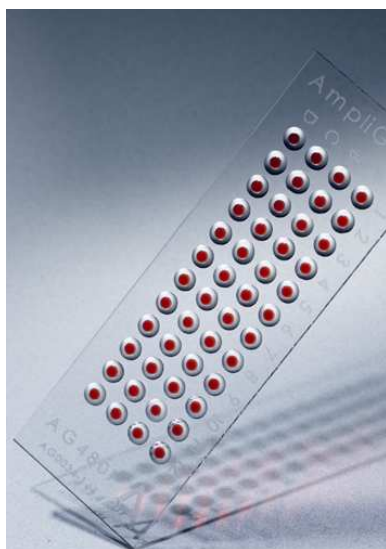


RT-PCR on total RNA and single cells using AmpliGrid AG480F

This process instruction describes the processing of the RT-PCR for murine spleen cells or mouse spleen total RNA. In this RT-PCR system, DNA fragments of approximately 400 bp of length are amplified using mouse spleen total RNA or murine spleen cells as template and either the Qiagen OneStep RT-PCR Kit or the Promega AccessQuick™ RT-PCR system together with AmpliGrid slides.

1 AmpliGrid AG480F



Material:

RT-PCR:

- Template: Mouse Spleen Total RNA (Ambion) or single mouse spleen cells (staining with Hoechst dye recommended) deposited using FACS on the reaction sites of an AmpliGrid AG480F
- Optional: Fluorescence microscope Olympus BX 61 with OSIS cell detecting system for detection of deposited stained cells
- AmpliGrid AG480F (Advalytix)
- Primer: CH1 and VH

VH: 5'-GGGAATTCGAAGGTGCAGCTGCAGGAGTCTGG-3'

CH1: 5'-AGGGGGCTCTCGCAGGAGACGAGG-3'

RT-PCR Primermix containing 2 µM of CH1 and VH primer each

- Alternative 1: OneStep RT-PCR Kit (Qiagen)
 - RNase free water
 - Qiagen OneStep RT-PCR Buffer, 5x containing 12.5 mM MgCl₂
 - Q-Solution, 5x
 - Enzyme Mix
 - dNTP Mix, 10 mM each

- Alternative 2: Promega AccessQuick™ RT-PCR system
 - 2x AccessQuick™ Master Mix
 - AMV Reverse Transcriptase (5 U/µl)
 - Nuclease-free water
- RNasin® Ribonuclease Inhibitor 40 U/µl (Promega)
- Sealing Solution (Advalytix)
- AmpliSpeed slide cycler (Advalytix)
- Electronic multistep pipette

Protocol:

RNA TEMPLATE

- Prepare RNA solution of 10 ng/µl RNA total mouse spleen (storable at -80°C)

SINGLE CELL TEMPLATE

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

MASTER MIX

- Prepare master mix in a fresh PCR-clean tube according to table 1 or 2 when using total RNA as template or to table 3 or 4 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 multipipette about 10 µl)

Qiagen One-Step RT-PCR using total RNA:

A Table 1: RT-PCR setup with Qiagen reagents using total RNA as template

Reagent	1 Spot
Qiagen OneStep RT-PCR Buffer, 5x	0.2 µl
Primermix 2 pmol/µl CH1 and VH	0.3 µl
Q-Solution, 5x	0.16 µl
dNTP Mix, 10 mM each	0.04 µl
Enzyme Mix	0.04 µl
RNasin® 40 U/µl	0.02 µl
RNA, 10 ng/µl	0.1 µl
ddH ₂ O	0.14 µl
Total volume:	1 µl

Promega AccessQuick™ RT-PCR using total RNA:

B Table 2: RT-PCR setup with Promega reagents using total RNA as template

Reagent	1 Spot
AccessQuick™ Master Mix, 2x	0.5 µl
Primermix 2 pmol/µl CH1 and VH	0.3 µl
AMV RT 5 U/µl	0.02 µl
RNasin® 40 U/µl	0.02 µl
RNA, 10 ng/µl	0.1 µl
ddH ₂ O	0.06 µl
Total volume:	1 µl

Qiagen One-Step RT-PCR using single cells:

C Table 3: RT-PCR setup with Qiagen reagents using single cells

Reagent	1 Spot
Qiagen OneStep RT-PCR Buffer, 5x	0.2 µl
Primermix 2 pmol/µl CH1 and VH	0.3 µl
Q-Solution, 5x	0.16 µl
dNTP Mix, 10 mM each	0.04 µl
Enzyme Mix	0.04 µl
RNasin® 40 U/µl	0.02 µl
ddH ₂ O	0.24 µl
Total volume:	1 µl

Promega AccessQuick™ RT-PCR using single cells:

D Table 4: RT-PCR setup with Promega reagents using single cells

Reagent	1 Spot
AccessQuick™ Master Mix, 2x	0.5 µl
Primermix 2 pmol/µl CH1 and VH	0.3 µl
AMV RT 5 U/µl	0.02 µl
RNasin® 40 U/µl	0.02 µl
ddH ₂ O	0.16 µl
Total volume:	1 µl

- Mix gently and spin down shortly
- Distribute 1 µl of master mix on each reaction site
- Immediately cover with 5 µl sealing solution
- Transfer AmpliGrid slide onto thermal cycler
- Run PCR programme (table 5 or 6)

2 AmpliSpeed ASC200D



E Table 5: RT-PCR programme using Qiagen reagents

Temperature	Time	Cycles
58 °C	30 min	
94°C	10-15 min	
94 °C	30 sec	
57°C	60 sec	40
72°C	60 sec	
72°C	10 min	
Ambient	hold	

F Table 6: RT-PCR programme using Promega reagents

Temperature	Time	Cycles
45 °C	45 min	
94°C	5 min	
94 °C	30 sec	
57°C	60 sec	40
72°C	60 sec	
72°C	10 min	
Ambient	hold	

Analysis:

- Store AmpliGrid slides ≤ 8°C until further processing
- Add 4 µl of loading dye on top of each reaction site
- Analyse recovered samples by PAGE and silverstaining

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