

# Quick-guide for Cancer Focus microRNA PCR panels

(per panel, 1x 96-well plate or 1/4x 384-well 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 37 for details).

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

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

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 39 for details).

- Immediately before use, dilute your cDNA 100x: 495 μl water and 5 μl cDNA, and add 500 μl 2x SYBR® Green master mix. Add passive reference dye if recommended by instrument manufacturer.
- Dispense 10 μl to each well of the focus panel.
- 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 55).



## Workflow for Cancer Focus microRNA PCR panels (per sample)

### Phase I: Prepare RNA sample

See protocol page 51 for recommendations



### Phase II: cDNA synthesis

See protocol page 37.

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

*Illustration for a 96-well plate set-up*

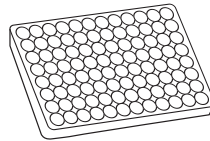


### Phase III: real-time PCR amplification

See protocol page 39.

- 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.**

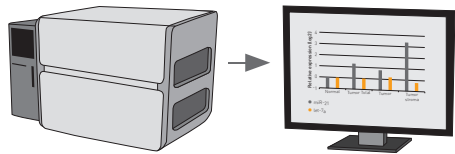


### Phase IV: Data analysis

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](http://www.exiqon.com/sds).



[exiqon.com/mirna-pcr](http://exiqon.com/mirna-pcr)

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