AmpliCell

12 well culture-to-PCR slide for multiple *in situ* assays.

Ref.-No.	Product	Description
OAX04531	AC480	12 well cell culture-to-PCR slide (5pcs./pck.), incl. 5x500 µL sealing solution, fibronectin coated
OAX04532	AC480	12 well cell culture-to-PCR slide (15pcs./pck.), incl. 15x500 µL sealing solution, fibronectin coated
OAX04533*	AC480	12 well cell culture-to-PCR slide (5pcs./pck.), incl. 5x500 µL sealing solution, Collagen IV coated
OAX04534*	AC480	12 well cell culture-to-PCR slide (15pcs./pck.), incl. 15x500 µL sealing solution, Collagen IV coated
OAX04535*	AC480	12 well cell culture-to-PCR slide (5pcs./pck.), incl. 5x500 µL sealing solution, Poly-L-Lysin coated
OAX04536*	AC480	12 well cell culture-to-PCR slide (15pcs./pck.), incl. 15x500 µL sealing solution, Poly-L-Lysin coated

*available on request

Shipping & Storage Conditions

All components of the AmpliCell system are shipped at ambient temperature. Please store the components in the original package at 4°C. After opening the packaging all components should be used immediately to avoid contamination. Do not use any of the components after expiry date.

Quality Control

All AmpliCell products are manufactured and packed in a state-of-the-art clean room to ensure superior product quality. Each lot is quality controlled for sterility, physical and biological performance.

Product Information

Conventional cell culture, treatment and extraction for gene expression analysis may induce cellular stress responses which affect gene expression. In addition, current methods require high volume culture flasks and spin-column extractions which are time-consuming and costly. The innovative AmpliCell system integrates culturing, treatment and PCR on a single platform without extraction. Minimize cellular stress and save time and reagents for transfection, microRNA assays, and methylation analysis.

The AmpliCell slide consists of AmpliGrid, a glass slide for 1µL PCR reactions, plus a removable upper structure that sits tightly sealed on top of the slide and separates 12 growth chambers. After cell expansion, remove the upper chamber structure, wash off excess cells, and run up to 4 independent (RT)-PCR reactions and optional subsequent qPCR on the cells in each chamber. Amplification takes place in 1 µL master mix volumes on each reaction site. Reactions are covered with 5 µL of sealing solution to prevent evaporation during thermal cycling on the AmpliSpeed slide cycler.

Applications

- Cultivation of adherent cells and cells in solution
- IF stainings
- Direct sequencing from cell populations
- High resolution fluorescent microscopy
- Transfection assays
- siRNA assays
- Methylation assays
- High throughput gene expression analysis

Customer Support

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Product use limitations, Warranty, Disclaimer

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The AmpliCell system is intended for research applications and is not approved for clinical use!

User Guide

Materials required but not provided

- Powder free gloves.
- General cell culture equipment.
- Cell culture media.
- Amplification reagents (Advalytix GoTaq® PCR Kit or Advalytix One-Step RT-PCR Kit).
- AmpliSpeed slide cycler (e.g. ASC100D, OAX04101).
- Electronic Multi-step pipettes (e.g., Eppendorf Multipette™ Stream or Rainin AutoRep™ E).
- Pipette tips for multi-step pipette covering the range of 1- 5 µL.
- Analysis system (e.g., qPCR system, capillary electrophoresis, polyacrylamide or agarose gel electrophoresis, UV-spectrometer).

Precautions before Use

- It is recommended to work in a clean environment as dust particles may interfere with the reaction and may be a source of contamination for cell culture and PCR.
- Standard precautions and good laboratory practice are necessary to avoid cross contamination throughout all steps of the experiment.
- Each reaction drop should be covered with sealing solution as soon as possible. The loaded reaction volume (1 µL per reaction site) may dry depending on room temperature, humidity etc. Inadequate volumes will compromise the interpretation of results. A divided workflow might be advisable.
- Wear powder free gloves and use filter pipette tips.

Experimental Procedure

1. Dispense 200-300 µL cell growth media into the chambers of the AmpliCell.
2. Seed approximately 2-10x10^3 cells per well. The optimal starting amount of cells depends on the application and may vary.
3. Place the lid onto the AmpliCell to prevent from media evaporation and contamination.
4. Incubate AmpliCell under optimal cell growth conditions for 24-48 h.
5. Check the current cell growth state using a microscope. If the desired density has been reached continue with step 6.

6a. adherent cells: remove the cell growth media by pipetting.

6b. non-adherent cells: spin down (~500xg) the cells using a cytospin or a slide adaptor for microplate swing out buckets. Remove the cell growth media by pipetting.

7. Optional: setup a cell assay of interest e.g., transfection, methylation, microRNA, IF staining. The reagent volume can be downscaled to 200-300 µL for an optimal workflow in the AmpliCell. Please contact the Advalytix application support for more details on integrating assays to the workflow.
8. Remove the silicon chamber.
9. Wash the slide carefully for 10 seconds in 1x PBS, for 10 seconds in 0.05x PBS and for 2 seconds in dH_2O. Do NOT rinse to minimize loss of cells. Dry the slide by putting it into a laminar flow bench.
10. Remove the blue tape from the slide surface. Do NOT remove the tape if the slide is wet!
11. Put the AmpliCell slide on a dark surface to ensure good visualization of the engraved circles marking the reaction sites.
12. Pipet 1 µL of PCR or RT-PCR reaction mix onto each reaction site of the AmpliCell. Due to the hydrophilic structure of the reaction sites aqueous liquids will automatically move to the center and cover the cultivated cells on the surface.
13. Cover each droplet with 5 µL of sealing solution.

    NOTE: Ensure that there is no evaporation of the reaction mix before covering with the sealing solution. A divided workflow is advisable.
14. Incubate the AmpliCell on the AmpliSpeed slide cycler according to your required thermal cycling profile specifications. It might be necessary to adapt established thermal profiles due to the superior performance of the AmpliSpeed instrument.
15. Retrieving sample from the AmpliCell:
    - Remove the AmpliCell from the AmpliSpeed slide cycler.
    - Gel analysis: Add loading dye by gently touching the top of the sealing solution with the drop of loading dye (e.g. 4 µL). The loading dye will move through the sealing solution and merge with the PCR sample. Retrieve the liquid by piercing the pipette through the sealing solution into the aqueous phase, aspirate the complete aqueous phase (4 µL loading dye + 1 µL sample) and load the gel.
    - Other analysis: Pipette the aqueous phase into a new repository by piercing through the sealing solution and aspirating the aqueous phase (1 µL sample). Add buffers for downstream processing and continue with analysis (e.g., CE Sequencing, qPCR).

      NOTE: Alternatively add 4 µL of buffers for downstream processing or nuclease free water to the sample as described for the gel analysis procedure. By increasing the volume before retrieving the sample from the AmpliCell pipetting gets easier and pipetting errors are minimized.

Product Information AmpliCell AC480, #1/2009

Advalytix
Part of Beckman Coulter

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