

Quick-guide for Human and Mouse&Rat microRNA PCR panels (per plate)

Before starting the experiment

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 51-52 for tips).

Phase II (Steps 1-5): cDNA synthesis (see page 30 for details).

- Adjust each template RNA to 5 ng/μl
- For each sample, prepare and mix:

Reagent	Panel I Volume (μl)	Panel I+II Volume (μl)
5x Reaction buffer	4	8
Nuclease-free water	9	18
Enzyme mix	2	4
Synthetic spike in, optional replace with H ₂ O if omitted	1	2
Template total RNA (5 ng/μl)	4	8
Total volume	20	40

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 32 for details).

- Immediately before use, dilute your cDNA 100x add 1980 μL H₂O pr 20μL RT. Add passive reference dye if recommended by instrument manufacturer.
- Mix 2x SYBR® Green MasterMix and diluted cDNA 1:1.
- Dispense 10 μl to each well of the 384-well PCR 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)
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 55).



Workflow for Human and Mouse&Rat microRNA PCR panels (per sample)

Phase I: Prepare RNA sample

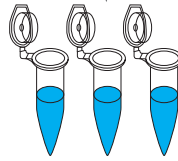
See protocol page 51 for recommendations



Phase II: cDNA synthesis

See protocol page 30.

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

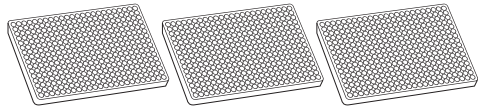


Phase III: real-time PCR amplification

See protocol page 32.

- 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.

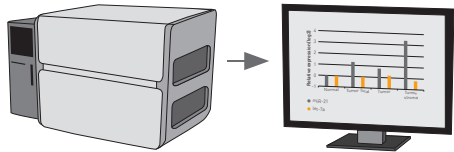


Phase IV: Data analysis

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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