

### Reverse transcription (RT)

Reverse transcription (RT) reactions are performed using TaqMan miRNA Reverse Transcription Kit (Applied Biosystems, Germany) and miRNA-specific RT primers from Applied Biosystems, Germany.

Reactions are carried out in a scaled-down multiplex (4 target miRs) reaction with final volume of 7.5  $\mu$ l.

Reagent:		Volume ( $\mu$ l)
RNase/DNase-free water		3,08
10x RT-buffer		0,75
100mM dNTPs		0,075
RT-primers (n=4)	(0,5 $\mu$ l for each miR)	2
RNase-Inhibitor		0,095
Multiscribe RTase		0,5
miRNA sample		1

The number of miRs in the multiplex reaction is variable. If you add another target miR, then deduct this volume from the volume of water and vice versa. Also, you can add more (mi)RNA template (if you expect to have low RNA concentrations)...

RT was carried out in a thermal cycler under the following conditions: 16°C for 30 min, 42°C for 30 min and 85°C for 5 min, followed by a hold at 4°C.

### Real-time PCR

TaqMan real-time PCR reactions are performed in triplicates in scaled-down reactions comprising 2.5  $\mu$ L TaqMan2x Universal PCR Master Mix with No AmpEraseUNG (Applied Biosystems, Germany), 0.25  $\mu$ L 20x miRNA-specific primer/probe mix (Applied Biosystems, Germany) and 2.25  $\mu$ L of the reverse transcription product (diluted 1:5).

Real-time PCR is carried out in a LightCycler 480 thermal cycler (Roche, Germany) under the following conditions: 95°C for 10 min, then 40 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s, followed by a hold at 4°C.