

# CellASIC:Y16 Microfluidic Plate Operating Instructions

For Serial Numbers starting with 08-

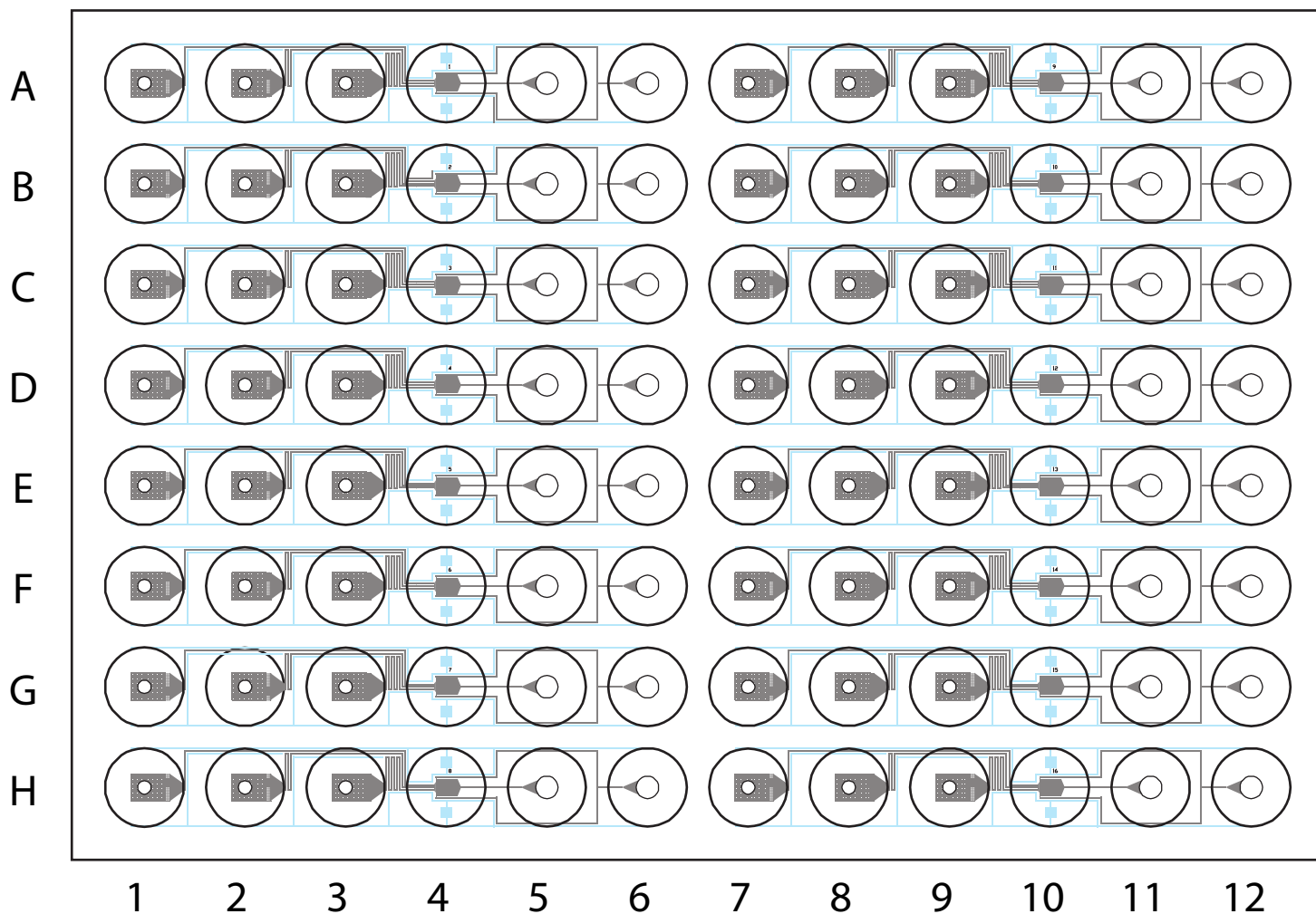


Figure 1 Well Layout

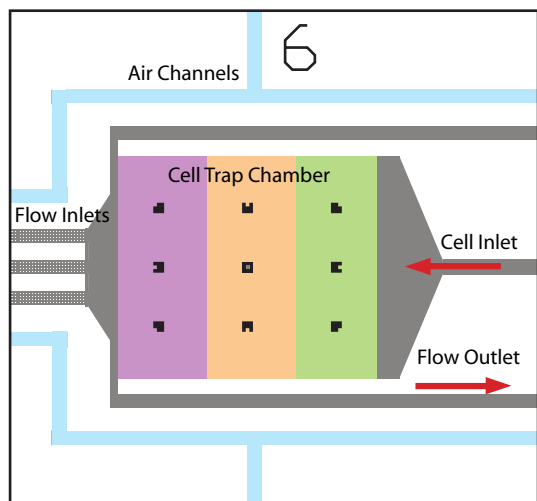


Figure 2 Trap Area

The Y16 is designed for multiplexed perfusion imaging of yeast 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 1.75 x 1.5 mm in size, divided into 3 trapping regions of 4.0, 4.5, and 5.0 micron in height for trapping of yeast cells into a single focal plane. Nine position markers are etched in the trap area. A unit marker (1-16) is written near each chamber.

A thin cover glass bottom allows for optimum image quality.

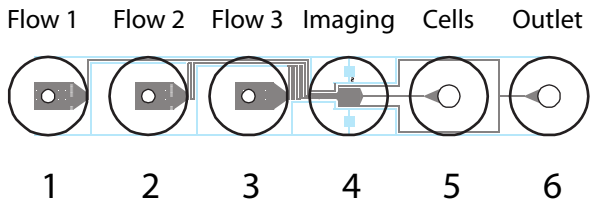


Figure 3 Single Unit Well Layout

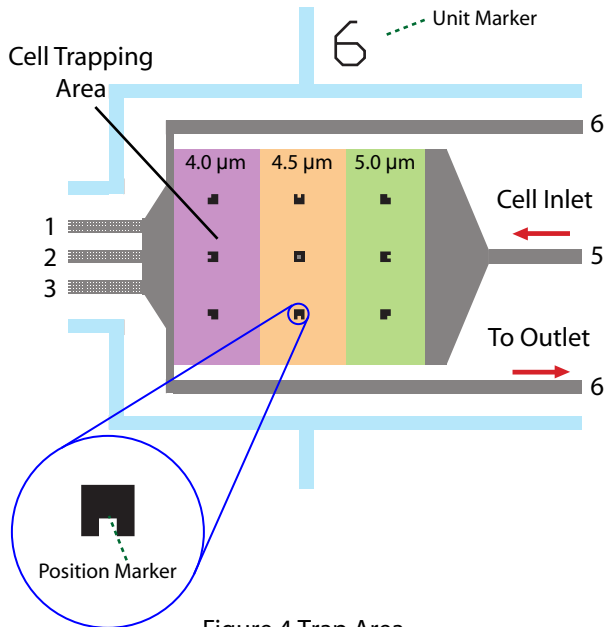


Figure 4 Trap Area

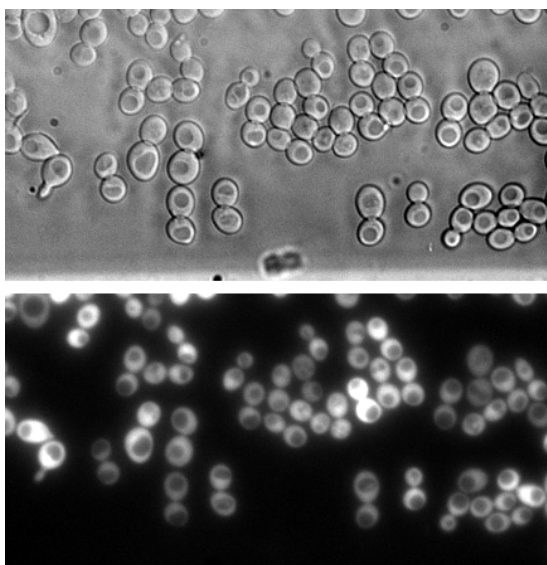


Figure 5 Cell Microscopy

1. The Y16 microfluidic unit consists of 16 identical flow units as depicted in Figure 3. Each unit has 3 inlets, an imaging area, a cell loading, and an outlet. The plate is shipped pre-filled with sterile dl water containing penicillin/streptomycin, which can be replaced with a buffer of choice prior to experiment. Plate performance guaranteed up to the 6 month expiration date.

### Cell Loading and Perfusion Operation

2. Fill the three sets of flow inlet wells with up to 300  $\mu\text{l}$  of solution. These solutions will be exposed to the cells during long term culture.
3. Fill the cell inlet wells with 10-50  $\mu\text{l}$  of cell suspension. A density of  $1\text{-}20 \cdot 10^6$  cells/ml is recommended depending on desired trapping density.
4. To seal the microfluidic plate to the manifold: Clean the clear rubber gasket of the manifold with 70% ethanol and let dry. Place the microfluidic plate on a flat surface. Align and set the manifold over the wells of the plate. Turn on the vacuum on the ONIX system and push down on the manifold with slight force for  $\sim 5$  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.** Leave the vacuum on during the course of the experiment.

(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.)

5. Place assembly on an inverted microscope. Focus on the center of the "imaging" area to find the cell trapping chamber. The trapping region is  $1.75 \times 1.5 \text{mm}$  in size, divided into three rectangular traps of 4.0, 4.5, and 5.0 micron ceiling heights in series as depicted in Figure 4.
6. (Optional) Using the ONIX FG software, prime flow channels by turning on all 3 flow inlets for  $\sim 5$  minutes at 6 psi to flush out shipping solution. It is recommended that this be followed by a 1 minute wash with only the first solution. Turn off all flow switches after washing.
7. Activate cell loading (via ONIX FG software) to transport cells into the trapping region. The suggested loading protocol is at 6-8 PSI for 10 seconds. The loading profile will depend on cell density, cell size, and desired trapping density. When loaded, the trapping region will look similar to Figure 5. Repeated pulsing of the cell load button will push more cells into the trapping chamber. The cell trapping mechanism is depicted in Figure 6.
8. Turn on flow to the first exposure solution. This will remove cells that are not trapped. After  $\sim 5$  minutes of flow, the remaining cells will be those firmly held in x,y,z for imaging.

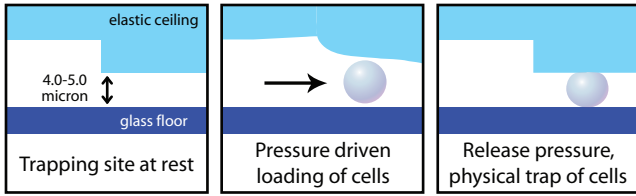


Figure 6 Cell Trap Schematic

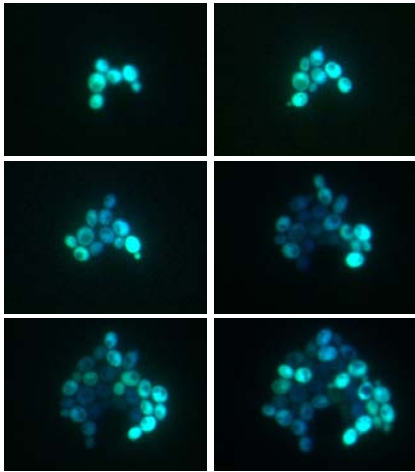


Figure 7 Perfusion Culture

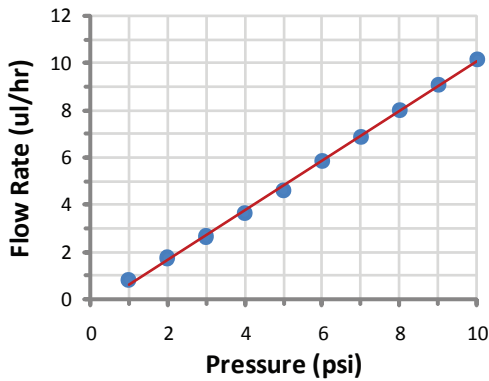


Figure 8 Flow Rate

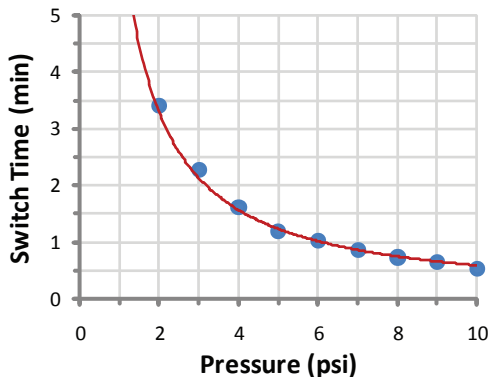


Figure 9 Switch Time

- Use the ONIX FG computer program to schedule flows to the cells. Flow rates are given in Figures 8 and 9 . 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 ~4 psi is recommended.

## ADDITIONAL PROTOCOLS

### 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 6 with the wash solution and flowing at high pressure from well 6. This will wash the chamber contents out to well 5. Note: the flow rate through well 6 is over 50x faster than through 1,2,3.

### Surface Coating

The culture chamber can be exposed to standard 2D surface coatings prior to cell loading. Add 50-300  $\mu\text{l}$  of coating solution to well 5 (cell loading inlet) and perfuse through the chamber at 2 psi for 15-60 minutes. Repeat as necessary. (The flow rate at 2 psi is ~100  $\mu\text{l/hr}$ .)

### Re-using the Y16 Plate

In some cases, not all of the 16 chambers will be utilized in a single experiment. The unused chambers can be saved for future experiments as follows. After the experiment is complete, check to ensure all the wells of the unused chambers still contain liquid. If necessary, add buffer to the wells to prevent drying out. Store the plate at 4 °C until next use. For longer term storage, apply a plate sealing film (such as the foil that is shipped with the plates) to prevent evaporation.