

MicroRNA profiling in blood serum and plasma

miRCURY LNA™ Universal RT microRNA PCR

At a glance

- Profiling of up to 742 human microRNAs using only 40ng total RNA – no pre-amplification necessary
- Highly sensitive – suitable for serum/plasma, FFPE material and other challenging samples. Reliable quantitation from as little as 1pg total RNA
- Superior specificity – LNA™-enhanced primers enable specific quantification of microRNAs that differ by only one nucleotide
- Easy to use – protocol completed in 3 hours
- Step-by-step data analysis solution

MicroRNA profiling from blood serum and plasma

MicroRNAs are emerging as a new class of blood-based biomarkers. However, their small size and the limiting amounts of sample available, present a significant challenge for the sensitivity of the detection system. The unmatched sensitivity of miRCURY LNA™ Universal RT microRNA PCR system makes it possible to profile up to 742 microRNAs from just 40ng total RNA. This enables high quality microRNA expression analysis in samples that contain very little total RNA, such as serum or plasma (figure 1). Pre-amplification of the cDNA is not required.

Individual microRNAs may be accurately and reliably quantified from just 1pg total RNA starting material, allowing investigation of microRNA signatures in very small amounts of serum and plasma.

A unique system for microRNA profiling

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

1. 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.
2. LNA™ enhanced PCR – 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 quantification of very low microRNA levels and 2) highly specific assays that allow discrimination between closely related microRNA sequences.

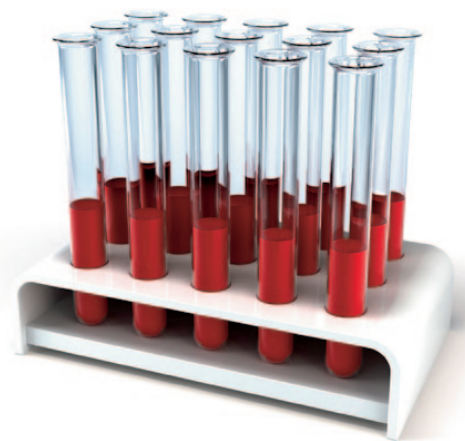


Figure 1

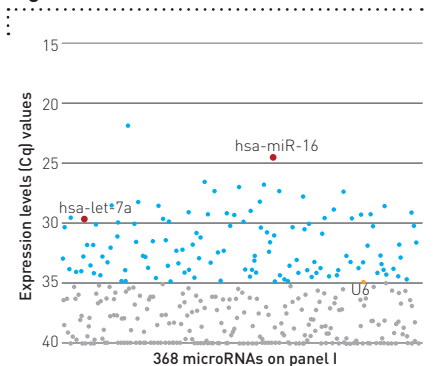


Figure 1. Expression profiling of 368 microRNAs using total RNA from 35µl serum. Real-time PCR was performed using triplicate RT reactions on total RNA purified from serum. Average Cq (quantification cycle) values from the microRNA ready-to-use PCR panel I are shown (368 microRNAs). Over 120 microRNAs showed robust expression with Cq values below 35. hsa-miR-16 (known to be highly expressed in serum/plasma) and hsa-let-7a are indicated in red. The U6 reference gene is indicated in orange.

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. The ready-to-use microRNA PCR panels enable simple and convenient expression profiling. 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 even from challenging samples such as serum or plasma (figure 2).

Product coverage

We offer solutions for both large-scale microRNA expression profiling and for quantification of individual microRNAs:

- Ready-to-use panels – contain pre-aliquoted PCR primer sets in 384-well PCR plates. Just add cDNA and PCR master mix to the wells and run your real-time PCR profiling. Two panels covering 742 human microRNAs are available. Six reference genes, one control primer set and triplicate inter-plate calibrators are included in all panels. The panels are compatible with most real-time PCR instruments.
- Individual assays – validated real-time PCR primer sets for quantification of about 742 human microRNAs and 9 endogenous reference genes are available.
- Other reagents needed for first-strand cDNA synthesis and real-time PCR: miRCURY LNA™ Universal RT cDNA synthesis kit
miRCURY LNA™ SYBR® Green master mix.
- NEW: Create your own panels of PCR assays for specific microRNA signature screening
- NEW: Exiqon GenEx software for easy and comprehensive data analysis.

See figure 3 for an overview of the miRCURY LNA™ Universal RT microRNA PCR products.

Exiqon Services

We offer comprehensive microRNA PCR Services for both large-scale microRNA expression profiling experiments and for quantification of individual microRNAs. We also offer RNA Isolation from serum or plasma samples using a proprietary optimized protocol.

For more information and to order products, please go to www.exiqon.com/mirna-pcr.

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Concerning miRCURY LNA™ Universal RT microRNA PCR:

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For life science research use only. Not for use in diagnostic procedures.

Figure 2

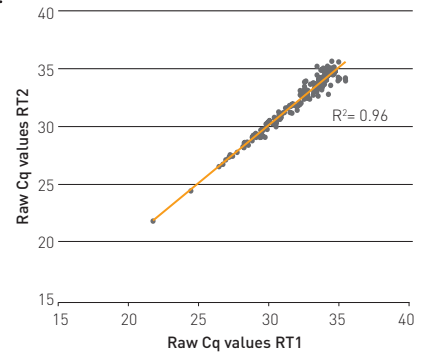


Figure 2. Excellent reproducibility between RT reactions on total RNA from serum.

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).

Figure 3

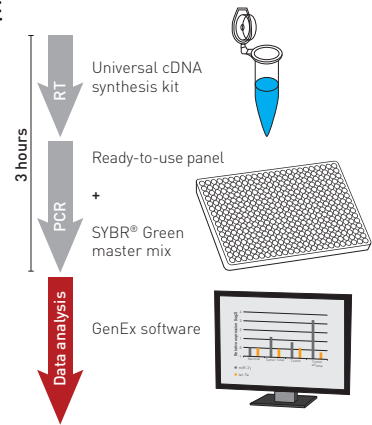


Figure 3. Overview of the miRCURY LNA™ Universal RT PCR workflow.

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.