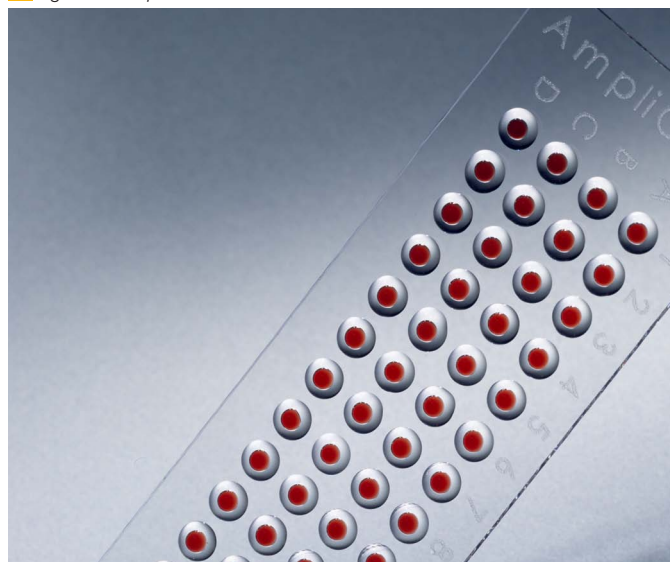


# TaqMan® microRNA Assay on single cells using AmpliGrid AG480F

This process instruction describes an experiment using the TaqMan® microRNA Assay (Applied Biosystems) together with the AmpliGrid slide.

1 Figure 1: AmpliGrid AG480F



## Material

- Template: single HeLa cells (human HPV18 positive epithelial cells from a cervix carcinoma) deposited on the AmpliGrid AG480F (Figure 1, Advantix) - stained with Hoechst dye
- Positive control RNA (total extracted RNA from HeLa cells)
- TaqMan® MicroRNA Reverse Transcription Kit, Applied Biosystems cat no: PN 4366596
- TaqMan® 2x Universal PCR Master Mix, No AmpErase® UNG (qPCR) Applied Biosystems cat no: PN 4324018
- TaqMan® MicroRNA Assay miR-24, Applied Biosystems cat no: 4373072 (includes 5x primer for RT and 20x primer for qPCR including the FAM-labeled probe)
- Sealing Solution (Advantix, e.g. OAX04207)
- AmpliSpeed slide cycler (Advantix, e.g. OAX04101)
- Electronic multistep pipette

## Notes

- Perform all steps on ice.
- Thaw all reagents on ice.
- The miRNA Assay must be carefully thawed on ice and afterwards aliquoted. Aliquots should be stored frozen until use and should be used only once when thawed.

- RT primers are used in a final concentration of 0.5fold.

## Protocol

### RNA TEMPLATE

- Prepare RNA solution of 10 ng/μL RNA (storable at -80°C)

### SINGLE CELL TEMPLATE

- Check Hoechst dye stained cells with cell detection system at the fluorescent microscope in order to verify their presence on the reaction sites

### REVERSE TRANSCRIPTION

- Prepare master mix in a fresh PCR-clean tube according to table A when using total RNA as template or to table B when using single cells as template.
- Make sure to prepare enough master mix for all the reactions, taking pipetting errors into account (dead volume of electronic multistep pipette about 10 μL)

A Table A: reverse transcription master mix using total RNA as template

Component	1 reaction
dNTP (100 mM)	0.01 μL
10x RT buffer	0.1 μL
RNase inhibitor	0.01 μL
5x RT primer	0.1 μL
Reverse transcriptase	0.07 μL
RNA (10 ng/μL)	0.1 μL
Nuclease free water	0.61 μL
<b>Total Volume</b>	<b>1 μL</b>

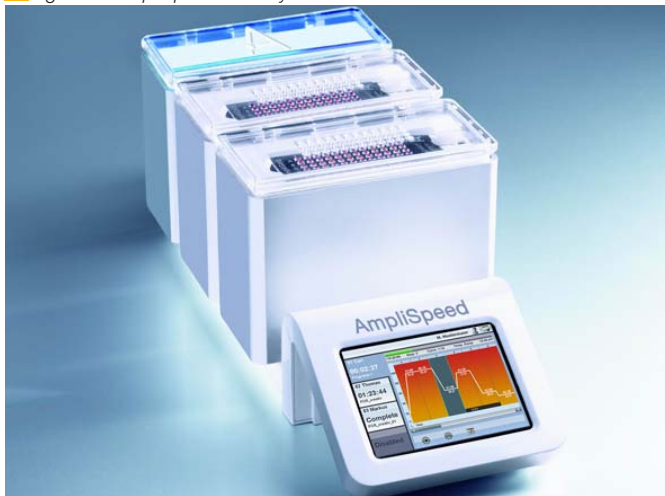
B Table B: reverse transcription master mix using single cells as template

Component	1 reaction
dNTP (100 mM)	0.01 μL
10x RT buffer	0.1 μL
RNase inhibitor	0.01 μL
5x RT primer	0.1 μL
Reverse transcriptase	0.07 μL
Nuclease free water	0.71 μL
<b>Total Volume</b>	<b>1 μL</b>

- Incubate the RT master mix on ice for 3 min.
- Add 1 μL of RT mix onto one reaction site of the AmpliGrid (on ice/cooled) and immediately cover with 5 μL sealing solution.
- Incubate the slide for 30 min at room temperature.

- Transfer the AmpliGrid onto the AmpliSpeed slide cycler (Figure 2) and incubate further 30 min at 50°C.

**2** Figure 2: AmpliSpeed slide cycler ASC200D



**qPCR MASTER MIX**

- Prepare the qPCR master mix as described in table C on ice.

**C** Table C: qPCR master mix

Reagent	1 reaction
20x TaqMan® MicroRNA Assay	1 µL
2x TaqMan® Universal PCR master mix	10 µL
Nuclease free water	8 µL
<b>Total Volume</b>	<b>19 µL</b>

- Pipette 19 µL qPCR master mix in each qPCR tube.
- In each well add 1 µL sample out of reverse transcription to the qPCR mix.
- Vortex gently and centrifuge the plate shortly to collect the solution at the bottom.
- Start qPCR program according to table D (use FAM filter for quantitative measurement)

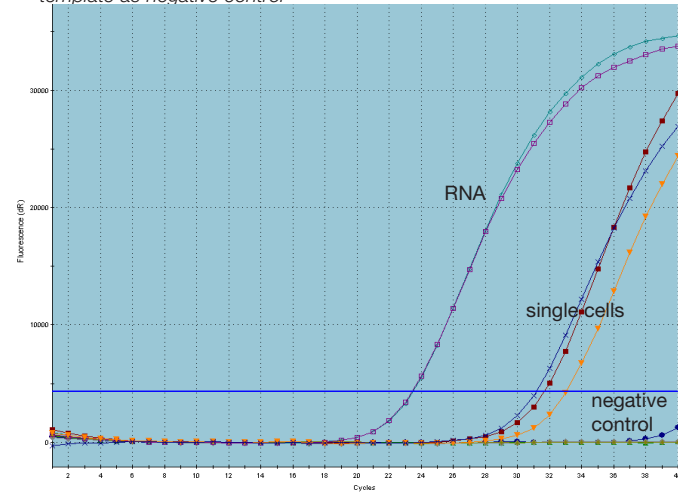
**D** Table D: qPCR program

Temperature	Time	Cycle
95°C	10 min	
95°C	15 sec	40 cycles
60°C	1 min	

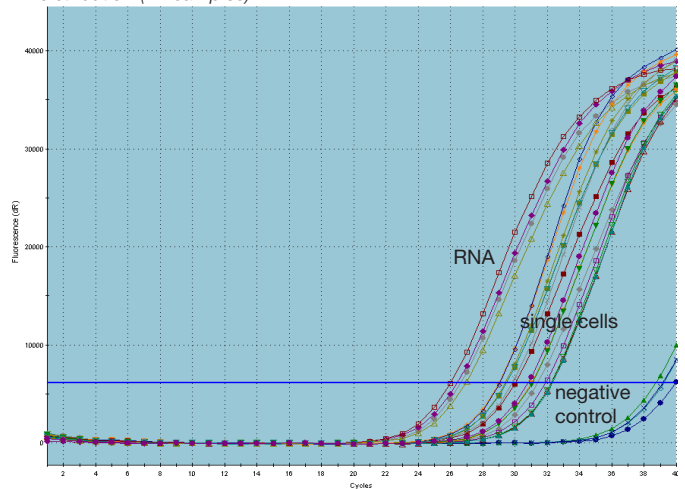
**Analysis**

- Analyse data using the real-time cycler software

**3** Figure 3: Example 1: experiment performed on single cells (three independent single cell reactions); extracted RNA as positive and no template as negative control



**4** Figure 4: Example 2: experiment performed on single cells; curves of samples with single cells (dependent on cell cycle) show normal distribution (12 samples)



As shown in Figure 3 and 4, the microRNA “miR-24” can be amplified in single HeLa cells using a TaqMan® microRNA assay on the AmpliGrid system.

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