

microRNA profiling from exosomes

From biofluid sample to data analysis

**All you need for successful microRNA profiling from exosomes.
Get a complete microRNA profile from your biofluid sample in
just one day.**

- Exosome isolation available for most biofluids including serum, plasma, CSF and urine
- Fast 2 hour exosome isolation protocol with excellent exosome recovery
- microRNA profiling using high-performance miRCURY LNA™ microRNA PCR panels

A complete work-package for microRNA profiling of exosomes

Exosomes are important for many cellular processes and disease states, which make them interesting targets for biomarker studies. Among the biomolecules found in exosomes, microRNAs are the most promising candidates for biomarker use. However, the small amounts of microRNAs available in biofluids make them challenging targets.

Exiqon has extensive experience in microRNA profiling from biofluids. Our RNA isolation kits offer excellent performance coupled with a fast and convenient workflow and our LNA™-enhanced microRNA qPCR system has taken microRNA PCR sensitivity and specificity to a whole new level.

Recently, we have added exosome isolation kits to our line of products. They offer a fast and easy way to purify exosomes from a wide range of biofluids with minimal loss. With this addition, we now offer a complete work-package for microRNA profiling of exosomes:

- Exosome isolation using a miRCURY™ Exosome Isolation Kit
- RNA isolation using a miRCURY™ RNA Isolation Kit
- microRNA qPCR profiling using a miRCURY LNA™ Universal RT microRNA PCR panel

1. Exosome isolation

2. RNA isolation

3. microRNA qPCR

1. Exosome isolation

The first step of an exosome microRNA profiling experiment is isolating the exosomes. The miRCURY™ Isolation Kits for exosome isolation are optimized for efficient isolation of exosomes from biofluids and for seamless integration with the miRCURY™ RNA Isolation Kits. Exosomes can be isolated from various biofluids such as cell conditioned media, urine, CSF, serum or plasma.

We currently offer two exosome isolation kits:

- **miRCURY™ Exosome Isolation Kit – Serum and plasma**
- **miRCURY™ Exosome Isolation Kit – Cells, urine and CSF**

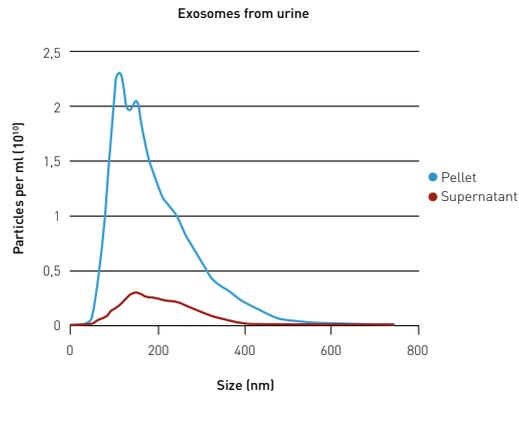
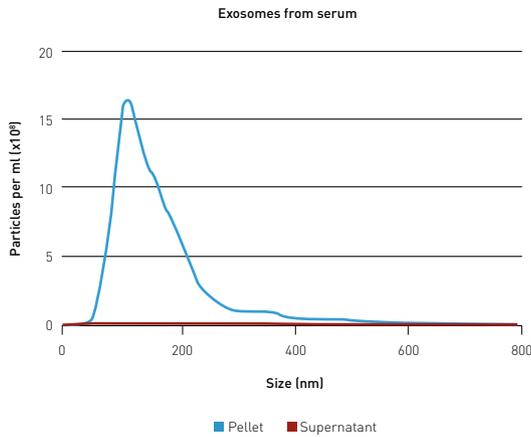
Both kits are designed to efficiently isolate exosomes in just two hours without any ultracentrifugation or phenol/chloroform steps.

An added benefit of the kits is their ability to concentrate samples that normally contain very small amounts of microRNA, such as urine or CSF. This allows the user to start with higher volumes than would normally be possible.

The isolated exosomes can be studied further by use of functional or antigenic assays as well as microscopic methods or NanoSight® measurements to characterize size distribution or membrane components. For microRNA profiling, the RNA from the exosomes will need to be purified using a method that preserves small RNAs, such as a miRCURY™ RNA Isolation kit.

Product	Product no.
miRCURY™ Exosome Isolation Kit - Serum and Plasma	300101
miRCURY™ Exosome Isolation Kit - Cells, urine and CSF	300111

Read more at: www.exiqon.com/exosome-isolation-kits



High recovery rate of exosomes from biofluids. NanoSight® measurements from exosomes isolated from serum (left) and urine (right) show that the vast majority of the exosomes are found in the pellets and only tiny amounts are lost to the supernatant. Data kindly provided by Ken Howard, NanoPharmaceutical Lab, iNANO, Aarhus, Denmark.

2. RNA isolation from exosomes

The miRCURY™ RNA Isolation Kits are ideally suited to isolate RNA from exosomes extracted using the miRCURY™ Exosome Isolation Kits. These kits offer a fast and convenient way of purifying high quality total RNA without the use of toxic phenol and chloroform. The whole process takes as little as 20 minutes.

Product	Product no.
miRCURY™ RNA Isolation Kit - Cell & Plant (50)	300110
miRCURY™ RNA Isolation Kit - Biofluids (50)	300112

Two kits are available:

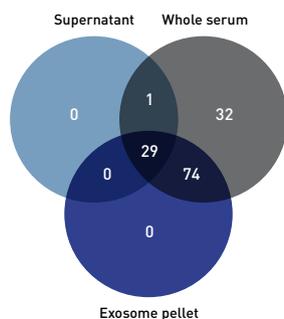
- **miRCURY™ RNA Isolation Kit - Cell & Plant:** Use this kit for RNA extraction of exosomes derived from cells, urine and CSF.
- **miRCURY™ RNA Isolation Kit - Biofluids:** Use this kit for RNA extraction of serum/plasma derived exosomes.

Read more at: www.exiqon.com/rna-isolation

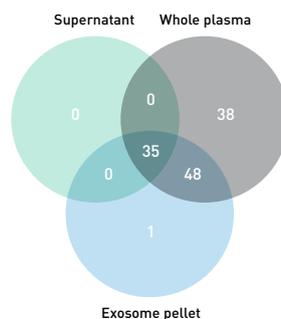
Best-in-class RNA isolation

In a study comparing Exiqon's biofluid kit to competing commercial kits, it was found that Exiqon's kit offers the best combination of performance and ease-of-use. Reference: McAlexander *et al.* Front. Genet. 2013.

(A) Serum

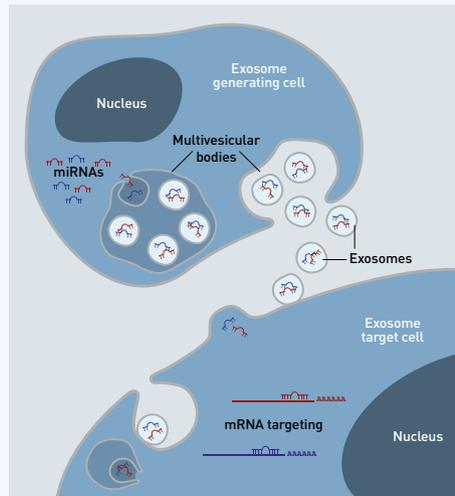


(B) Plasma



microRNA contents in sub-fractions of serum and plasma. This Venn diagram shows the number of microRNAs in serum (A) and plasma (B) found in the pellet and the supernatant after exosome isolation, compared to the number found before extraction.

What are exosomes?

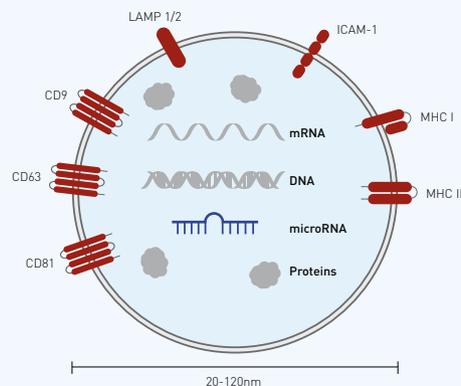


Exosomes are cell-derived membranous particles ranging in size from 20 to 120 nm, approximately the same size as viruses but considerably smaller than microvesicles. They are excreted from cells into the surrounding media and can be found in many if not all body fluids. Exosomes are believed to function as intercellular hormone-like messengers, transferring biomolecules such as proteins and RNA between cells.

Exosomes are believed to be involved in a number of important functions, including:

- Immune regulation
- Blood coagulation
- Cell migration
- Cell differentiation
- Cell-to-cell communication

The importance of exosomes for normal cell function and disease progression has made them tempting targets for use as non-invasive biomarkers.



Workflow for microRNA profiling from exosomes

Time	Cultured cells, urine, CSF or other biofluids	Serum or Plasma
2 hours	miRCURY™ Exosome Isolation Kit - Cells, urine and CSF	miRCURY™ Exosome Isolation Kit - Serum and Plasma
20 minutes	miRCURY™ RNA Isolation Kit - Cell and Plant	miRCURY™ RNA Isolation Kit - Biofluids
3 hours	miRCURY LNA™ Universal RT microRNA PCR	

3. microRNA profiling of exosomes

Once your RNA has been purified, it is time for microRNA expression profiling. Exiqon's miRCURY LNA™ Universal RT microRNA PCR system offers excellent sensitivity and specificity for the low RNA levels available from biofluids. Get a complete profile in just 3 hours without the need of a pre-amplification step. The system is available in individual assay format or in a wide range of panels, including miRNome panels, Focus panels and custom Pick-&-Mix panels. The panels include reference genes and controls and are compatible with most real-time PCR instruments.

For the initial screening, we recommend using a microRNA Ready-to-Use PCR miRNome panel or a dedicated focus panel covering microRNAs typically found in exosomes from urine, cerebrospinal fluid or serum/plasma.

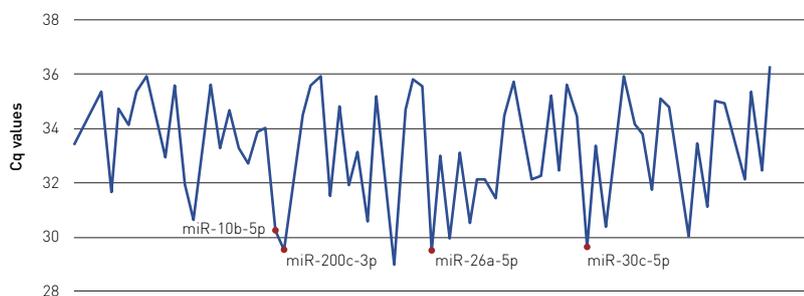
Analyze your qPCR data using the Exiqon GenEx qPCR software suite. It contains powerful pre-processing and statistical tools as well as fast and convenient wizards for data import from Exiqon's qPCR panels. Download a free trial from our website to get started.

Product	Product no.
Universal cDNA Synthesis Kit II, 8-64 rxns	203301
Exilent SYBR® Green Master mix, 20 ml	203420
Exilent SYBR® Green Master mix, 2.5 ml	203402
microRNA Ready-to-Use PCR, Human panel I+II, V3	203611-12
Urine Exosome Focus microRNA PCR Panels	Varies
CSF Exosome Focus microRNA PCR Panels	Varies
Serum/Plasma Focus microRNA PCR Panels	203836-43
Exiqon GenEx 6 Pro	207013-4
Exiqon GenEx 6 Enterprise	207015-6

Read more at: www.exiqon.com/mirna-pcr

Independent study recommends Exiqon's qPCR system

In a study comparing commercial microRNA profiling products, Exiqon's system was found to offer better sensitivity and linearity than competing technologies. Reference: Jensen *et al.* BMC Genomics 2011, 12:435.



Urine microRNA profile. Cq values of microRNAs isolated from 5 mL normal urine supernatant using miRCURY™ Exosome Isolation Kit – Cells, urine and CSF followed by an RNA extraction using miRCURY RNA Isolation Kit – Cell and Plant. Samples were profiled on a Urine Exosome Focus microRNA PCR Panel.

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