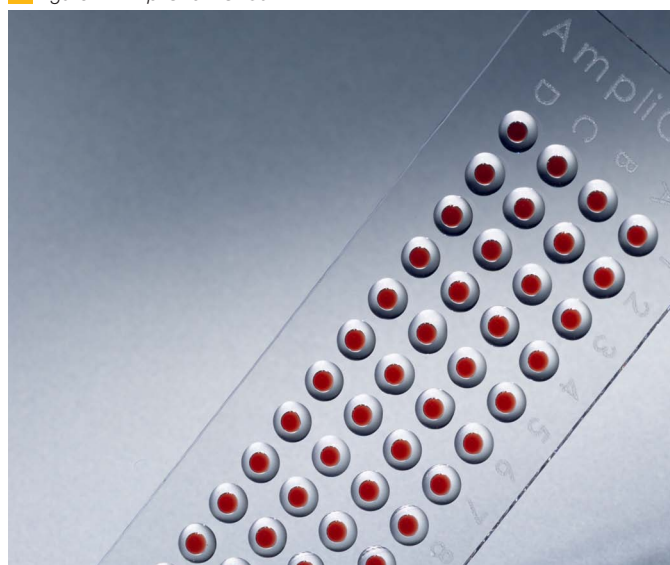


RT-PCR on human total RNA and single cells using AmpliGrid AG480F

This process instruction describes a **One-Step RT-PCR** for human lymphocytes or human total RNA. In this RT-PCR system, b2m and Calm fragments are amplified using the Single Cell One-Step RT-PCR kit together with AmpliGrid slides.

1 Figure 1: AmpliGrid AG480F



Material

PCR

- Template: human total RNA or single human lymphocytes (staining with Hoechst dye recommended) deposited on the reaction sites of an AmpliGrid 480F
- Fluorescence microscope with DAPI filter
- AmpliGrid AG480F incl. sealing solution (Advalytix, e.g. OAX04503)
- Primer: (product size)
 - Calm (189bp)
 - b2m (139bp)
 - GAPDH (110bp)
- RT-PCR Primermix containing 10 μ M of primer each.

Each Primer Set can be used separately (singleplex) or combined as multiplex.
- Single Cell One-Step RT-PCR Kit (Advalytix, OAX04515)
- Optional: DNase digest reagents

NOTE: Please refer to the Product Information of the Single Cell One-Step RT-PCR system.
- RNasin® Ribonuclease Inhibitor 40 U/ μ l (Promega)

- AmpliSpeed slide cycler (Advalytix, e.g. OAX04101)
- Electronic multistep pipette

Protocol

Cell Deposition

- Deposit single cells in 1x PBS on the AmpliGrid reaction sites (e.g., using cell sorting, micromanipulation or laser capture microdissection)

NOTE: Do not exceed a deposition volume of 100 nL as PBS will inhibit enzymatic reactions. In case of higher volumes needed for cell deposition dilute PBS (max. dilution 0.05 x PBS)

- Air dry the cells
- Optional: Perform a visual QC of cell deposition using a microscope. We strongly recommend to take advantage of this key benefit of the AmpliGrid platform as it enables you to correlate RT-PCR results with template presence or absence.
- Continue with reaction setup either with or without on-slide DNase digestion.

NOTE: DNase digestion can be skipped if using an intron spanning primer design to eliminate DNA amplicons or to distinguish between RNA and DNA amplicons.

RNA preparation

- Include RNA into the master mix (for positive control, we recommend to use 1 ng total extracted RNA per reaction)

One Step RT-PCR without DNase I digestion

NOTE: Primers for RT-PCR can either be contained in the mastermix or pre-deposited on the AmpliGrid reaction sites. For pre-deposition primers have to be dissolved in nuclease free water at a suggested concentration of 0.6 μ M each. Let 1 μ L of the primers air dry at room temperature or at 37°C before adding the master mix.

- In a sterile, nuclease-free microcentrifuge tube, combine the following components on ice:

A Table A: Composition of master mix

Component	Volume (1 reaction)	Volume (48 reaction)
2x Single Cell RT Reaction Buffer	0.50 µL	30.0 µL
RNase inhibitor (10 U/µL)	0.02 µL	1.20 µL
5x Single Cell RT Enhancer	0.15 µL	9.00 µL
Optional: Primer mix (10 µM each)	0.06 µL	3.60 µL
Single Cell RT Enzyme Mix	0.04 µL	2.40 µL
Optional: total RNA control (10 ng/µL)	0.10 µL	6.00 µL
Nuclease-free water	ad 1.00 µL	ad 60.00 µL
Total volume	1.00 µL	60.00 µL

- Mix gently by vortexing and spin down shortly.
- Pipette 1 µL of the RT-PCR master mix to each of the AmpliGrid reaction sites.
- Cover each droplet with 5 µL of sealing solution.

NOTE: Ensure that there is no evaporation of the master mix before covering with the sealing solution. A divided workflow might be advisable.

- Perform RT-PCR on the AmpliSpeed slide cycler like shown in table B (it is recommended to preheat the cycler to 42°C before inserting the AmpliGrid slide).

B Table B: One-Step RT-PCR program.

Temperature	Time	
42°C	10 min	Start One-Step RT-PCR
50°C	10 min	
58°C	30 min	
95°C	10 min	
94°C	30 sec	
60°C	75 sec	40 cycles
72°C	75 sec	
72°C	10 min	
ambient	hold	

Analysis & Storage

- For storage please transfer the AmpliGrid slide to an appropriate slide holder, e.g. the Advalytix slide tray and keep it at 4°C until further processing.
- For gel analysis please add 4 µL of a 1.5x concentrated gel loading buffer on top of the sealing solution.
- Aspirate the 5 µL sample volume by piercing the sealing solution with a pipette tip and transfer the samples to a gel (Agarose / PAA).

NOTE: After adding additional volume to the AmpliGrid please do not move the slide as the surface structure will not hold 5 µL volumes reliably.

- For other downstream analysis methods please add 4 µL of ddH₂O instead of the gel loading buffer. It is also possible to retrieve the 1 µL sample but increasing the volume significantly reduces pipetting errors.

2 Figure 2: AmpliSpeed slide cycler ASC200D

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