (red) were used to detect miR-38 in mouse hippocampus. DNA is labeled with DAPI (blue). Image kindly provided by Dr. Javier Martinez, IMBA, Vienna, Austria.
**Figure 35.** MicroRNA detection in zebrafish. Detection of miR-122a (top), miR-20a (middle) and miR-124a (bottom) using LNA™ probes in whole mount zebrafish embryos. Image kindly provided by Dr. Ronald Plasterk, Hubrecht Laboratory, The Netherlands.

Other modifications are also available. Learn more at www.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.

**Table 1:** Ordering information

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<td>UA Positive Control, 250 pmol</td>
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<td>Sense miR-159, 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 are also available. Learn more at www.exiqon.com/oligonucleotide-modifications

**Figure 36.** MicroRNA detection in chick. Specific detection of miR-20a in a Gallus gallus embryo using an LNA™ probe. miR-20a is detected in myotomal muscle cells (Ason et al. 2006).

**Figure 37.** This microRNA is clearly up-regulated in the tumor (A) compared to normal tissue (B). Moreover, it is specifically located in the stromal fibroblast compartment. The miRCURY LNA™ microRNA Detection Probe is FITC labeled (green) and nuclei are counterstained in DAPI (blue).

**Figure 38.** Double DIG labeling is more sensitive than single DIG labeling. hsa-miR-21 detection in 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).
miRCURY LNA™ microRNA
ISH Optimization Kit (FFPE)

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 and a minimum 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 super 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 39]. 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 28].

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 [see table on next page]. These 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 40-42].

Product coverage
• 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 for optimizing ISH experimental settings [Figures 40-42].

Figure 39. Overview of the procedure. First, the tissue is “opened” using Proteinase K. In the hybridization step, the double DIG-labeled LNA™ probe binds specifically to its target microRNA. Alkaline phosphatase (AP)-conjugated anti-DIG antibodies are then added. This step is followed by NBT-BCIP development and optional counter-staining with Nuclear Red.
Selecting the appropriate miRCURY LNA™ microRNA ISH Optimization Kit.
The table indicates the tissue(s) and cell types in which each of the kits has been validated.

<table>
<thead>
<tr>
<th>Kit 1</th>
<th>Kit 2</th>
<th>Kit 3</th>
<th>Kit 4</th>
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**Selected publications**
Jørgensen et al. Methods 2010, 52: 375-81

For updated product information, please visit www.exiqon.com/mirna-ish-kit

**Ordering information** (Details on page 48).

<table>
<thead>
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<th>microRNA ISH Optimization Kit</th>
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<tbody>
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<td>Kit 2 (miR-21) Includes controls and buffer</td>
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<td>Kit 8 (miR-205) Includes controls and buffer</td>
<td>90008</td>
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<tr>
<td>Kit 9 (miR-223) Includes controls and buffer</td>
<td>90009</td>
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</table>

**Other reagents**

<table>
<thead>
<tr>
<th>Product no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>microRNA ISH Buffer 25ml (1000 slides)</td>
</tr>
<tr>
<td>microRNA ISH Buffer and Controls kit</td>
</tr>
</tbody>
</table>

**Figure 40.** miR-126 detection in colon wall. Kit 5 can be used to detect microRNAs in inflamed colon FFPE tissue. Here, it was used to detect miR-126. Staining was performed with NBT-BCIP (blue). Sections were counterstained with nuclear red.

**Figure 41.** miR-145 detection in human colon. Kit 7 can be used to detect microRNAs in colon FFPE tissue. Here, miR-145 is detected in a human colon wall with underlying muscle layers. Staining was performed with NBT-BCIP (blue). Sections were counterstained with nuclear red.

**Figure 42.** miR-205 detection in human breast carcinoma. Kit 8 can be used for detection of microRNAs in breast cancer FFPE tissue. Here, it was used to detect miR-205. Staining was performed with NBT-BCIP (blue). Sections were counterstained with nuclear red.

Did you know?
microRNA ISH kits can also be used for fresh frozen samples. Go to www.exiqon.com/ish for more information.
miRCURY LNA™ microRNA

Inhibitors and Power Inhibitors


At a glance
- Unmatched efficacy even for AU-rich microRNA targets
- Inhibitors available for specific suppression of all microRNAs in miRBase and our proprietary miRPlus™ microRNAs
- Excellent silencing at low concentrations with minimal risk of off-target effects
- Superior specificity and biological stability for long-lasting antisense activity
- Family Inhibitors available for the inhibition of all microRNAs within a family

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

All microRNA inhibitors were developed using novel advanced design algorithms that identify the optimal combination of length, sequence and LNA™ positioning. This ensures that the inhibitors offer a high uniform potency combined with excellent specificity and biological stability, while keeping self-complementarity to a minimum.

Coverage and content
Five product categories are available:
- miRCURY LNA™ microRNA Inhibitors. are our well known pre-designed inhibitors with phosphodiester backbone. Using a sophisticated design algorithm, the microRNA inhibitors have been Tm-normalized and optimized with respect to potency and stability. Learn about Tm-normalization on page 7.
- miRCURY LNA™ microRNA Power Inhibitors. Offers the highest potency on the market. These inhibitors come with a phosphorothioate (PS) backbone which makes them highly resistant to enzymatic degradation. As a result, they offer superior potency and prolonged stability (Figure 43). In all other respects, this class of inhibitors is identical to our regular line of microRNA inhibitors.
- miRCURY LNA™ microRNA Family Inhibitors are a novel class of inhibitors 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.
- In vivo miRCURY LNA™ microRNA inhibitors. We offer custom designed microRNA inhibitors optimized for in vivo use. Our in vivo inhibitors have fully PS modified backbone and are shorter than our regular inhibitors to facilitate cellular uptake and reduce toxicity problems. In addition we inactivate CpG motifs (by methylation of C) in order to alleviate potential problems of immunostimulatory effects.

All microRNA inhibitors are available with fluorescein (6-FAM) labels or as ready-to-label (unlabeled) oligonucleotides. They are delivered HPLC-purified and desalted in tubes containing 5 nmol dried-down oligonucleotide ready for transfection or electroporation using standard techniques. Our in vivo inhibitors are purified for use in laboratory animals and are available in large amounts.

Figure 43. Power inhibitors offer even higher potency. HeLa cells were transfected with different concentrations of microRNA Inhibitors and Power Inhibitors targeting miR-16. Reporter gene expression was measured 48 h after transfection.
Unmatched high and uniform potency
The miRCURY LNA™ microRNA Inhibitors have been optimized with a sophisticated design algorithm resulting in high and uniform potency (Figures 44 & 45). By varying the length of the oligonucleotides along with the number and positions of LNA™ bases, the melting temperatures of the inhibitors have been normalized to an optimal temperature.

These design features mean that the inhibitors will have the same high efficacy against all microRNAs regardless of the GC-content of the targeted microRNA. This is important because the GC-content of microRNAs varies between 5 - 95%. Since the binding affinity of traditional antisense inhibitors (2'-O-Me oligonucleotides) is essentially dictated by the GC-content of their microRNA targets, they will typically have poor or no efficacy against AU-rich microRNA. The dramatic effect of intelligently placed LNA™ bases in the inhibitor oligonucleotide sequence to increase and normalize their $T_m$ is shown in Figure 5 on page 8. More information on $T_m$ normalizing can be found on page 7.

Excellent specificity
Intelligent LNA™-spiking also ensures excellent discrimination between closely related microRNA family members, which means that any biological effects seen with the inhibitors can be safely attributed to the antisense inhibition of the targeted microRNA and not to unspecific binding (Figure 46, page 35).

Minimal toxicity and off-target effects
The high potency of both miRCURY LNA™ microRNA Inhibitors and Power Inhibitors means that they can be used at low concentrations minimizing risk of undesired secondary effects unrelated to the antisense activity.

An added benefit of the inhibitor design is that LNA™ bases are distribution throughout the entire length, which ensure that LNA™ inhibitor/RNA duplexes are not recognized as substrates for RNase H. As a consequence, there will be minimal off-target effects on mRNAs.

Finally, the miRCURY LNA™ microRNA Inhibitors and Power Inhibitors are HPLC purified to ensure that they are not contaminated by synthetic bi-products.

Selected publications
Hansen et al. Genes Dev. 2010
Zaragosi et al. Genome Biology 2011
Bonn et al. Circ Res. 2011

For more publications and updated product information, please visit [www.exiqon.com/mirna-inhibitor](http://www.exiqon.com/mirna-inhibitor)

Ordering information (Details on page 48).

We also offer miRCURY LNA™ Target Site Blockers that inhibit microRNA binding to a specific mRNA target. Please contact us for more information: [www.exiqon.com/contact](http://www.exiqon.com/contact)
miRCURY LNA™ microRNA Inhibitor Libraries

LNA™ oligonucleotide libraries for easy and efficient high-throughput antisense inhibition of human and mouse microRNAs.

At a glance
• LNA™-enhanced microRNA inhibitors for high-efficacy silencing of human and mouse microRNAs
• Excellent coverage: All miRBase v.12 human and mouse microRNAs are covered
• Potency-normalized oligonucleotides with high affinity and minimal self-annealing
• Delivered in convenient 96 well plates for easy handling

Product coverage
The miRCURY LNA™ Inhibitor Libraries are available in three different versions:
• A human library covering 980 microRNAs.
• A mouse library covering 739 microRNAs.
• A combined human and mouse library covering all the microRNAs above.

All libraries are delivered in 96-well plates containing 0.25 nmol, HPLC purified and desalted, dried-down miRCURY LNA™ microRNA Inhibitor per well.

Potency normalized antisense inhibitors
When performing large screening projects, it is not practical to optimize the transfection conditions for each of the ~1000 microRNA inhibitors. Our libraries represent a simple solution to this problem, as they feature our optimized miRCURY LNA™ microRNA Inhibitors which are Tₘ-normalized and have high uniform potency (see page 7). Transfection conditions optimized for a single highly expressed miRNA can therefore safely be adopted for screening of the entire library.

Minimal off-target effects
In addition to the high specificity of the LNA™ microRNA inhibitors for their target microRNAs (Figure 46), the use of LNA™ in the inhibitors ensures that potential mRNA-inhibitor duplexes are not recognized as RNase H targets. This minimizes any potential off-target effects and reduces the risk that any observed biological phenotype is caused by factors other than the antisense activity of the inhibitors.

The high potency of the inhibitors means that smaller concentrations can be used, which further minimizes the risk of negative side effects. This risk is also reduced by the high purity offered by the HPLC purified and desalted oligonucleotides.

Biological stability
LNA™ antisense inhibitors are very stable, which means that in addition to offering unrivaled specificity and potency, they also offer long lasting antisense activity.

miRPlus™ microRNAs
The human library includes inhibitors targeting 37 miRPlus™ microRNAs. These inhibitors target proprietary microRNA candidates that have been identified by Exiqon using cloning and sequencing of human normal and diseased tissue.

Before being added as miRPlus™ microRNAs, the sequences have been subjected to strict quality control procedures to ensure that they are truly different from annotated microRNAs and that they are found in multiple clones. Once added, the miRPlus™ sequences give scientists unique information about microRNAs otherwise not available.

Important note about validation
When evaluating your transfection, you should not use microRNA qPCR only. Always use a functional read out like Western blots or Luciferase reporter assays.

For updated product information, please visit www.exiqon.com/mirna-inhibitor
Ordering information (Details on page 48).

<table>
<thead>
<tr>
<th>miRCURY LNA™ microRNA Inhibitor Library</th>
<th>Product no.</th>
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<tr>
<td>Human</td>
<td>190102-2</td>
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<tr>
<td>Mouse</td>
<td>190202-00</td>
</tr>
<tr>
<td>Human &amp; Mouse</td>
<td>190302-2</td>
</tr>
</tbody>
</table>

**Figure 45.** miRCURY LNA™ microRNA Inhibitors are more efficient than competing technologies. The expression of the pMIR-21 luciferase reporter is upregulated compared to the no-oligonucleotide control when MCF7 cells are transfected with antisense inhibitors directed against miR-21. This effect is significantly stronger in cells transfected with LNA™ oligonucleotides than cells transfected with DNA (Product A and B) or 2’-O-Me oligonucleotides.

**Figure 46.** Mismatch discrimination of miRCURY LNA™ microRNA Inhibitors. The pMIR-21 luciferase reporter is upregulated compared to the no-oligonucleotide control when HeLa cells are transfected with perfectly matched and mismatched (MM) antisense inhibitors targeting miR-21. There is a sharp drop in luciferase expression between the perfectly matched inhibitor and the single (1MM) and double (2MM) mismatched inhibitors, indicating that they are very specific and can distinguish between single nucleotide mismatches.
Exiqon Services

microRNA Services

Send us your samples and let us perform your microRNA experiments. We offer high-quality RNA isolation and microRNA profiling using microarray and qPCR. All experiments are performed by microRNA experts in state-of-the-art laboratories using the latest generation of Exiqon’s miRCURY LNA™ products.

At a glance
- Based on our highly specific and sensitive miRCURY LNA™ products
- Performed in state-of-the-art laboratory facilities ensuring excellent reproducibility
- Tailored to suit your research needs and budget
- Performed by highly skilled and experienced scientists and technicians
- Consult with our microRNA experts throughout the process
- Rigorous quality control in all steps
- Industry-leading service reports and support
- Preferred supplier for pharmaceutical companies

Exiqon’s highly skilled and talented team of researchers has extensive experience with microRNA profiling and data analysis. We perform all analyses in our state-of-the-art laboratory facilities using the latest generation of miRCURY LNA™ products ensuring unmatched specificity and sensitivity.

Contact Exiqon for a microRNA Service project tailored to your requirements and budget.

Overview of Exiqon Services.

History and benefits of Exiqon’s services
As the first commercial microRNA service provider, Exiqon Services has since 2006 profiled over 15,000 samples of various types and delivered high quality services to more than 1000 customers in pharma, biotech and academia.

We offer full microRNA profiling and our team takes pride in ensuring that you get the best service throughout the project; from initial consultation and tailored experimental setup to data analysis and scientific follow-up.

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.

miRCURY™ RNA Isolation Kits
- Preparation of high quality total RNA
- Clinical samples, biofluids, cell lines, and custom requests

MicroRNA Array profiling
- miRCURY LNA™ microRNA Arrays
- Rigorous quality control in all steps
- Industry-leading service reports and support

MicroRNA qPCR profiling
- miRCURY LNA™ Universal RT microRNA PCR
- Rigorous quality control
- Industry-leading service reports and support
- Ideal for samples with limited RNA content
Exiqon Services

RNA Isolation

Just send us your biological samples and we will prepare high quality total RNA suitable for microRNA profiling with array and PCR. 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 isolation and microRNA experts
- Standard Operating Procedure (SOP) protocols for optimal quality and efficiency
- Optimized protocol for samples with minute RNA content such as bio-fluids and FFPE samples
- High quality RNA extraction suitable for Exiqon’s microRNA Array and PCR Services
- Consultation with microRNA experts throughout the process

Consultation and sample submission

Exiqon can perform the initial RNA isolation for your microRNA array and PCR service projects. We can isolate high quality RNA suitable for microRNA research from many different sample sources.

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

If your samples are of animal origin then please remember to mention this when you contact us.

Sample handling and total RNA extraction

All RNA Isolation Service projects are carried out in our state-of-the-art service facilities. 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 if applicable.

RNA isolation from a broad range of sample types

We offer RNA isolation on the following types of samples:

- Serum/plasma samples and other bio-fluids
- Clinical tissue and FFPE samples
- Cell lines
- Other sample types upon request – for example blood PAXgene® blood RNA tubes

Superior for microRNAs in serum and plasma

Exiqon Services have vast experience with profiling of circulating microRNA in serum and plasma samples.

Learn more!

For more information and ordering please visit
www.exiqon.com/rna-isolation-services
Exiqon Services

microRNA microarray profiling

Exiqon offers a comprehensive microRNA profiling service based on our miRCURY LNA™ microRNA Arrays with unrivaled accuracy and sensitivity. Let our microRNA microarray experts perform your analysis in state-of-the-art automated laboratories with rigorous quality control, fast turn-around times and advanced data analysis 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 30 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 procedure and data analysis is optimized for each individual project

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 who will ensure that you get 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 47 shows the workflow for the microRNA 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 and experimental design
   Tailored experimental design to suit your research needs and budget

2. RNA sample submission
   Only a small amount of isolated RNA is needed for full microRNA profiling

3. RNA sample quality control (QC)
   RNA integrity, quantity and possible contaminations are assessed for each sample

4. Labeling, hybridization and scanning
   Highly efficient labeling, automated hybridization and highly sensitive scanning of arrays

5. Data analysis and normalization
   Technical and biological quality assessment along with a thorough data analysis appropriate for the experiment at hand

6. Report and final consultation
   The service project ends with an extensive report and follow up scientific discussion

Next, we complete a detailed sample submission form making sure that all experimental details and subsequent analyses are clearly defined and understood by both parties.

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 37).

Due to the sensitivity of our microarrays, we can perform analysis with low RNA input. We normally use between 30 - 1000ng 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. You will receive a report with the results of these analyses prior to the array profiling.

4. Labeling, hybridization and scanning

Following quality control, your RNA samples will be labeled using our new miRCURY LNA™ microRNA 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.

Arrays are scanned with highly sensitive equipment and image analysis is then performed to digitize the data. All projects include spike-in and negative control samples to determine background levels.

Let our microRNA 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. Analysis
After scanning the arrays, we perform a technical quality assessment of the data based on the results from the 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.

Highly reproducible results
We have invested heavily in our laboratory facilities to provide fast, high-throughput service. Our laboratories use Tecan HS4800™ Pro automated hybridization stations and Agilent G2505B Microarray Scanners operated in an ozone-free environment to avoid bleaching of the fluorescent labels.

This provides optimal conditions for performing single and dual color microRNA array profiling with a minimum of technical variation (Figure 49).

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. 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 can be organized in case you have questions to your final report or wish to discuss the significance of your results further.

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.

Figure 49. Exiqon’s miRCURY LNA™ microRNA Array platform generates highly reproducible results. The following experiments were repeated on two separate days: Total RNA from lung and brain was labeled in 10 separate reactions with Hy3™ and Hy5™, respectively. Pairs of labeled lung and brain RNA were hybridized on 10 arrays. Correlation coefficients for Hy3™ and Hy5™ signals (raw data) were calculated for all possible combination of pairs among the 20 arrays. The arrays were very reproducible with an average correlation coefficient of $R^2 = 0.987$. 
The microRNA Array Profiling report consists of:
RNA sample quality control (QC) report

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

A material 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 – and Box-plots

Learn more!
For more information and ordering please visit
www.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 PCR experts throughout the process
- System flexibility and sensitivity ensures cost-efficient experimental setup
- Experiments performed by expert scientists in state-of-the-art laboratories
- Rigorous quality control in all steps
- Normalization procedure optimized for each individual project

A complete microRNA 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 and easy-to-read final report with publication-grade illustrations.

Figure 50 shows the standard workflow for a microRNA PCR service project.

1. Consultation and experimental design
When you engage in a microRNA PCR 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. Following this we will complete a detailed sample-submission form making sure that all experimental details and subsequent analyses are clearly defined and understood by both parties.

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 universal RT reaction and 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 genome profiling and RNA corresponding to just 16μl plasma for profiling with our Serum/Plasma Focus microRNA PCR Panels.

A complete microRNA profiling service using Exiqon’s highly sensitive and specific microRNA PCR system - performed by those who know the system best. Our expert scientists perform your microRNA profiling in our state-of-the-art automated laboratories with rigorous quality control and maximum reproducibility tailored to your research needs and budget.
3. RNA sample quality control (QC)
After receiving your RNA samples our specialists can assess the integrity of each sample. In this case, we offer PCR-based QC tests to assess the performance of samples prior to microRNA qPCR profiling. Samples are tested in PCR assays for the amplification of selected microRNAs and 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 (see info box below).

4. microRNA qPCR
All PCR reactions are performed using our miRCURY LNA™ Universal RT microRNA PCR products which give superior experimental flexibility and allow you to identify and focus on the microRNAs that carry information: full genome profiling with miRNome Panels, Focus Serum/Plasma, Cancer, Toxicology Panels and custom-defined Pick-&-Mix Panels (see page 20).

Did you know?
Our miRCURY LNA™ Universal RT microRNA PCR system is ideally suited for the detection of microRNA in difficult samples such as FFPE and biofluids including serum and plasma.

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.

Did you know?
The flexibility of Exiqon’s qPCR system allows us to identify and focus on the microRNA that carry information.
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. Melt 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 analysis 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. 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 can be organized in case you have questions to your final report or wish to discuss the significance of your results further.

Did you know?

Exiqon’s microRNA PCR Services are well suited for biomarker development projects. Read about Exiqon’s biomarker discovery project and the benefits of our flexible PCR platform for biomarker studies on page 46.
The microRNA PCR Service report consists of:
RNA sample quality control (QC) report

An easy-to-read summary report
• Quality assessment of sample and data
• Plots showing the effect of normalization
• PCA plots and heat maps
• Heat maps based on unsupervised and supervised 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

A material and methods section
• Ready to use for publication purposes

Excel file with all raw data
• Raw and normalized Cq values
• Statistical analysis and heatmaps according to customer specifications

Remember!
Report examples are available online.
Contact us for more information (contact details page 49).

Learn more!
For more information and ordering please visit
www.exiqon.com/microRNA-pcr-services
Exiqon Services

Experts in microRNA biomarker discovery

The miRCURY LNA™ Universal RT microRNA PCR system offers excellent performance for difficult samples such as FFPE and serum/plasma.

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 microRNA qPCR platform (Table 3).

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 51.

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

Table 3. Experimental details of our colorectal cancer biomarker study.

<table>
<thead>
<tr>
<th>Experimental stage*</th>
<th>Study size</th>
<th>MicroRNAs studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genome wide screening</td>
<td>50 controls, 59 CRC patients</td>
<td>730 microRNAs screened</td>
</tr>
<tr>
<td>Identify set of candidate microRNAs</td>
<td>76 controls, 151 CRC patients</td>
<td>378 custom defined microRNAs screened</td>
</tr>
<tr>
<td>Validation set for microRNA signatures</td>
<td>1000 patients</td>
<td>Defined microRNA signature</td>
</tr>
</tbody>
</table>

* See Figure 51

Figure 51. Biomarker discovery workflow using Exiqon’s microRNA PCR system. Pilot genome-wide screenings on experimental groups with a limited number of individuals are performed with our Pre-defined miRNome 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 in custom Pick- &-Mix panels. Final biomarker validation can be performed on large groups of individuals with a small set of microRNA assays organized in custom Pick- &-Mix panels.
Our uniquely flexible PCR panel formats allow us to conduct cost effective biomarker screening projects by focusing only on the microRNAs with biomarker potential.
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 50 and 51.

1. Finding a product number
We recommend visiting our website for the latest update 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.

2. 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: +1 718 376 4152
Rest of the World: +45 4566 1888

Order by Phone:
To place an order by phone, call:
North America: +1 718 376 4150
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.

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.
Contact us

Outside North America

Business hours
8:30 a.m. - 4:30 p.m.
Central European Time

Mailing address
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