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. Add this volume to the table volumes.
Keep all reagents on ice while setting up.

Phase I: prepare RNA sample (see Tips section, page 57-58 for tips).
Phase II (Steps 1-5): cDNA synthesis (see page 34 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 RNA spike ins, optional replace with H2O if omitted	1
Template total RNA[1)]	4
Total volume	20

[1)] 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 36 for details).

- Immediately before use, dilute your cDNA 50x: 980 µl water and 20 µl cDNA, and add 1000 µL 2x PCR 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 cycler, and run according to the following settings:

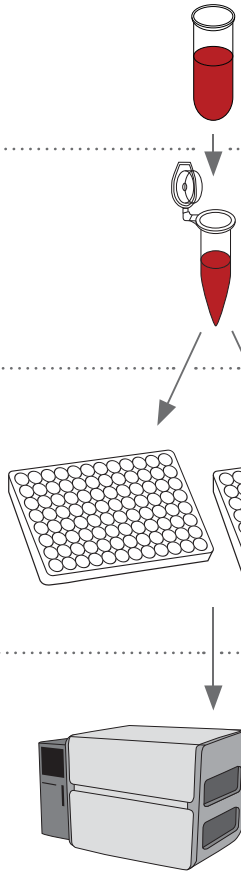
Polymerase Activation/Denaturation	95˚C, 10 min
40 amplification cycles*	95˚C, 10 s 60˚C, 1 min, ramp-rate 1.6˚C/s (100% standard on ABI instruments) Optical read
Melting curve analysis	

*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 61).

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



Phase I: Prepare RNA sample
See protocol page 57 for recommendations

Phase II: cDNA synthesis
See protocol page 34.
- 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 36.
- Mix cDNAs with PCR Master mix
- Add cDNA:PCR Master mix to PCR plates

ROX: The ExiLENT 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 7500, 7500FAST, StepOne Plus, 7900 and 7900 HT: 96- and 384-well sds template files with pre-defined cycling conditions and analysis settings are available at www.exiqon.com/sds.

exiqon.com/mirna-pcr

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