

M04S Microfluidic Cell Culture Plate

M04S-03

Description: The M04S plate contains 4 independent chambers, each with 5 upstream inlet channels. Each chamber is 2.8 mm in diameter with a 120 micron height and designed for long term, high magnification, live cell perfusion imaging with solution switching.

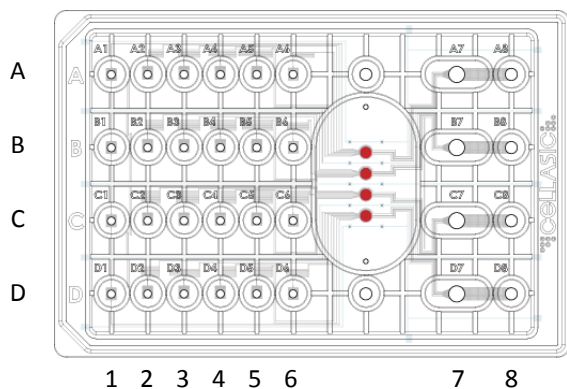
Applications:

- Time-lapsed, high magnification imaging of adherent cells (up to 50 um diameter)
- Long term continuous perfusion experiments (3 days typical, up to 14+ days)
- Solution exchange experiments (induction, inhibition, drug dosing, etc.)
- Automated immunostaining in the micro-chamber and “on-demand” fixing of live cells
- Comparing up to 4 different cell types or exposure conditions in parallel
- Temperature and gas atmosphere control (temperature shift, anoxic, etc.)

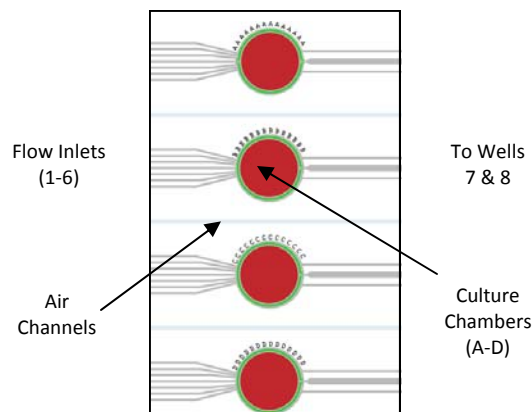
Product Specifications:

- Use with ONIX-262 system and F84 manifold
- SBS standard footprint frame (fits to typical 96 well stage holders)
- #1.5 thickness (170 um) glass slide bottom (in contact with cells)
- Capillary driven cell loading using only a pipette (10 uL sample per chamber)
- Laminar flow rates of 1-100 ul/hr (typical chamber refresh times of 20-60 seconds)
- Microfluidic gas exchange channels

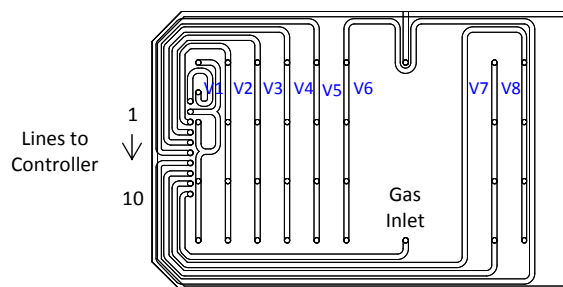
Plate Design:



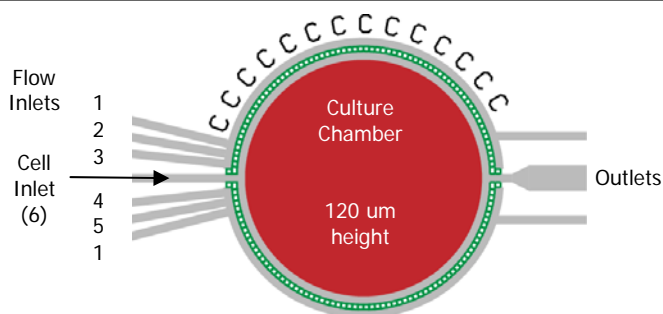
The M04S has 4 independent units (A-D), each with 5 inlet wells (1-5), cell inlet (6), and flow outlet (7). All four culture chambers are located under a single imaging window.



The 4 culture chambers are located under a large imaging window for high magnification phase objectives and to minimize travel distance. All channels are resistance matched for uniformity.



The F84 manifold has 8 pressure channels (V1-V8) to control flow rates through the microfluidics. A vacuum line is used to seal the plate to the manifold, and a gas line enables atmosphere control.

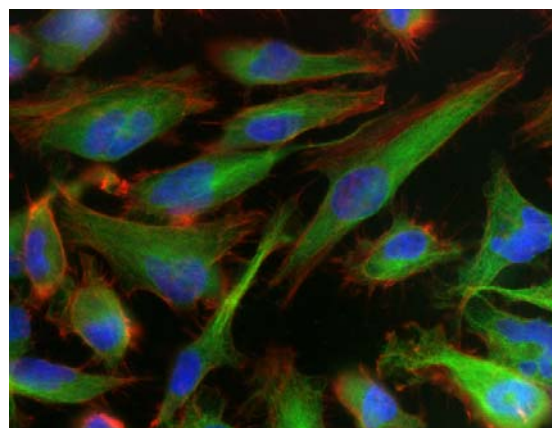


The culture chamber is 2.8 mm in diameter with a height of 120 microns (0.9 ul). Inlet 1 allows for gravity driven perfusion during pre-culture, and inlets 2-5 allow switching between 4 medias during cell imaging.

Highest Quality Live Cell Imaging

CELLASIC's microfluidic cell culture technology delivers unmatched quality for live cell imaging.

- Stress-free cell loading using a pipette inside your sterile hood via our capillary flow method.
- Gravity driven perfusion lets you "pre-culture" cells for many days in a standard incubator.
- The #1.5 thickness (170 μm) glass coverslide floor is matched to immersion and high NA objectives and amenable to surface coating.
- A large imaging window allows light to pass freely for optimal phase contrast quality.
- The four microchambers are located within a 1.6 cm area, minimizing stage travel and focus drift.

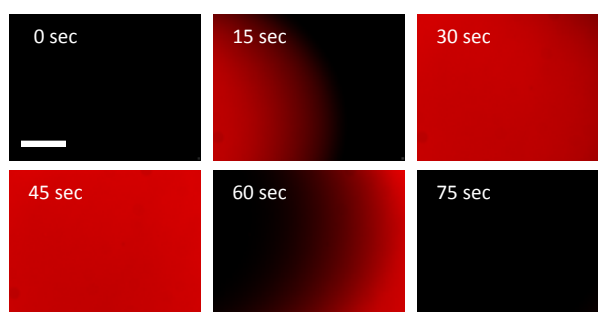


HeLa cells immunostained in the microchamber after perfusion culture.

Solution Switching

Our advanced perfusion control enables experiments not possible with existing instrumentation.

- Five switchable inlet wells for each chamber.
- Highly laminar flow provides uniform exposure profiles and sharp transition boundaries.
- Low shear ensures minimal stress on cells.
- Rapid solution exchange rates and <10 nL dead volume for fine time resolution experiments.
- Perfusion rates of $\mu\text{l/hr}$ allow continuous multi-day experiments on your microscope.

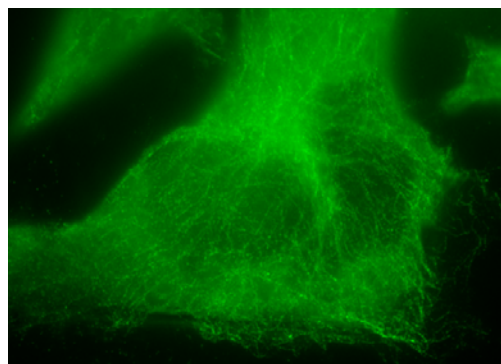


Solution exchange profile between a fluorescent dye and buffer solution in a 10X field of view. Scale bar = 200 μm .

On-Demand Immunostaining

The M04S plate allows *in situ* immunostaining of cells monitored in the microfluidic chambers.

- Automate staining and washing protocol using up to 6 inlet solutions.
- Laminar flow mechanics provide excellent washing and staining kinetics with minimal reagent usage.
- Fix cells "on-demand" by flowing fixative into chamber during cell imaging. Fix cells when they reach a desired state (mitosis, apoptosis, response to drug, etc.) to obtain precise detail on single cell biology.

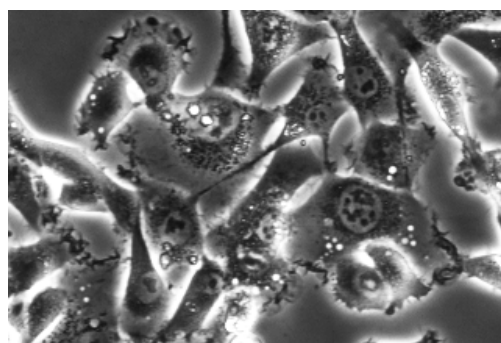


α -tubulin staining in the microfluidic chamber. HeLa cell, 100X

Long Term Cell Culture

The innovative microfluidic plate is designed for long term cell happiness.

- Continuous perfusion ensures fresh medium and waste removal, even at confluent cell densities.
- Small chamber areas allow uniform temperature control using only an objective heater.
- The sealed manifold delivers 5% CO_2 through specialized microfluidic gas diffusion channels.
- Gravity driven medium channel allows pump-free culture in any incubator.



HT-1080 breast cancer cells growing at high density after 6 days continuous perfusion.

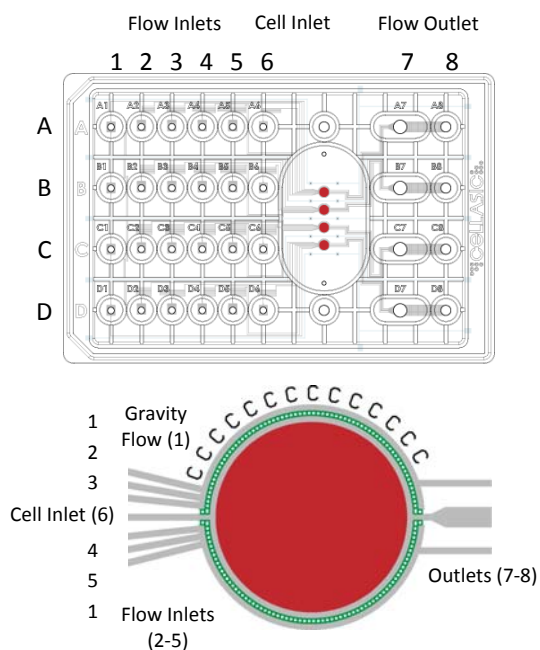


Figure 1. Plate Layout

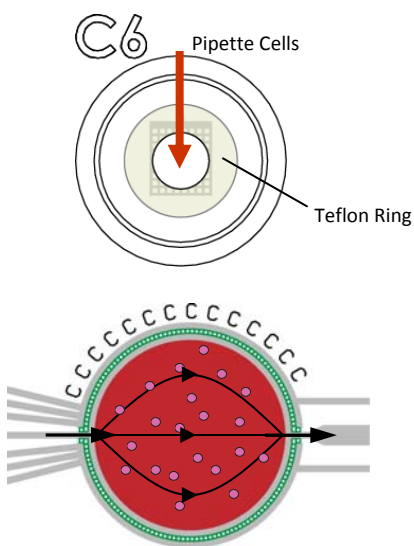


Figure 2. Capillary Cell Loading



Figure 3. Manifold Sealed to Plate on Microscope Stage

Operation Instructions

1. The M04S microfluidic plate contains 4 parallel chambers, each with a gravity flow inlet (1), 4 solution inlets (2-5), a cell inlet (6) and two shared outlets (7 & 8, Figure 1). Each row of wells (A-D) addresses the corresponding chamber. The plate is shipped pre-primed with sterile PBS, which can be replaced with a buffer of choice prior to experiment.

Cell Loading

2. Prepare a cell suspension of $1-5 \times 10^6$ cells/ml. Aspirate the cell inlet well 6, including the hole at the bottom of the well. The inner hole has a Teflon ring on its surface (Figure 2). Pipette 10 μ l of cell suspension into the hole on well 6. Without delay, aspirate the liquid in the outlet wells (7 & 8), including the holes at the bottom of the well. This will cause capillary action to pull the cells into the chamber in 3 minutes, as depicted in figure 2. Check loading density on a microscope. If more cells are desired, repeat this step.
3. Optional Pre-Culture. Remove excess cells from well 6. Pipette 300-400 μ l of culture medium into well 1 and 50 μ l of liquid into wells 7 and 8 to initiate gravity perfusion of the medium. Place the plate in an incubator for cell attachment and growth. Replace the medium in well 1 (and empty 7 & 8) every 2-3 days for long term culture.

Live Cell Imaging

5. Fill the four sets of flow inlet wells (col 2-5) with up to 400 μ l of solution. If less than 4 units will be used, fill the unused inlet wells with buffer. If wells 1 & 6 will not be used, fully aspirate the liquid from these wells.
6. Seal the microfluidic plate to the F84 manifold: Clean the manifold gasket with 70% ethanol and blot dry. Place the microfluidic plate on a flat surface. Align and set the manifold over the wells of the plate. Turn on the vacuum switch on the ONIX box and push down on the manifold with slight force for ~ 5 seconds to ensure uniform contact during sealing. When a proper seal is formed, the green "sealed" light will be lit. Make sure a proper seal is formed before proceeding. Leave the vacuum on during the course of the experiment. (If the seal is unsuccessful, turn off the vacuum switch and wait for the blue "ready" light to turn back on. Repeat the protocol above. If a seal cannot be formed, please contact CellASIC.)
7. Place assembly on an inverted microscope (Figure 3). Focus on the center of the imaging area. Each culture region is 2.8 mm in diameter with a 120 micron ceiling height.
8. (Optional) Turn on CO₂ flow to the manifold. The CO₂ will fill the air channels in the microfluidic plate and diffuse through the gas permeable chamber walls.
9. Open the ONIX FG software on a computer attached to the USB line from the control box. Select the "M04" tab from the front page. If you do not see the tab, make sure you have an updated version of the software (www.cellasic.com/Products-downloads) or contact CellASIC.

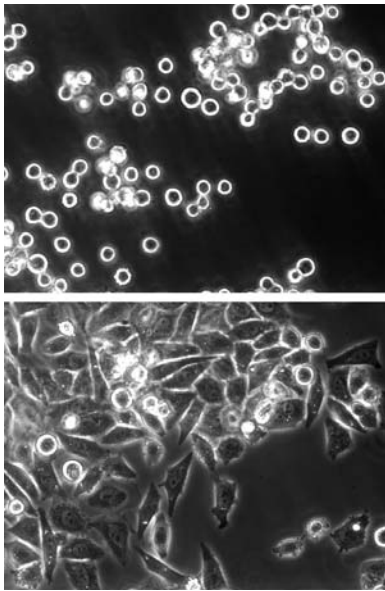


Figure 4. Cells Attaching to Glass After Overnight Perfusion

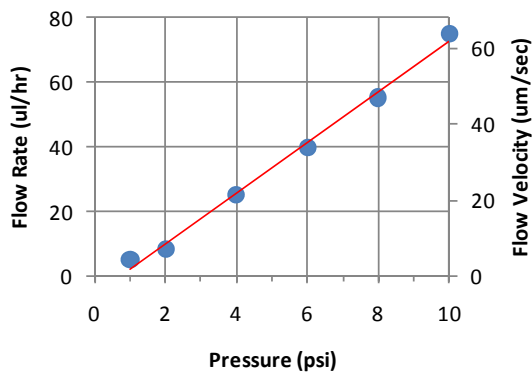


Figure 5. Flow Rate and Chamber Flow Velocity

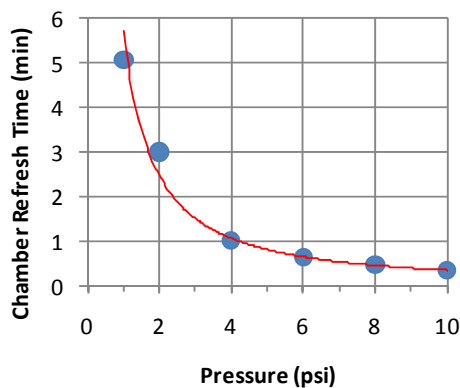


Figure 6. Total Chamber Switch Time

Operation Instructions (cont.)

- Flow properties are given in Figures 5 and 6. Figure 5 shows the total flow rate out of each inlet well. The 2nd y-axis, flow velocity, is of the laminar front moving through the chamber, or the local switch rate for cells in the chamber. Figure 6 gives the time for the laminar front to move through the entire chamber. If more than one channel is open at a time, multiply the flow rate/velocity by the number of open channels. Note: flow of liquid from wells 1 or 6 are roughly 100x faster than wells 2-5.
- Use the tabs on the right side of the software control window (Figure 8) to set up your experiment.
- For custom flow scheduling, use the "Protocol" tab to enter text based commands. The commands are given in Figure 8.
- Click "Run" to run the perfusion program.
- Acquire images using your standard microscope methods.

Pressure Driven Cell Loading

- Alternately, cells can be loaded using the ONIX flow controller by filling 50 ul of cell suspension into well 6, and flowing well 6 at 0.2 psi for ~10 seconds. Cells will exit to well 7. In general, this requires a higher density of cells compared with the capillary method.

Rapid Solution Exchange

- For experiments requiring rapid solution exchange, the following techniques can be used.
- Flow at high pressure for the initial transition, then reduce flow for long term exposure.
- For symmetric flow switching between 2 solutions, use inlets 2 & 5 for the first solution and 3 & 4 for the second solution.
- For extremely fast washing, utilize the gravity flow well (column 1). Replace the liquid in column 1 with the desired switching solution. Flow 1 at the desired pressure. The switch time will be ~100x faster than listed in Figure 6. For example, at 2 psi, the chamber will be replaced in approximately 2 seconds (~20 ul/min). Make sure not to flow well 8 for extended periods of time in order to avoid emptying the well and introducing air bubbles.
- For additional protocols, contact CellASIC.

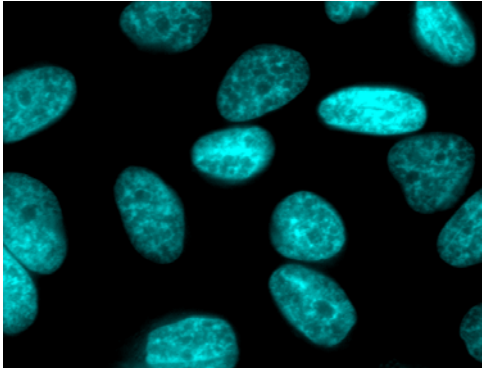


Figure 7. Nuclear staining in live cells (63X)

Operation Tips

21. During capillary cell loading, empty the bottom holes using a standard pipette to avoid introducing air bubbles. A vacuum aspirator can be used, but be careful to quickly and gently empty the liquid.
22. For long term cell perfusion, a pressure of 0.5-1 psi generally provides adequate nourishment with minimal stress.
23. For longer term experiments requiring less than 6 solutions, use multiple wells for each solution.
24. For some cell types, pre treating the chambers with medium or coating solutions may be necessary.

Software Operation

Valve on/off Buttons

Regulator Setpoints

Note: Flow X controls V1 and V2, Flow Y controls V3-V8

Status Bar

Function Tabs

Protocol Wizard

	Valve:								Flow (psi):	Duration (min):
	1	2	3	4	5	6	7	8		
1	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2	240
2	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2	240
3	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2	240
4	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2	240
5	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1	60
6	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1	60
7	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	1	240
8	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	0.25	0
9	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	0.25	0
10	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	0.25	0
11	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	0.25	0
12	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	0.25	0
13	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	0.25	0
14	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	0.25	0
15	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	0.25	0

Figure 8. ONIX FG M04 Interface

Text Commands:

- setflow X n** ; where $n = 0.25-10$ (psi) ; sets flow rate on X (orange)
- setflow Y n** ; where $n = 0.25-10$ (psi) ; sets flow rate on Y (blue)
- open V1** ; V1, V2, ..., V8, all ; opens pneumatic valve
- close V1** ; V1, V2, ..., V8, all ; closes pneumatic valve
- wait n** ; n is minutes ; holds current condition until next step
- end** ; ends the program ; shuts off all valves and resets regulators
- %** ; Put at the beginning of line for comments

```

1 % Sample Protocol
2
3 setflow Y 2
4 open V3
5 open V5
6 wait 10
7 setflow X 4.5
8 open V1
9 wait .5
10 close all
11 end
12

```