

# CellASIC:M16 Microfluidic Plate Operating Instructions

For Serial Numbers starting with 12-

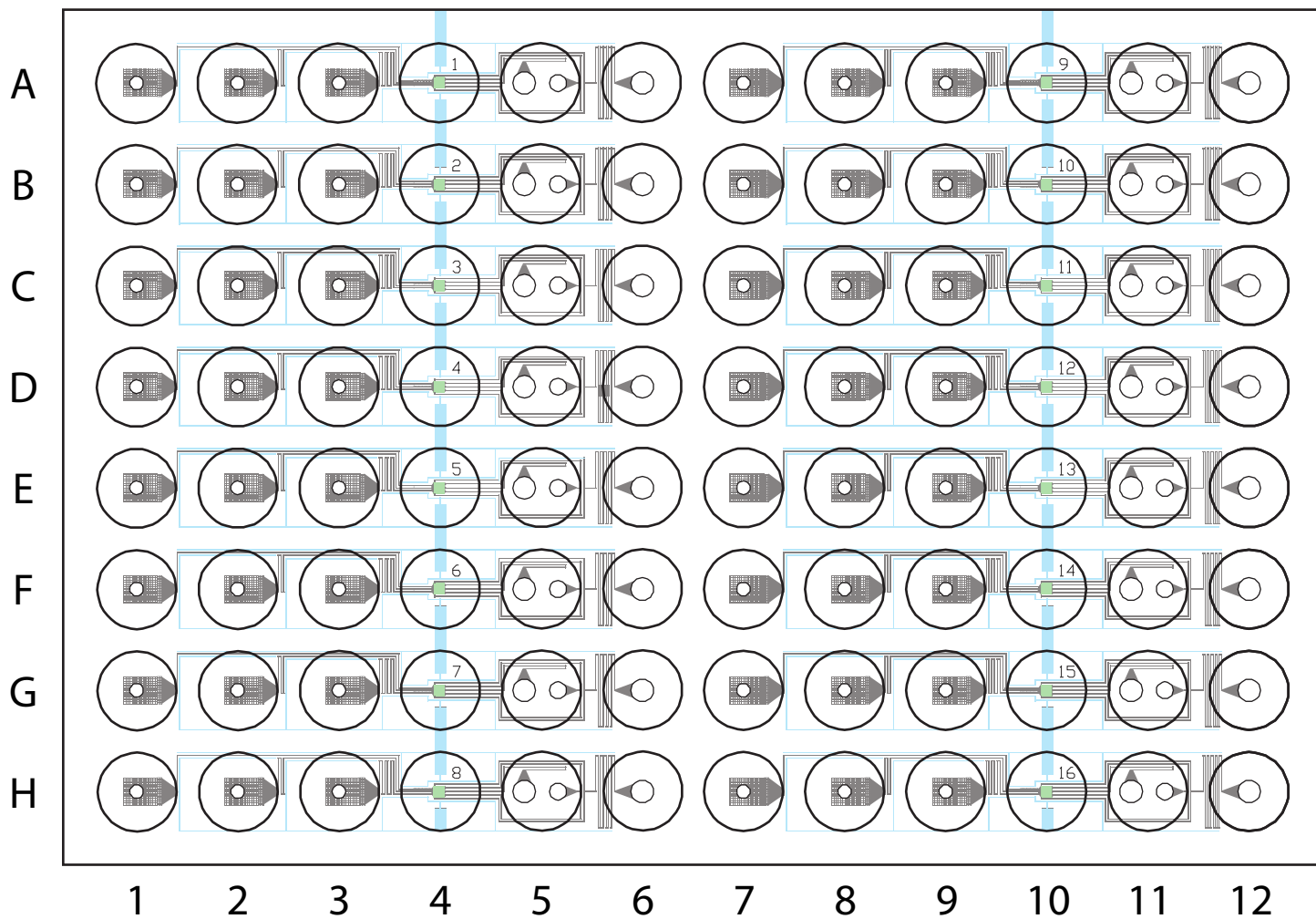


Figure 1 Well Layout

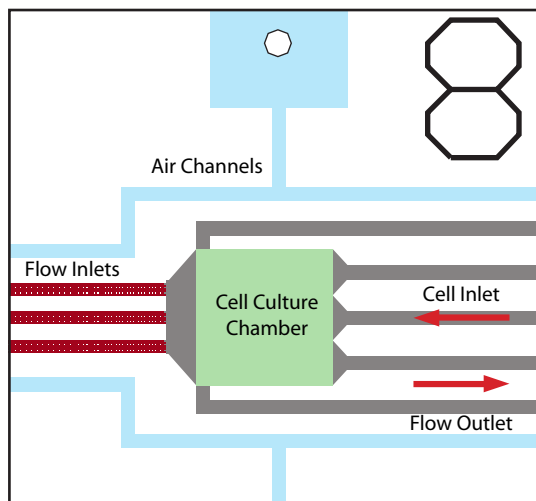


Figure 2 Trap Area

The M16 is designed for multiplexed perfusion imaging of mammalian cells on 16 different cell samples at once, each with 3 upstream solution inlets.

Each row of the plate (A-H) contains 2 fully independent flow units (6 wells each), consisting of 3 solution inlets (col 1, 2,3 and 7,8,9), a cell culture/imaging well (col 4 and 10), cell inlet wells (col 5 and 11), and an outlet (col 6 and 12).

The perfusion inlets are designed to allow laminar flow switching between the three solutions with precise kinetics.

Each chamber is 1.0 x 1.0 x 0.12 mm in size for culture of adherent or non-adherent cells. A unit marker is etched near the chamber, indicating the chamber number (1-16).

A thin cover glass bottom allows for optimum image quality.

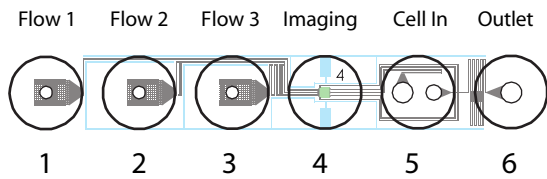


Figure 3 Well Layout

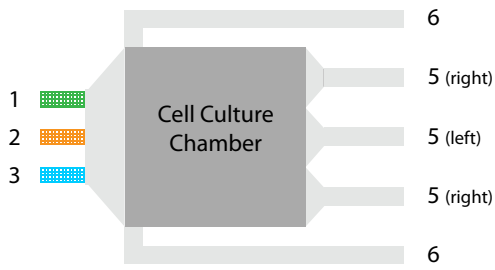


Figure 4 Culture Area

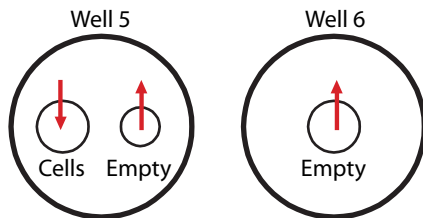


Figure 5 Cell Loading Step

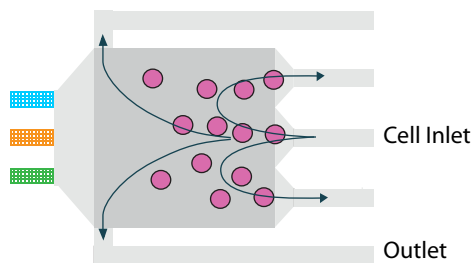


Figure 6 Cell Loading Flow Profile

1. The M16 microfluidic plate consists of 16 identical flow units. The layout of a single unit is depicted in Figure 3. Each unit has 6 well positions: 3 flow inlets (1-3), an imaging area (4), a cell inlet (5), and an outlet well (6). The 1.0x1.0x0.12 mm cell culture chamber is located under well 4, with the design depicted in Figure 4. The plate is shipped pre-primed with 100  $\mu$ l PBS containing Penicillin/Streptomycin/Amphotericin B in each well, which can be replaced with a buffer of choice prior to experiment. The plates have a guaranteed shelf-life of 6 months.

### Cell Loading

2. Prepare a cell suspension of  $0.5\text{-}5 \times 10^6$  cells/ml. Completely aspirate the cell loading well (5) and outlet well (6), **making sure to carefully remove the liquid from the 2mm diameter inner holes at the bottom of the wells** (see Figure 5). *Caution:* Excessive aspiration may introduce air bubbles into the microfluidics.
3. Pipet 2-6  $\mu$ l of cell suspension into the **left** 2mm hole at the bottom of the cell inlet well (5). Capillary force will pull the liquid through the microfluidics, and cells will accumulate in the culture area over ~2-5 minutes (Figure 6). Verify cell loading density by viewing the culture chambers under a phase contrast microscope. If desired, re-aspirate the right hole to re-establish capillary flow and increase cell numbers. In general, cells will divide more quickly when loaded at higher density (see Figure 7).

### Cell Culture in Incubator (optional)

4. Cells can be cultured in the plates with a standard incubator prior to microscopy (see Figure 8). Wash and replace well 5 with 300  $\mu$ l of cell culture medium. Add 50  $\mu$ l of PBS or medium to well 6. This will cause the fresh medium to perfuse the loaded cells by a gravity flow from 5-->6 at a rate of 20  $\mu$ l/day. Place the plate in a standard incubator for attachment and growth. Aspirate liquid from 6 and add medium to 5 every 3-5 days to maintain perfusion flow.

### To Perform Flow Switching Experiment

5. Turn on the ONIX Perfusion System and warm up any necessary temperature/CO<sub>2</sub> controllers. Thoroughly clean the manifold gasket with 70% ethanol to sterilize and improve sealing quality.
6. Replace the PBS in the Flow wells (1,2,3) with up to 300  $\mu$ l of the desired solutions for flow switching. For best results replace all other wells with 50  $\mu$ l of buffer.
7. Seal the microfluidic plate to the ONIX Perfusion System manifold: 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 System 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 on the ONIX front panel will turn on. **Make sure a proper seal is formed before proceeding.** If sealing is unsuccessful, turn off the vacuum switch until the blue "Ready" light turns on and repeat protocol above. Leave the vacuum on during the course of the experiment.

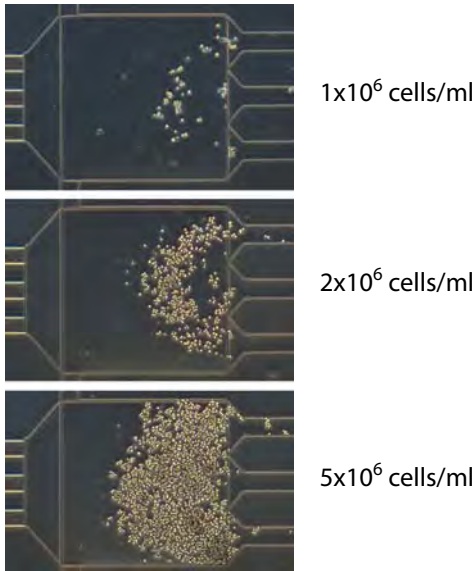


Figure 7 Cell Loading Density

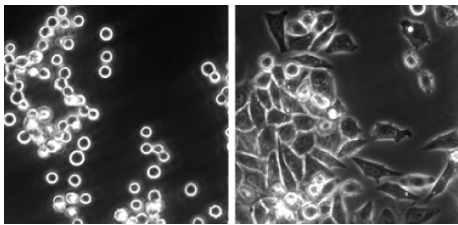


Figure 8 Cell Adhesion and Culture

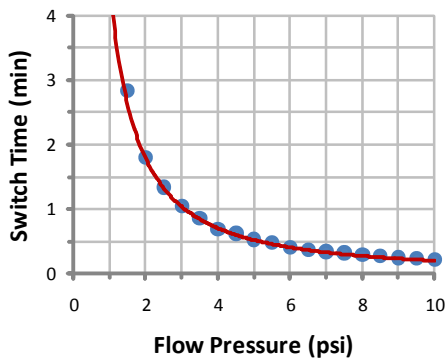


Figure 9 Flow Switching

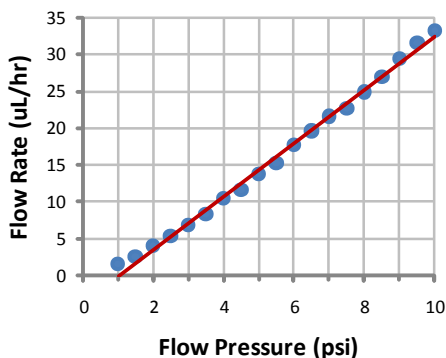


Figure 10 Flow Rate

(Note: All plates are pre-screened to maintain a proper seal. If you experience plates that are unable to seal, please contact us for replacement.)

- Place assembly on an inverted microscope. The plate frame will fit to any standard 96 well plate holder. Focus on the center of the "imaging" well (4). The cells will be adhered to the #1.5 thickness (170  $\mu$ m) glass coverslide, which forms the bottom surface of the culture unit.
- Use the ONIX FG computer program to schedule flows to the cells. Flow rates are given in Figures 9 and 10. The switch time is defined as the time to completely refresh the chamber medium volume. It will take about 2-3 minutes to flush out the residual PBS in the upstream channels the **first time** a channel is used. Except in cases where rapid switching is desired, flow at 2-3 psi is recommended.

## ADDITIONAL PROTOCOLS

### Creating a Spatial Gradient

A unique feature of the M16 microfluidic design is the ability to maintain stable spatial gradients. When 2 or more solutions are flowed through the chamber in parallel, they will form defined boundary interfaces. Molecular diffusion across these interfaces will create a spatial gradient. Because this is a flow based gradient, the concentration profile is stable for long periods of time as long as the flow rate is kept steady.

Since different solutes have different diffusion rates, it is best to first measure the profile of your gradient at different flow rates using appropriate fluorescent dyes.

### Pressure Driven Cell Loading

As an alternative to the capillary driven cell loading process, it is possible to use the ONIX flow controller to load cells into the culture chamber. Pipet 50  $\mu$ l of cell suspension into well 5 (cover both holes) and flow at 0.5 psi.

### Fast Washing of Culture Chamber

In some cases, it may be desired to pressure wash the cell culture chamber. This can be done by replacing the liquid in well 5 with the wash solution and flowing at high pressure from well 5. This will wash the chamber contents out to well 6. Note: the flow rate through well 5 is over 100x faster than through 1,2,3.

### Surface Coating

The culture chamber can be exposed to standard 2D surface coatings prior to cell loading. For shorter coating times (<20 min), use the capillary method described in step 3. For longer coating times, follow this with gravity perfusion by adding 300  $\mu$ l of coating solution to well 5 with 50  $\mu$ l of buffer in well 6.