

Pearl: Microfluidic Perfusion Array for Hepatocytes

H32M-19

Description: The Pearl plate contains 32 independent microfluidic hepatocyte culture units in a 96 well plate format. Each unit contains ~30,000 cells with a separate inlet and outlet well. The biomimetic design simulates the liver acinus and promotes long term primary hepatocyte function with continuous perfusion.

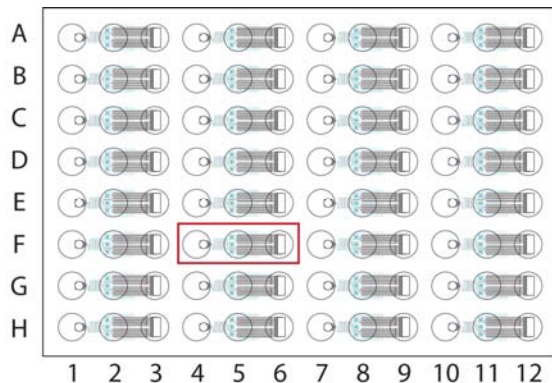
Applications:

- Long term “liver-like” primary hepatocyte culture
- Continuous perfusion experiments
- Metabolite analysis over multi-day exposures
- Monitoring cell response to drug dosing
- High content analysis and live cell imaging
- Automating primary hepatocyte screening protocols

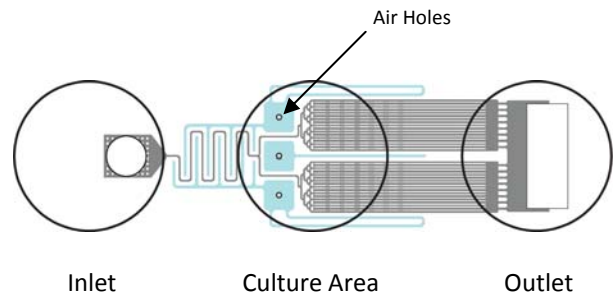
Product Specifications:

- Use with Pearl Cell Loading System
- SBS standard 96 well plate dimensions, 32 flow units per plate
- #1.5 thickness (170 μm) glass slide bottom
- Cells cultured in a parallel network of $80 \times 55 \times 3,000 \mu\text{m}$ cords
- Gravity driven flow rates of ~100 μl per day
- Long term cell culture in any standard incubator

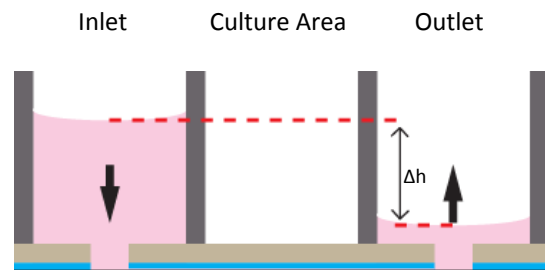
Plate Design:



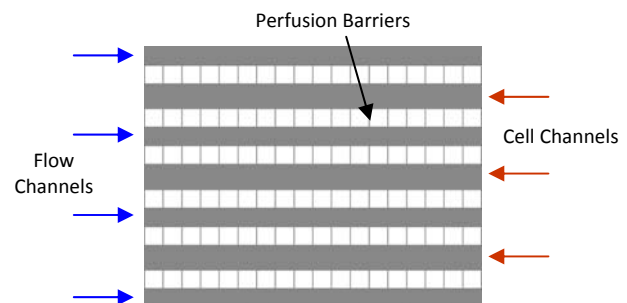
The Pearl has 32 independent perfusion units tiled on a 96 well plate. Each unit has an inlet well (col 1, 4, 7, 10), a cell culture well (col 2, 5, 8, 11), and an outlet well (col 3, 6, 9, 12).



The single flow unit consists of 3 well positions. Air channels (blue) enable rapid gas diffusion to/from the cells. 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.

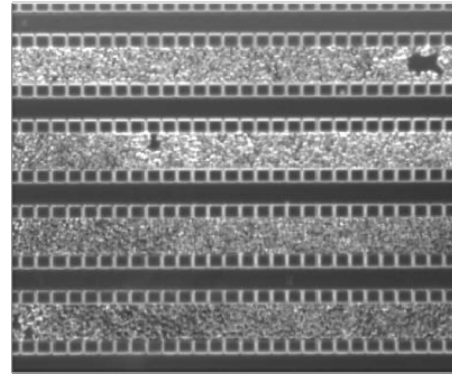


Cells are cultured in elongated microfluidic channels ($80 \times 55 \mu\text{m W} \times \text{H}$), alternating with flow channels ($60 \times 55 \mu\text{m W} \times \text{H}$) separated by perfusion barriers with $4 \mu\text{m}$ pores.

Biomimetic Design

The Pearl plate uses an innovative design that replicates key aspects of the liver microstructure.

- Hepatocytes are loaded into 3D cords, maximizing cell-cell contact and medium exposure.
- Continuous flow medium exposure through sinusoid channels.
- Perfusion barrier dimensions effectively separate cells from flows without limiting mass transport.
- Microfluidic air channels enhance oxygen diffusion directly to the cultured cells.
- Alternating cell/flow channel design packs 30,000 cells in a single flow unit.

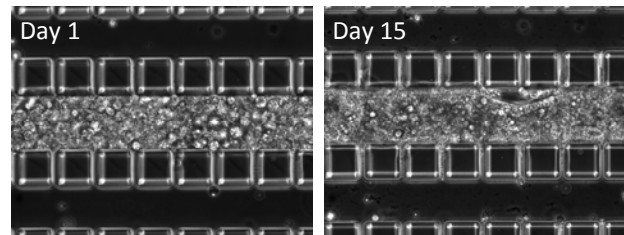


A section of the hepatocyte perfusion array showing alternating cell/flow channels separated by perfusion barriers.

Long Term Hepatocyte Function

CellASIC's microfluidic cell culture technology delivers unmatched quality for long term hepatocyte culture studies.

- Hepatocytes self-organize into stable tissue structures within a few days in microfluidic perfusion culture.
- CYP450 activity and induction potential maintained for over 28 days.
- Validated with freshly isolated and cryopreserved hepatocytes from human and rat origin.
- The #1.5 thickness (170 μm) glass coverslide floor enables imaging with high NA objectives.

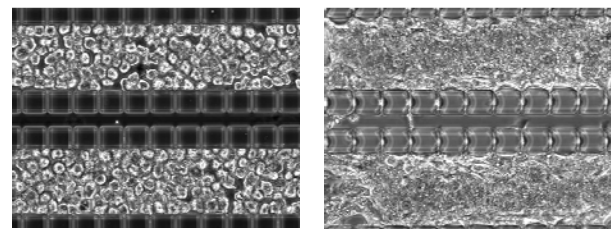


Human cryopreserved hepatocytes after 1 day and 15 days culture in the microfluidic array.

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 $\mu\text{l/day}$ during normal operation.
- Chamber design optimized for cell health.
- Maintain flow by refilling inlets and emptying outlets every 24-48 hours.



No Flow

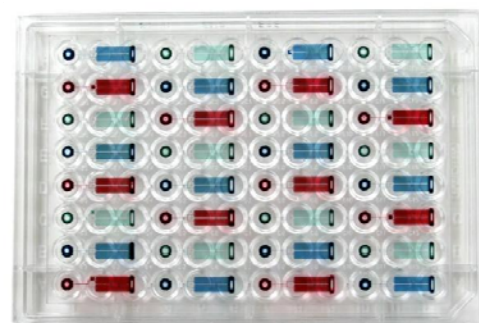
Perfusion

Continuous perfusion causes hepatocytes to form tissue structures with long term viability and liver activity. Rat hepatocytes shown.

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 Pearl is built on an industry standard 96-well plate.

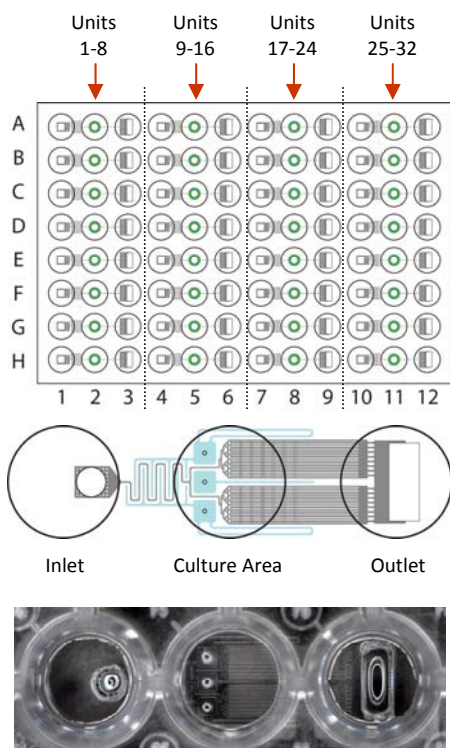


Figure 1. Well Layout

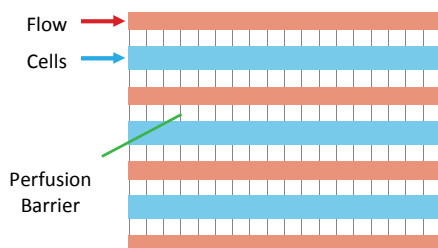


Figure 2. Schematic of Culture Area

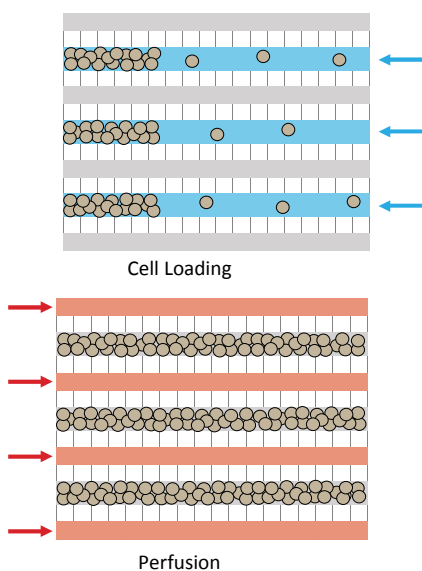


Figure 3. Cell Loading and Perfusion

Operation Instructions

1. The Pearl microfluidic plate contains 32 independent chambers on a 96 well plate. Each chamber has 3 wells: an inlet, a culture area, and an outlet (Figure 1). The plate is shipped pre-primed with sterile PBS.

Cell Loading

2. Cells are loaded into the liver-sinusoid structures via pressure driven flow from the outlet to the inlet. Refer to the Cell Loader instructions for a detailed protocol.
3. Prepare a suspension of hepatocytes (7 million cells/ml for human; 5 million cells/ml for rat). Each plate will require approximately 2 million cells.
4. Aspirate the priming solution from all wells of the plate.
5. Seal the Loader manifold to the Pearl Plate.
6. Fully aspirate the outlet wells, including the rectangular cutout at the bottom of the well.
7. Pipet 8 μ l of hepatocyte suspension into the rectangular cutout under the outlet well.
8. As soon as cells are pipette into an entire column of the plate, turn on the vacuum switch for that column (set at 20 inches W.C. on the gauge) for approximately 15 seconds. This will drive the cells into the microfluidic structures (Figure 3).
9. Check loading quality on a phase contrast microscope to ensure uniformity and density. Hepatocytes should be closely packed within the culture channels.
10. Repeat for all 4 columns of the plate.
11. De-seal the manifold from the plate.

Perfusion Culture

12. The plates are fed via gravity perfusion from the inlet to the outlet wells. Medium will continuously flow through the perfusion channels (Figure 3) allowing exchange with the cultured cells.
13. Add 300 μ l of culture medium to the inlet well. The liquid height difference between the inlet and outlet well will drive perfusion flow at approximately 100 μ l/day.
14. Place in a 37°C/5% CO₂ incubator for long term culture.
15. Every 24 to 48 hours, empty the outlet well and refill the inlet well to maintain continuous flow. For best results, add 125 μ l of fresh medium every 24 hours.
16. Typically, after 2-4 days of perfusion culture, hepatocytes will adopt an aggregate morphology and recover metabolic (CYP450) activity (Figure 5). If the cells are free from contamination and fed daily, viability and activity will persist for over 1 month.

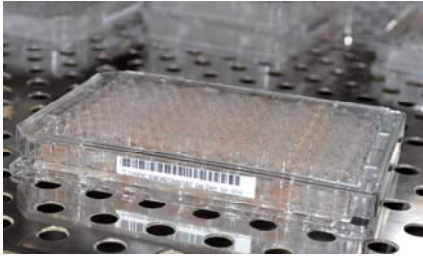


Figure 4. Gravity Fed Perfusion in Incubator

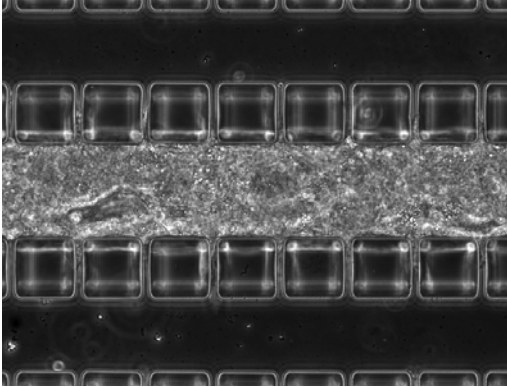


Figure 5. Human hepatocytes cultured after 7 days with perfusion

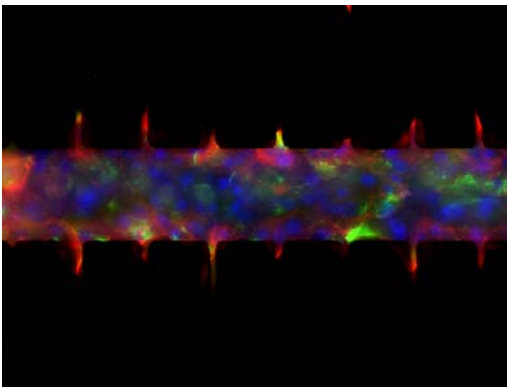


Figure 6. Immunostaining of hepatocytes (nucleus, actin, tubulin)

Operation Instructions (cont.)

Metabolite Analysis

17. Metabolites of substrate and drug solutions can be collected from the outlet stream after flow exposure to hepatocytes from hours to days.
18. When the cells are stabilized (typically day 4-6), empty the inlet well and replace with 300 μ l of substrate or drug solution.
19. Incubate in an incubator for the desired time period. For multi-day exposures, replenish the substrate/drug solution every 24 hours.
20. Collect the flow through from the outlet well at the desired time points. This solution can be prepared for analytical methods such as HPLC/MS.

Cell Assay

21. 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 by flowing reagents through the chamber (inlet to outlet). Cells can also be recovered or lysed directly from the open-chamber.
22. For additional protocol details, please contact CellASIC.