

B04A Microfluidic Bacteria Plate: 0.7-3.0 μm

B04A-02

Description: The B04A plate contains 4 independent chambers, each with 5 upstream inlet channels. Each chamber has a 6-tier microfluidic trap of 0.7, 0.9, 1.1, 2.0, 3.0 and 4.0 micron heights to hold cells in a single focal plane during long term, high magnification, perfusion imaging.

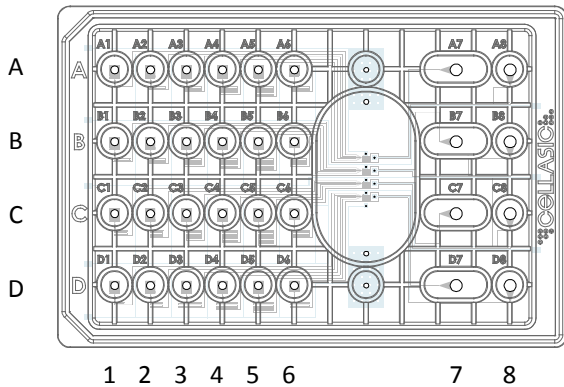
Applications:

- Time-lapsed, high magnification imaging of bacteria cells
- Long term continuous perfusion experiments (24+ hours)
- Solution exchange experiments (induction, inhibition, drug dosing, etc.)
- Comparing up to 4 different cell types or exposure conditions in parallel
- Cell division tracking (follow mother/daughter cells over generations)
- Temperature and gas atmosphere control (temperature shift, anoxic, etc.)

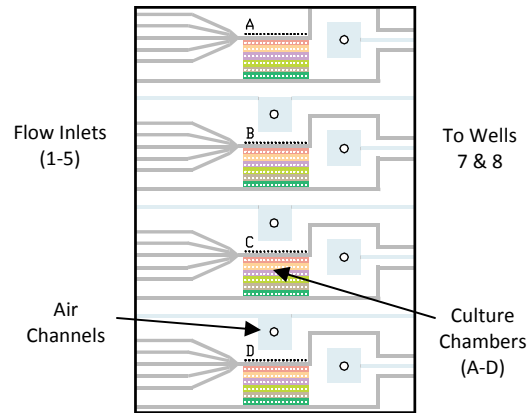
Product Specifications:

- Use with ONIX-262 system and F84 manifold
- SBS standard footprint frame (fits to typical 96 well stage holders)
- #1.5 thickness (170 μm) glass slide bottom (in contact with cells)
- Six trap heights per chamber (0.7, 0.9, 1.1, 2.0, 3.0, 4.0 micron)
- Laminar flow rates of 1-20 $\mu\text{l/hr}$ (typical chamber refresh times of 10-60 seconds)
- Microfluidic gas exchange channels

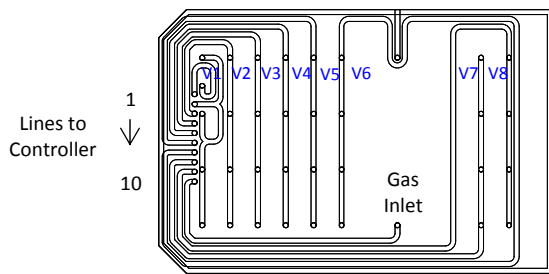
Plate Design:



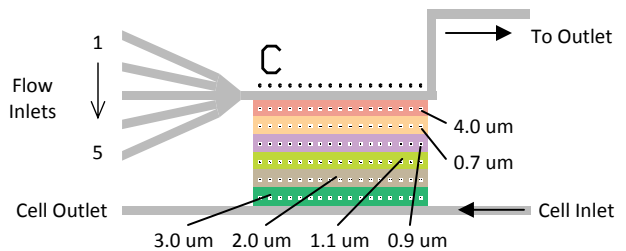
The B04A has 4 independent units (A-D), each with 5 inlet wells (1-5), a cell inlet (8), and a large outlet well(7). All four culture chambers are located under a single imaging window.



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.

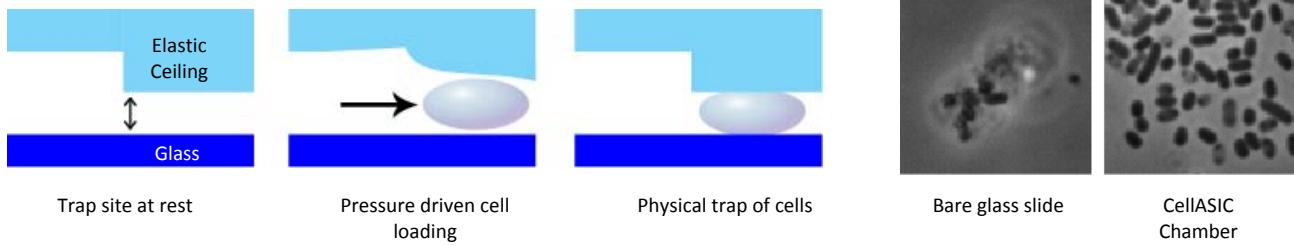


The F84 manifold has 8 pressure channels (V1-V8) to control flow rates through the microfluidics. A vacuum line is used to seal the plate to the manifold, and a gas line enables atmosphere control.



The culture chamber is 2.0 x 1.2 mm in area with heights of 4.0, 0.7, 0.9, 1.1, 2.0, and 3.0 micron as indicated. Support posts (with position markers) maintain a uniform ceiling height.

Cell Trapping Mechanism

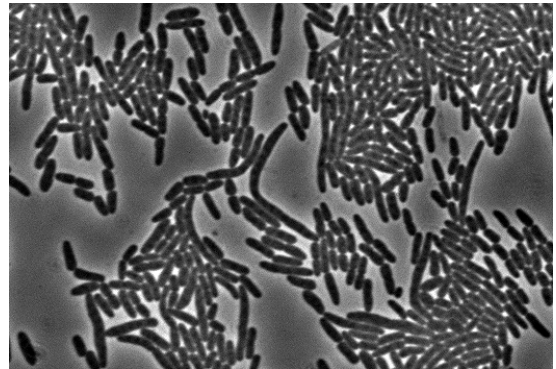


The microfabricated chamber gently holds cells against the glass imaging surface to maintain a single focal plane during perfusion imaging experiments. The B04A has 6 trap heights of 0.7, 0.9, 1.1, 2.0, 3.0, and 4.0 micron.

Imaging Quality

The B04A plate is designed for optimal image quality for high end microscopes.

- The cells are held against a #1.5 thickness (170 um) glass coverslide floor, making the plate compatible with immersion and high NA objectives
- The large imaging window allows light to pass freely for optimal phase contrast quality
- The four microchambers are located within a 1 cm area, minimizing stage travel and focus drift

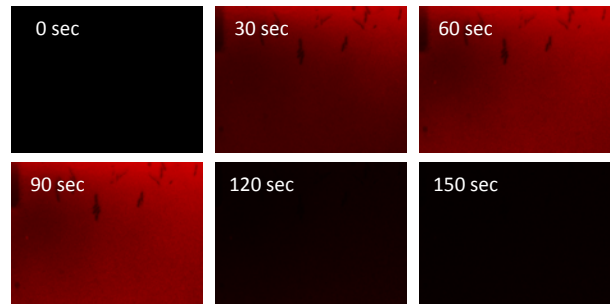


Phase contrast image of *E. coli* cells in the 0.7 um trap of the B04A plate (100X objective).

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 ul/hr allow continuous multi-day experiments on your microscope

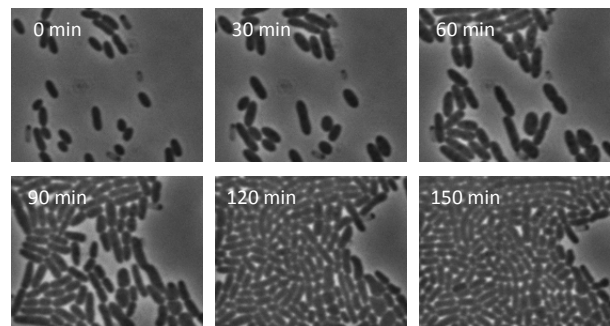


Solution exchange profile between a fluorescent dye and buffer solution in a 100X objective field of view.

Long Term 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 and microfabricated air diffusion channels allow user control of the gas atmosphere in equilibrium with imaged cells



E. coli (BL21) cells tracked over a few cell division cycles in the CellASIC B04A chamber.

Cells courtesy the Lu Lab, MIT

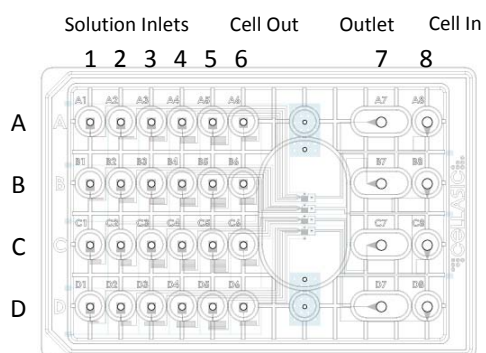


Figure 1. Plate Layout

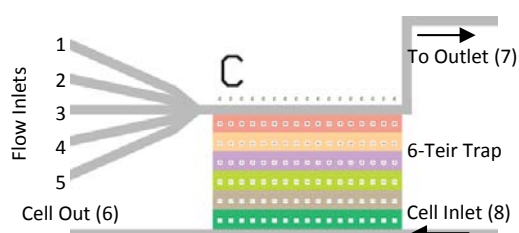


Figure 2. Unit Layout

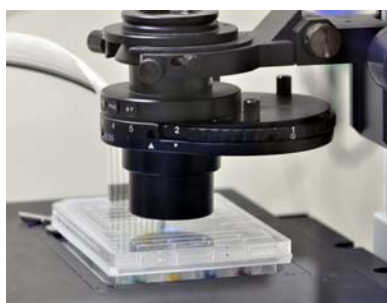


Figure 3. Plate with Manifold on Stage

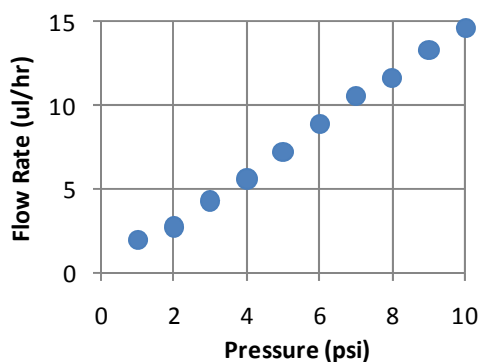


Figure 4. Flow Rate

Operation Instructions

1. The B04A microfluidic plate contains 4 parallel chambers, each with 5 solution inlets (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.
2. Fill the five sets of flow inlet wells (col 1-5) with up to 300 μ l of solution. If less than 4 units will be used, fill the unused inlet wells with buffer. Empty the cell outlet (col 6) and flow outlet well (col 7).
3. Fill the cell inlet wells (col 8) with 50 μ l of cell suspension, making sure to cover the hole at the bottom of the well. Alternately, 10 μ l of cell solution can be dispensed directly into the bottom hole of the well. A density of $1-20 \cdot 10^6$ cells/ml is recommended depending on desired trapping density.
4. 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 step 4 above. If a seal cannot be formed, please contact CellASIC.)
5. Place assembly on an inverted microscope (Figure 3). Focus on the center of the imaging area. The trapping region is 3×1.2 mm in size with 4.0, 0.7, 0.9, 1.1, 2.0, and 3.0 micron ceiling heights in series (from top to bottom) as depicted in Figure 2. A series of posts in the chamber maintain uniform ceiling height. The posts are numbered (1-6) in descending order.
6. Open the ONIX FG software on a computer attached to the USB line from the control box. Select the "B04" tab from the "Other" page. If you do not see the tab, make sure you have an updated version of the software (www.cellasic.com/ONIX-downloads) or contact CellASIC.
7. On the control window (see figure 6), set "Cell Load" parameters to 8 psi and 5 seconds. Click the "Load Cells" button. Depending on your cell type/strain and desired cell density, modify and repeat the loading process as necessary. Make sure wells 6 and 8 are empty to ensure proper solution exchange during perfusion.
8. Wash un-trapped cells by flowing one or more inlet solutions at 4 psi for 5 minutes. This will also serve to prime the specified inlet channel by flushing out the shipping solution. Cells remaining in the trap regions are now firmly held in x,y,z for imaging.
9. Figure 3 shows the total flow rate out of each inlet well (col 1-5) at the specified pressures. Note: flow rates from channels 6, 7, and 8 will be over 10X those of the inlets.

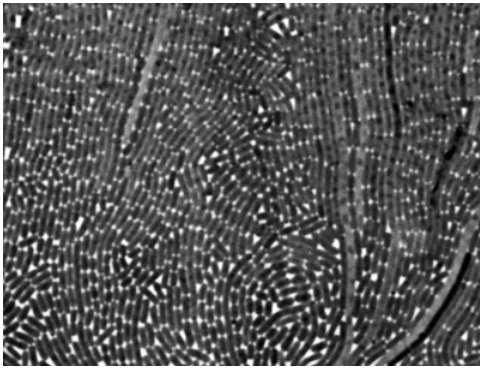


Figure 5. High density bacterial (*E. coli*) monolayer in the microfluidic chamber.

Cells courtesy of the Lu Lab, MIT

Operation Instructions (cont.)

10. Use the tabs on the right side of the software control window to set up your experiment. Manual operation is possible by clicking on the valve buttons and dragging or typing in values on the flow bars on the left of the software control window.
11. For custom tuning of flow scheduling, use the "Protocol" tab to enter text based commands. The commands are given in Figure 6.
12. Click "Run" to run the perfusion program.
13. Acquire images using your standard microscope methods.

Software Operation

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

Figure 6. ONIX FG 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

Cell Load	Gradient	Switch	Protocol
1			% Example Program:
2			setflow Y 2
3			open V3
4			open V5
5			wait 10
6			setflow X 4.5
7			open V1
8			wait .5
9			close all
10			end
11			
12			