

AmpliGrid Cell Extraction Kit

This reagent kit has been especially designed for the use with the Advalytix AmpliGrid System and is only available through Advalytix.

Order Information

Ref.-Nr.	Product	Description
OAX04523	Cell Extraction Kit	One step lysis & DNA extraction kit (sufficient for 5 AG480F)

Contents

Part Nr.	Component	Volume
OAX04244	Lysis Enzyme	6 µL
OAX04245	10 x Lysis Buffer	40 µL

Storage

Store all components at -20°C.

Please avoid frequent freeze and thaw cycles as this might reduce activity of the lysis enzyme.

Ship components from -20°C to ambient temperature.

Quality control

All components are tested for lysis activity in a biological assay using a multiplex PCR assay on human lymphocytes.

Beckman Coulter certifies that all reagents are free of detectable bacterial DNA, free of detectable endonuclease and exonuclease activity, free of detectable human DNA and free of detectable PCR inhibitors. All components of the lysis enzyme mix are tested for product purity on an SDS-PAGE and with MALDI-TOF analysis.

For further information please refer to the corresponding quality control certificate.

Manufacturer

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User Guide

The AmpliGrid Cell Extraction Kit has been optimised for one step DNA extraction from cells or tissues in ultra low reaction volumes on the AmpliGrid AG480F slide.

Using the unique enzyme mix the cells are lysed and the DNA gets available for downstream amplification reactions with standard PCR systems as DNA associated proteins are digested. Please refer to the AG480F user guide for further information about the AmpliGrid system before using this reagent kit.

Materials and Reagents not supplied

- AmpliGrid AG480F DNA free microliter reaction slide, 48 reaction sites each, incl. sealing solution (Advalytix AG, e.g. OAX04201)
- AmpliSpeed slide cycler (Advalytix AG, OAX04101 - OAX04103)
- Multi step and manual pipettes and pipette tips covering the range from 0.75 to 5 µL
- Nuclease free water

Experimental Procedure

- Deposit cells on the AmpliGrid slide with e.g., microdissection, cellsorting or capillary and let air dry. We recommend to use 0.2 - 0.05x PBS as sorting buffer. Please ensure that the transferred volume is in the range of 0.1 - 0.5 µL as higher volumes will increase salt concentration and inhibit the subsequent enzymatic reactions.
- Thaw all reagents completely prior to use and vortex shortly.
- In a sterile, nuclease free tube combine the components according to table 1.

Table 1: Composition of cell extraction reaction mix

Component	Volume (1 slide)	Volume (5 slides)
Lysis Enzyme	1 µL	5 µL
10x Lysis Buffer	6 µL	30 µL
Nuclease free water	53 µL	265 µL
Total volume	60 µL	300 µL

- Mix gently by vortexing and spin down shortly.
- After preparing the working dilution of the cell extraction kit keep it on ice and use within 6 hours. Do not store the diluted enzyme as this will rapidly decrease activity.
- Dispense 0.75 µL of the cell extraction reaction mix on the AmpliGrid reaction sites.
- Cover immediately with 5 µL of sealing solution.
- Place the AmpliGrid slide on the AmpliSpeed slide cycler and run the programme shown in table 2.

Table 2: Recommended incubation conditions

Temperature	Time
75°C	5 min
95°C	2 min
Ambient	∞

- Store AmpliGrid slides at 4°C after the lysis procedure.

Further Processing

- Prepare the PCR mix in a 2-fold concentration since this mix will be diluted by the lysis mixture on the slide.
- Do NOT remove the liquids on the AmpliGrid slide.
- Optional: Place the AmpliGrid slide back on the AmpliSpeed cycler before starting the pipetting process.
- Add 0.75 µL of the 2x PCR mix on top of the sealing solution droplet. The water based PCR mix will move through the sealing solution and merge with the lysis mixture on the reaction site.
- Continue with the specific PCR protocol on the AmpliSpeed slide cycler.