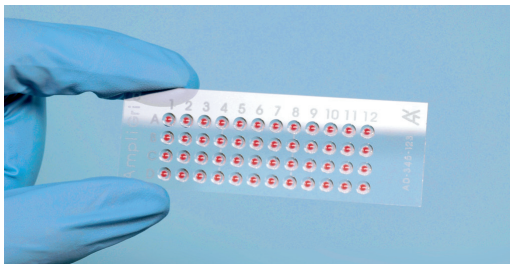


Advalytix

Part of Beckman Coulter



AG480F

Demo Kit DNA Amplification

Appendix Single Cell PCR

01 Introduction

The Demo Kit has been designed to amplify a 160 bp fragment from human chromosome 21. As an additional functionality the master mix can also be applied to single cells from a human source or cell culture.

For research use only. Not for use in diagnostic procedures.

02 The Demo Kit DNA Amplification

For kit content, required materials and storage information please refer to the Demo Kit manual delivered with the kit or download it from our website.

http://www.advalytix.com/images/downloads/DemoKit_Manual_V2.4_s.pdf

03 Cell deposition

Deposit single cells either using a:

- Flow cytometry sorter (e.g. Beckman Coulter MoFlo™): we recommend to use the Advalytix flow cytometry adaptor (OAX04118) in combination with the Advalytix flow cytometry calibration slide (OAX04120) for accurate alignment of the AmpliGrid to the cell sorter X/Y-stage. We recommend to use 1x PBS as sorting buffer. Please refer to the manual section of the Advalytix website for flow cytometry quick start guides for easy setup:

http://www.advalytix.com/manuals_362.htm

- Micromanipulation: deposit cells onto the AmpliGrid with any commercially available micromanipulator. We recommend to use 0.05x PBS as deposition buffer as higher PBS concentrations might inhibit PCR.
- Laser microdissection: isolate cells from tissue by using laser capture microdissection and deposit the cells directly on the AmpliGrid system (e.g., PALM LCM system). Please refer to the manual “Home made PALM adaptor” if you are using an older PALM system without RoboMover and a software version < 4.0. Best results will be obtained using fresh tissue.

http://www.advalytix.com/images/downloads/Example_Protocol_PALM_Adaptor_V1.0.pdf

- Pipette dilution series: dilute your cells to a theoretical concentration of one cell/μL using 0.05x PBS and dispense 1μL to each of the reaction sites and let air dry.

Please do not forget to leave some reaction sites empty for positive (extracted DNA) and negative (without template) controls.

04 Optical control of cell deposition

What you see is what you amplify! The AmpliGrid slide allows for the detection of the deposited cells prior to amplification using a standard microscope setup. This is one of the key benefits of the system. For further information on single cell staining and microscope requirements please refer to the following document:

http://www.advalytix.com/images/downloads/AR_Visual_inspection_V1.1_s.pdf

05 Experimental procedure

Set up

- Equilibrate sealing solution and slide to room temperature before opening the aluminium pouch.
- Deposit single cells as template on the AmpliGrid slide (see 03 Cell deposition)
- Prepare an amplification master mix in a PCR-clean reaction tube according to table 1.

Table 1: Composition of master mix

Component	Volume [μL]
10 x GoldStar Buffer	2
GoTaq® DNA Polymerase	0.4
Chr21 Primermix	2
AdvaGold 0.1 %	2
PCR-grade water	13.6
Total volume	20

- Mix gently by vortexing and spin down shortly.
- Place AmpliGrid slide on the black pad for better visualisation of the engraved markings.
- Prepare the human male DNA for positive controls:
 - Thaw the human male DNA and the PCR-grade water and mix thoroughly
 - Pipette 7.5 μL of PCR-grade water in a PCR-clean reaction tube
 - Add 2.5 μL of human male DNA (2ng/ μL)
 - Mix gently by vortexing and spin down shortly
- Add 1 μL of the diluted DNA solution onto the empty AmpliGrid reaction sites
- Let air dry the DNA solution
- Add 1 μL of the prepared master mix to each reaction site of the AmpliGrid slide without touching the slide surface.

NOTE: Wait until droplet becomes visible at the tip outlet before depositing on the slide surface.

- Immediately cover the master mix droplet with 5.0 μL sealing solution. We recommend to use an electronic multi-step pipette for pipetting the sealing solution as other pipettes may cause satellite droplets. Due to the high viscosity it is also advisable to pipette at a slow speed.
- Transfer the AmpliGrid slide to the AmpliSpeed slide cycler (see tab. 2 for program and run the amplification program.

Table 2: Amplification program

Temperature [$^{\circ}\text{C}$]	Duration	
95	3 min	
95	25 sec	
63	35 sec	40 cycles
72	20 sec	
72	1 min	
ambient	∞	

Document History

Version	Date	Remarks
1.0	26/02/2009	Creation of document
1.1	31/07/2009	Change of Logo



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