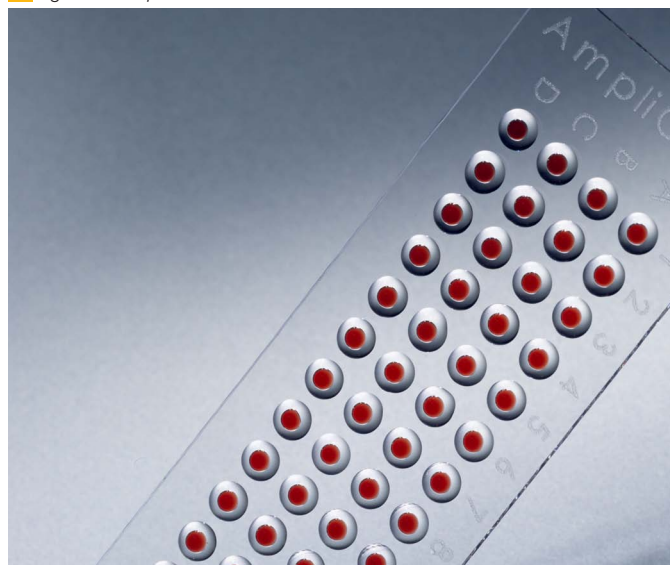


RT-PCR on human total RNA and single cells using Qiagen reagents and AmpliGrid AG480F

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 Qiagen OneStep 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 (Advantix, e.g. OAX04503)

- Primer: (product size)

Calm (93bp) 5'-TGACTGAAGAGCAGATTGCAG-3'

5'-CAGTTCCCAATTCTTTGTTG-3'

b2m (285bp) 5'-CTCTCTCTTTCTGGCCTGGAG-3'

5'-CAAAGTCACATGGTTCACACG-3'

GAPDH (219 bp) 5'-GACCCCTTCATTGACCTCAAC-3'

5'-CTTCTCCATGGTGGTGAAGAC-3'

RT-PCR Primermix containing 20 μ M of primer each.

Each Primer Set can be used separately (singleplex) or combined as multiplex.

- OneStep RT-PCR Kit (Qiagen)

- RNase free water
- Qiagen OneStep RT-PCR Buffer, 5x containing 12.5 mM $MgCl_2$
- Q-Solution, 5x
- Enzyme Mix
- dNTP Mix, 10 mM each
- RNasin® Ribonuclease Inhibitor 40 U/ μ l (Promega)
- AmpliSpeed slide cycler (Advantix, 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.

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

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: RT-PCR setup with Qiagen reagents using single cells

Component	Volume (1 reaction)
Qiagen OneStep RT-PCR Buffer 5x	0.20 µL
Q-Solution, 5x	0.16 µL
dNTP Mix, 10 mM each	0.04 µL
Enzyme Mix	0.04 µL
Optional: Primer mix (20 µM each)	0.03 µL
RNasin® 40 U/µL	0.02 µL
Nuclease-free water	ad 1.00 µL
Total volume	1.00 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 58°C before inserting the AmpliGrid slide).

B Table B: Qiagen OneStep RT-PCR program

Temperature	Time	Cycles
58°C	30 min	
94°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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