

Quick-guide for Serum/plasma Focus microRNA PCR panels

(per panel, 2 x 96-well plates or ½ x 384-well plate)

Before starting the experiment

Please note that this quick-guide refers to our Serum/plasma Instruction manual.

We recommend mixing your reagents with excess volume for pipetting. Typically, 10–25% excess is needed, depending on pipetting system. **Keep all reagents on ice while setting up.**

Phase I: prepare RNA sample (see Tips section, page 55–57 for tips).

Phase II (Steps 1–5): cDNA synthesis (see page 32 for details).

- For each sample, prepare and mix:

Reagent	Serum/plasma Focus Panel, Vol (µl)
5x Reaction buffer	4
Nuclease-free water	9
Enzyme mix	2
Synthetic spike in, optional replace with H ₂ O if omitted	1
Template total RNA [†]	4
Total volume	20

[†] The equivalent of 16 µl original serum/plasma is used per 20 µl reverse transcription reaction.

Incubate for **60 min at 42°C** followed by heat-inactivation of the reverse transcriptase for 5 min at 95°C. Immediately cool to 4°C. If not used immediately, store at 4°C or freeze.

Phase III (Steps 6–8): real-time PCR amplification (see page 34 for details).

- Immediately before use, dilute your cDNA 50x: 980 µl water and 20 µl cDNA, and add 1000 µl 2x SYBR® Green master mix. Add passive reference dye if recommended by instrument manufacturer.
- Dispense 10 µl to each well of the 96- or 384-well PCR plate(s) (two samples to a 384-well plate).
- Spin plate briefly in cooled centrifuge, wait 5 minutes while primers dissolve.
- Insert plate in cyclor, and run according to the following settings:

Polymerase Activation/Denaturation	95°C, 10 min
40 amplification cycles*	95°C, 10 s 60°C, 1 min, ramp-rate 1.6°C/s (100% standard on ABI instruments)
Melting curve analysis	Optical read

*45 amplification cycles are required for LC480 instruments to allow collection of data for Cp values up to 40.

Phase IV (Step 9): Data analysis

ABI instruments: use manual baseline and threshold settings (see Tip 10, page 59).

Workflow for Serum/plasma Focus microRNA PCR panels (per sample)

Phase I: Prepare RNA sample

See protocol page 55 for recommendations



Phase II: cDNA synthesis

See protocol page 32.

- Triplicate RT per sample is recommended
- a no enzyme RT negative control per study is recommended

Illustration for a 96-well plate set-up

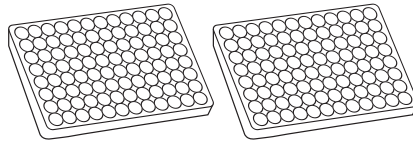


Phase III: real-time PCR amplification

See protocol page 34.

- Mix cDNAs with SYBR® Green
- Add cDNA:SYBR® Green mix to PCR plates

ROX: The SYBR® Green master mix, Universal RT does not include the ROX passive reference dye. Add as needed.

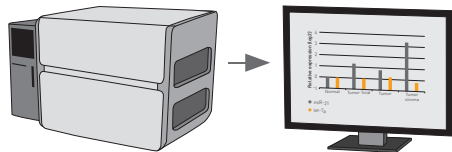


Phase IV: Data analysis

Export data for further analysis:

- See data analysis guide online
- Data pre-processing, normalization and statistical analysis

ABI 7900 and 7900 HT: sds template files with pre-defined plate layout, cycling conditions and analysis settings are available at www.exiqon.com/sds.



exiqon.com/mirna-pcr

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