miRCURY LNA™ Universal RT microRNA PCR

MicroRNA profiling in blood serum and plasma

At a glance

- Optimal coverage – Most comprehensive platform on the market for focused microRNA profiling in serum/plasma
- Excellent sensitivity – Profile all microRNAs on the panels using just 20 µl serum/plasma
- Exceptional accuracy – Provides the sensitivity and specificity required for reliable detection of microRNAs starting at just 5 microRNA copies - ideal for serum/plasma and other cell-free samples
- Proven track record – experimentally validated on thousands of clinical serum/plasma samples
- Compatibility – 96 and 384 well formats compatible with most real-time PCR instruments
- Fast and easy – Go from samples to results in just 3 hours without the need to pre-amplify

Why study microRNAs in serum and plasma?

MicroRNAs are present in small amounts in blood serum and plasma. They circulate in the blood in a highly stable, cell-free form, suggesting that they are protected from the RNA-degrading enzymes found in blood.

MicroRNA profiles have been shown to be altered in serum and plasma taken from patients suffering from cancers and many diseases (Figure 1). This makes microRNAs promising candidates for use as non-invasive biomarkers for a wide range of diseases, toxicology or injury studies. Blood samples are easy to obtain and carry little risk to the patient compared to biopsies and surgical procedures.

Challenges of microRNA profiling in serum and plasma

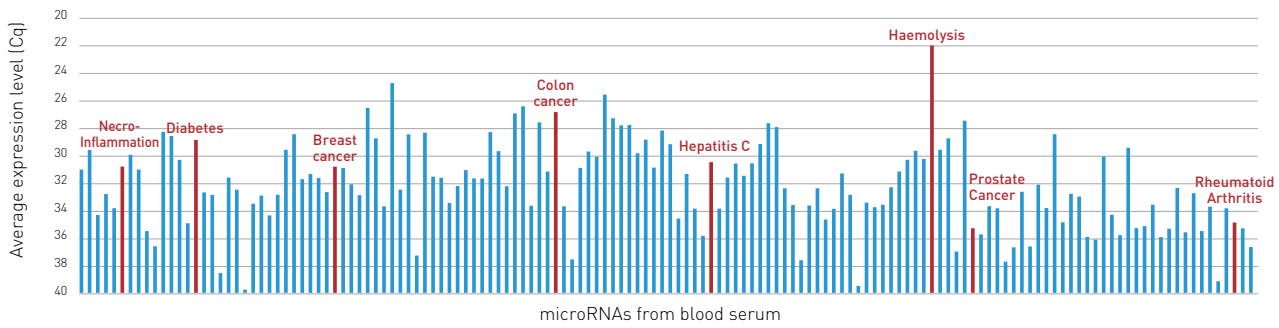
Serum and plasma only contain a fraction of the RNA found in whole blood. Most of the RNA is found in the buffy coat, which mainly consists of white blood cells and platelets. In order to get accurate microRNA profiles, it is important to ensure that the serum/plasma is not contaminated by RNA from blood cells as this will skew the results of the experiment.

There are several other challenges when it comes to profiling microRNAs in serum and plasma:

- Small amounts of RNA means that standard ways of RNA quality control cannot be used
- RNA from serum/plasma contains inhibitors of PCR enzymes
- Standard reference genes, such as U6, are not present in serum and plasma, which makes choosing good reference genes difficult

In order to overcome these challenges, it is important to use an extremely accurate microRNA qPCR system. The miRCURY LNA™ Universal RT microRNA PCR system has a proven track record of being very reliable for the detection of microRNAs in serum and plasma samples.

Figure 1. Serum microRNA biomarkers. Normal reference range for human serum profile with microRNA disease associated biomarkers highlighted in red.



LNA™

EXIQON
Seek Find Verify

A unique system for microRNA profiling

miRCURY LNA™ Universal RT microRNA PCR offers the best available combination of performance and ease-of-use in the microRNA real-time PCR market because it unites two important features:

- 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 in the laboratory
- LNA™ PCR amplification – Both PCR amplification primers (forward and reverse) are microRNA specific and optimized with LNA™. The result is 1) exceptional sensitivity as well as extremely low background enabling accurate quantitation of very low microRNA levels and 2) highly specific assays that allow discrimination between closely related microRNA sequences

Fast, easy and reproducible

Save time and effort in the laboratory with the 3 hour easy-to-follow protocol (Figure 2). 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. The number of pipetting steps is reduced to a minimum and technical variation is minimized. As a result, it is possible to achieve extremely high reproducibility from day-to-day and even site-to-site (Figure 3).

Product coverage

Exiqon's Serum/Plasma Focus microRNA PCR Panels are available in two formats:

- **96 well, Ready-to-Use** - Two 96-well PCR plates with a total of 168 LNA™ microRNA primer sets commonly found in human serum/plasma and 7 reference microRNAs. Compatible with most real-time PCR instruments
- **384 well, Ready-to-Use** - One 384-well PCR plate with 168 LNA™ microRNA primer sets commonly found in human serum/plasma and 7 reference microRNAs per plate. Compatible with most real-time PCR instruments

The plates are produced in Ready-to-use format which means that the primer sets are dispensed and lyophilized in the well in an amount sufficient for one 10 µL reaction per well.

Control assays

Finding good reference genes in serum and plasma samples can be challenging. Some of the common house-keeping genes such as U6 are not present in these samples and the overall RNA concentration is low. Exiqon's Serum/Plasma Focus microRNA PCR Panels contain 7 potential reference genes:

- miR-103, miR-191 and miR-423-5p. These are potential reference genes found on our miRNome panels and can be used by researchers migrating to the focus panels
- miR-93, miR-425. These potential reference genes are chosen as they are usually stably expressed in serum/plasma
- miR-451, miR-16. These microRNAs can be used as a control for hemolysis. If these microRNAs are found to be highly expressed it may be an indication of contamination of the serum/plasma samples

Exiqon Services

Exiqon has extensive in-house knowledge of microRNA profiling in serum and plasma. All 175 miRCURY LNA™ Universal RT 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 peer-reviewed published papers.

We offer comprehensive microRNA PCR Services for both serum and plasma focused panel experiments. We also offer RNA isolation from serum or plasma using a proprietary optimized protocol.

For more information go to
www.exiqon.com/services

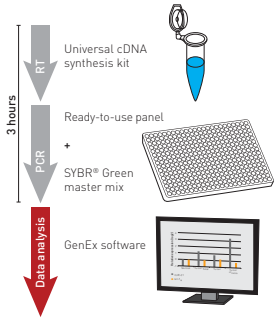


Figure 2. Overview of the miRCURY LNA™ Universal RT PCR workflow. The PCR primer sets have been designed for optimal performance when used with the miRCURY LNA™ SYBR® Green master mix. Use of other master mixes might affect the quality of the results. Ready-to-use panels can be replaced by individual PCR primer sets in this workflow.

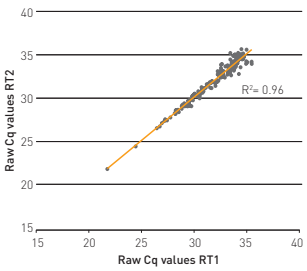


Figure 3. Excellent reproducibility between RT reactions on total RNA from serum. Raw Cq values from two separate RT reactions (RT1 and RT2) on total RNA purified from 65µl serum are shown. A total of 730 microRNAs were profiled. Only microRNAs with Cq values below 35 have been included (133 datapoints).

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