MiCA: Microfluidic Array for 3D Culture

M32P-02

Description: The MiCA plate contains 32 independent open-top perfusion chambers in a 96 well plate format. Each cell chamber is 2 mm in diameter with a separate inlet and outlet well. The innovative design allows perfusion culture of cells in 3D gel or 2D monolayer for long term cell culture experiments.

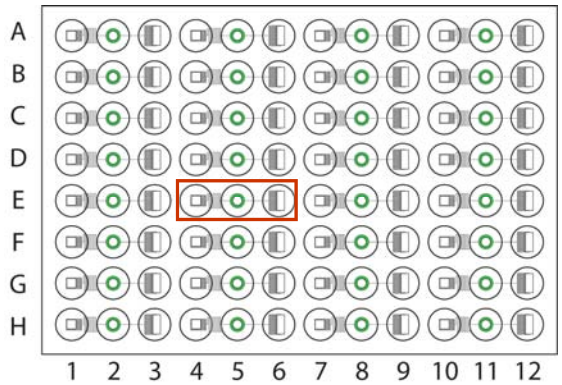
Applications:

- Multiplexed cell culture experiments in 3D matrix
- Long term continuous perfusion experiments
- Monitoring cell response to solution exchange (induction, proliferation, drug dosing, etc.)
- Comparing up to 32 different cell types, gels, or exposure conditions in parallel
- High content analysis and live cell imaging in 3D matrix
- Automating 3D culture screening protocols

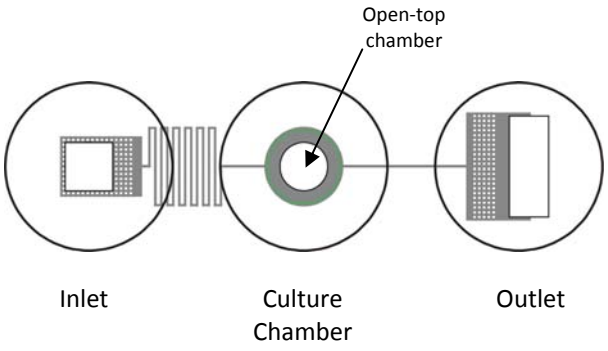
Product Specifications:

- Stand-alone gravity flow plates require no external instrumentation
- SBS standard 96 well plate dimensions
- #1.5 thickness (170 µm) glass slide bottom
- Direct access to open top micro-chamber (4 µl volume)
- Gravity driven flow rates of ~100 µl per day
- Long term cell culture in any standard incubator

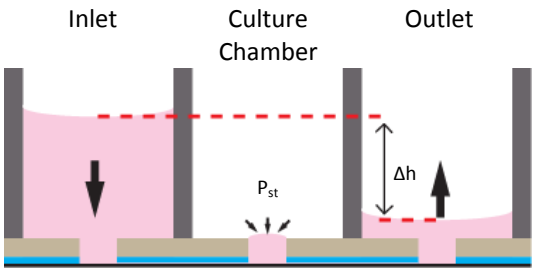
Plate Design:



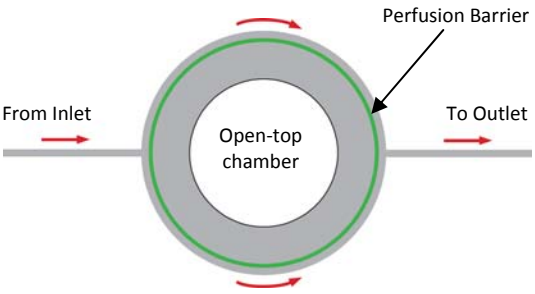
The MiCA has 32 independent perfusion units tiled on a 96 well plate. Each unit has an inlet well (col 1, 4, 7, 10), a cell culture well (col 2, 5, 8, 11), and an outlet (col 3, 6, 9, 12).



The single flow unit consists of 3 well positions. The culture chamber has an open top to allow direct liquid dispensing of cells/gels to the chamber. Perfusion flow is from the inlet to outlet wells.



Side view of the flow unit depicting gravity driven flow. The pressure drop from the inlet to outlet well drives flow past the culture chamber. The surface tension force (P_{st}) holds liquid in the chamber.



The open top culture chamber is 2 mm in diameter with a height of 1.4 mm. A perfusion barrier (green) surrounds the chamber to separate the cell/gel chamber from the flow channels.

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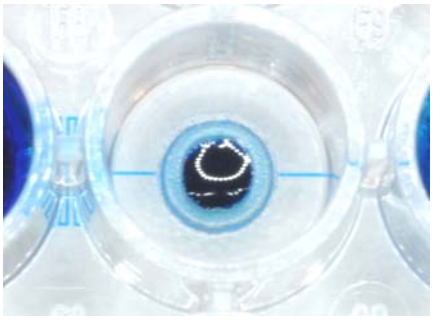
Open Top Design

The MiCA plate uses an innovative open top design that utilizes surface tension to maintain perfusion integrity.

- Directly pipet into the 2mm diameter opening. Ideal for viscous gels, small cell samples, difficult to culture cells, and primary cells.
- Microfluidic channels integrate with the open chamber, enabling laminar flow perfusion.
- The design of the microfluidic circuit directs flow to the outlet well without overflowing the open chamber.

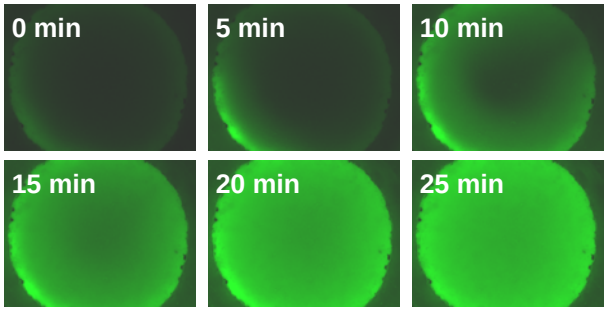


The 2mm diameter open top chamber allows direct dispensing of cells/gels from above.

Gravity Perfusion Culture

The fluidics maintain continuous perfusion for long term cell culture studies without any external equipment.

- Flow driven by liquid level difference between inlet and outlet wells.
- Set to 100 µl/day during normal operation.
- Chamber design optimized for cell health.
- Maintain flow by refilling inlets and emptying outlets every 48 hours.

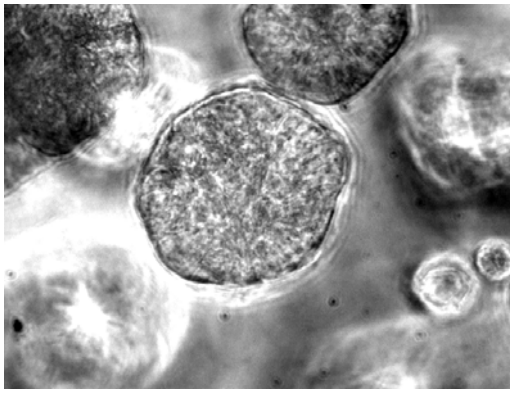


Perfusion of fluorescein conjugated dextran (3 kDa) in the 2mm diameter open chamber.

3D Cell Culture

CellASIC's microfluidic cell culture technology delivers unmatched quality for 3D cell culture studies.

- The open-top chamber allows easy dispensing of gel solutions using a pipette.
- Suitable for 3D embedded, overlay, sandwich, or no gel perfusion culture.
- Diffusion of nutrients into the chamber maintains long term cell health.
- The #1.5 thickness (170 µm) glass coverslide floor enables imaging with high NA objectives.
- Small volume culture chambers (4 µl) reduce cell/gel usage and cost.

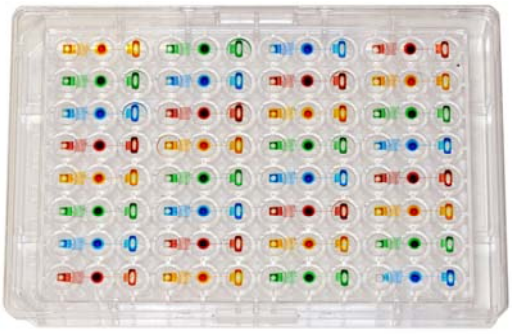


MCF10A breast epithelial cells in Matrigel after 7 days perfusion culture in the chamber.

Standard 96 Well Format

Industry standard format ensures compatibility with existing equipment and assays.

- Fluidics built into standard 96-well plate frame.
- Compatible with automated liquid dispensers and plate handling equipment.
- Cell culture in any standard incubator.
- Assay via commercially available kits— including fluorescence, luminescence, cell staining, lysis, etc.
- Run 32 independent perfusion units per plate.



The MiCA is built on an industry standard 96-well plate.

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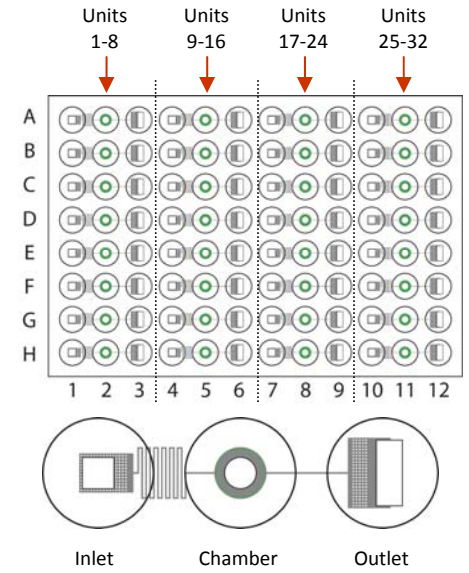

Figure 1. Well Layout

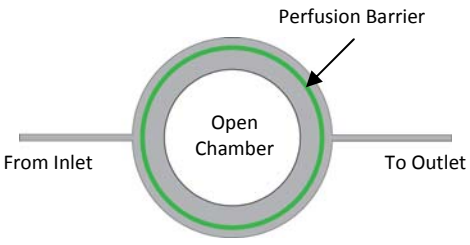

Figure 2. Culture Chamber

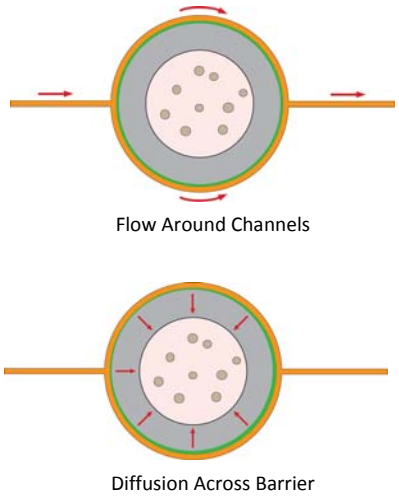

Figure 3. Perfusion Flow and Diffusion

Operation Instructions

1. The MiCA microfluidic plate contains 32 independent chambers on a 96 well plate. Each chamber has 3 wells: an inlet, a culture chamber, and an outlet (Figure 1). The cutouts at the bottom of the wells are different (square, circle, rectangle) for visual identification. The plate is shipped pre-primed with sterile PBS. Plate performance guaranteed up to the expiration date.

Cell Loading

2. Aspirate the priming solution from the chamber wells, leaving the liquid in the 2 mm hole at the bottom of the well. This is the open-top culture chamber, identified by a circular cutout surrounded with a hydrophobic ring. The ring prevents liquid from leaking out of the well during operation.
3. Aspirate the Inlet and Outlet wells to remove the priming solution. Avoid sucking out the liquid from the bottom holes, as this may introduce air bubbles.

3D Gel Embedded (Matrigel example)

4. Prepare a cell suspension of 2-6x10^6 cells/ml. On ice, mix cell suspension with Matrigel (BD Biosciences) 1:8 (one part cell to 8 parts gel).
5. Remove the liquid from the open-chamber (4 µl) and pipet in 4 µl of the cell/gel solution. Repeat with as many wells as desired.
6. Incubate the plate at 37°C for 15 minutes to polymerize gel. The gel will be localized by the microfabricated perfusion barrier surrounding the chamber. Depending on cell type and gel used, it may be necessary to optimize the cell density and cell/gel ratio.

3D Gel Overlay

7. Prepare a cell suspension of 0.25-1x10^6 cells/ml.
8. Remove the liquid from the open-chamber (4 µl) and pipet in 4 µl of the cell solution. Repeat with as many wells as desired.
9. After letting the cells settle to the bottom (10-30 minutes), carefully remove the liquid in the well and add 4 µl of gel solution over the cells.
10. Place the plate into a 37°C incubator for 15 minutes to polymerize the gel. Depending on cell type and gel used, it may be necessary to optimize the cell density and cell/gel ratio.

2D Culture

11. If desired, coat the glass bottom of the chamber by directly pipetting coating solution to the open chamber (e.g. poly-lysine, collagen, etc.). After a suitable incubation time, remove and wash with PBS or culture medium.
12. Prepare a cell suspension of 0.25-1x10^6 cells/ml.
13. Remove the liquid from the open-chamber (4 µl) and pipet in 4 µl of the cell solution. Repeat with as many wells as desired. Depending on cell type, it may be necessary to optimize the cell density and coating protocol.

Figure 4. Gravity Fed Perfusion in Incubator

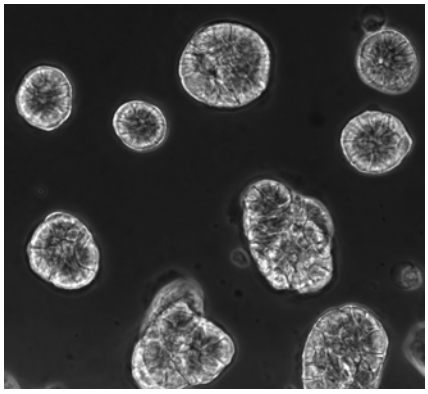

Figure 5. MCF10A Breast Epithelial Cell Culture in 3D Matrigel

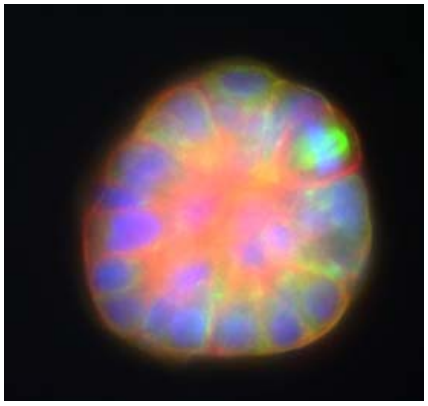

Figure 6. Immunostaining of cells in 3D gel

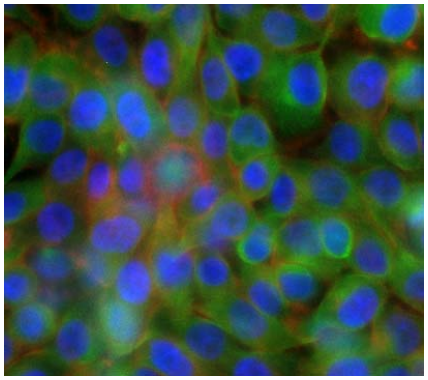

Figure 7. Immunostaining of cells in 2D culture

Operation Instructions (cont.)

Perfusion Culture

14. The array uses gravity driven perfusion to feed the cells. A perfusion barrier (figure 2) surrounds the cell/gel chamber. The 4 µm pores of the barrier allow free diffusion from the flow channels (figure 3). It takes approximately 0.5-1 hour for a new solution to diffuse into the culture chamber during perfusion.
15. To initiate perfusion culture, add 300 µl of medium to the inlet well and 30 µl to the outlet well. Gravity perfusion will flow at about 100 µl/day.
16. Place the plate into a standard cell culture incubator for long term culture (Figure 4).
17. Every 2 days, add 150 µl of medium to the inlet well, and empty the outlet well to maintain continuous flow.

Cell Assay

18. Cell based assays can be performed in a variety of formats. The thin cover glass bottom enable high quality cell imaging using microscopy. Additionally, plate reader based assays can be applied either by flowing reagents through the chamber (inlet to outlet) or by direct addition to the center well. Cells can also be recovered or lysed directly from the open-chamber.
19. For protocol details, please contact CellASIC.