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User Guide

CellASIC® ONIX Y04T-04 Microfluidic Plate

For research use only. Not for use in diagnostic procedures.

Introduction

The CellASIC® ONIX Y04T Microfluidic Plate is a 4-chamber cell culture plate designed for use with the CellASIC® ONIX2 Microfluidic System and ONIX2 Manifolds, enabling single cell trapping and generational monitoring in real time. This bio-inspired plate provides a controlled and dynamic microenvironment for cells, which when used in conjunction with the ONIX2 system, permits perfusion-based, long-term, live-cell analysis with automated solution switching. The easy-to-use format and superior technology redefine the standard for microfluidics-based experimentation.

Applications

- Single cell trapping and monitoring
- Time-lapse analysis of yeast cells including division tracking (follow mother/daughter cells over generations)
- Temperature and gas atmospheric control (temperature shift, anoxic conditions, etc.)
- Long-term continuous perfusion experiments
- Solution exchange experiments (induction, inhibition, drug dosing, etc.)
- Comparison of up to 4 different cell types or exposure conditions (media components) in parallel

Plate Description

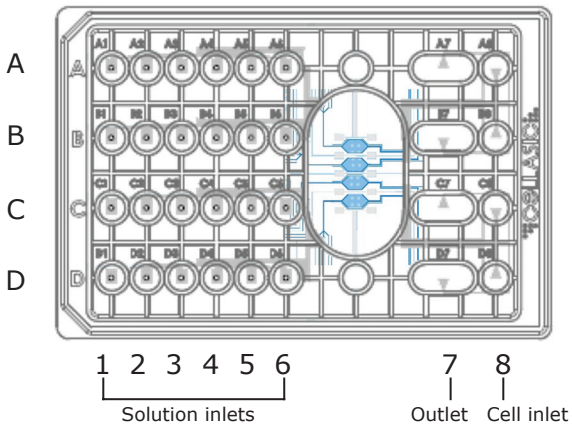

Figure 1. Plate configuration

The Y04T plate has 4 independent units (A–D), each with 6 inlet wells (1–6), a cell inlet (8), and a large outlet well (7). Each row of wells (A–D) addresses the corresponding culture chamber. The plate is shipped pre-primed with a PBS (phosphate-buffered saline) solution, which can be replaced with a buffer of choice prior to experiment. Each chamber has an array of 104 barrier trap pads 4.0 µm in height to hold cells in a single focal plane during long-term analysis. The plate is for single use only.

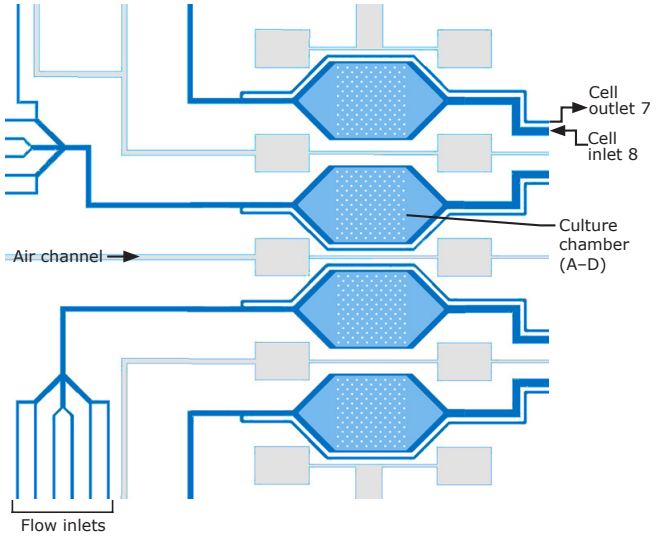

Figure 2. Chamber viewing window

All four culture chambers are located under a single viewing window to minimize travel distance for high-magnification phase objectives.

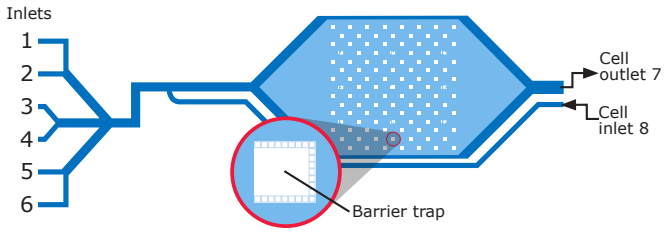

Figure 3. Culture chamber

The culture chamber hexagon marquise is 3.0 × 6.0 mm with a ceiling height of 15.5 µm. Within each chamber, the culture array area is 3.0 × 3.0 mm with barrier trap heights of 4.0 µm. Nine position markers indicate unit number and relative position.

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The inlet/outlet functions and minimum/maximum recommended volumes for each culture unit are listed below.

	Function	Minimum Volume (µL)	Maximum Volume (µL)
Inlet 1	Inlet for solution switching	50	300
Inlet 2	Inlet for solution switching	50	300
Inlet 3	Inlet for solution switching	50	300
Inlet 4	Inlet for solution switching	50	300
Inlet 5	Inlet for solution switching	50	300
Inlet 6	Inlet for solution switching	50	300
Outlet 7	Accepts flow-through from culture chamber	50	795
Inlet 8	Cell inlet for loading cells into culture chamber/Accepts flow-through from culture chamber	50	265

Cell Trapping Mechanism

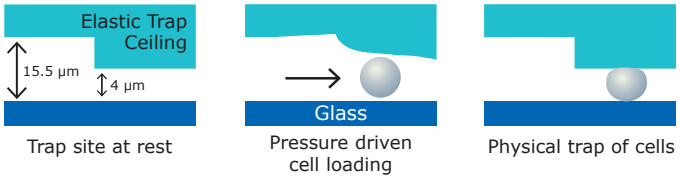
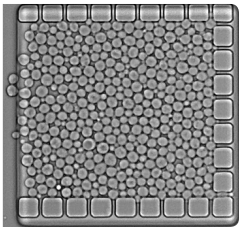


Figure 4. Cell trapping mechanism

The microfabricated chamber and polydimethylsiloxane (PDMS) barrier gently hold cells against the glass viewing surface to maintain a single focal plane during perfusion analysis experiments. The traps of the Y04T plate are 100 × 100 µm in size and 4.0 µm in height. Small pillars bracket each trap pad's perimeter on three sides and act to retain resulting daughter cells during long-term culture. To maximize capture efficiency, each trap is "open" on the side facing the direction of flow during cell loading.



Manifold Description

The CellASIC® ONIX2 heated (CAX2-MXT20) or basic (CAX2-MBC20) manifolds connect the microfluidic plate to the CellASIC® ONIX2 Microfluidic System.

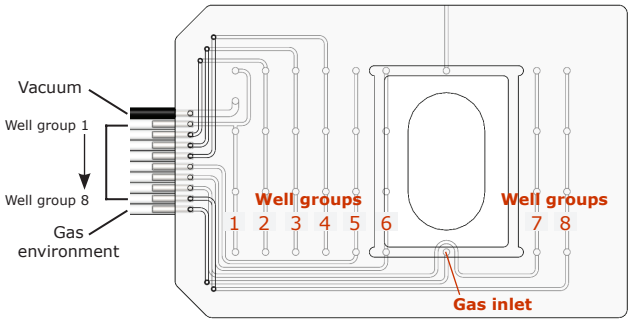


Figure 5. Lines to CellASIC® ONIX2 Microfluidic System

Flow control is achieved using air pressure above the liquid in each well. Multiple wells on a plate are grouped together and addressed by a single pneumatic line via the manifold. Each set of wells is called a "well group." A vacuum line is used to seal the plate to the manifold, and a gas line enables atmospheric control.

Flow Properties

Flow properties of wells 1–6 are shown in Figure 6. The figure shows the flow rate out of the well as a function of pressure. If more than one channel is pressurized, multiply the well flow rate by the number of pressurized channels to derive the overall flow rate.

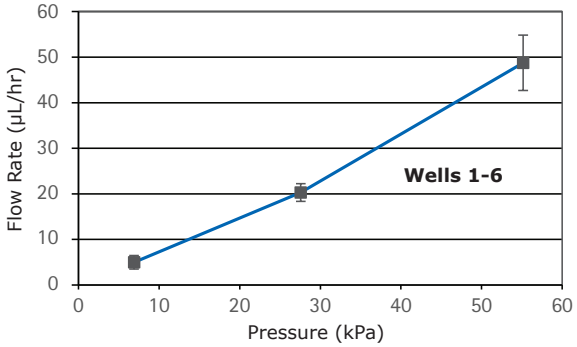


Figure 6. Flow rate for wells 1–6

Plate Storage

Store at room temperature. Do not store in direct sunlight.

Limitations

The plate is incompatible with acetic acid and organic solvents such as acetone, ethanol, and methanol. Plates should be tested for compatibility with other acids or organic solvents prior to use.

Plate Operation

If temperature control is needed, use the CellASIC® ONIX2 Manifold XT (CAX2-MXT20). Refer to the CellASIC® ONIX2 Microfluidic System User Guide for setup instructions.

Plate Priming (Optional)

1. Aspirate the PBS solution from wells that will be used for the experiment and add 250 µL of your solution/medium to these wells. Make sure that the unused solution inlet wells are filled with buffer.
2. **Steps 2–4 are optional:** If your experiment requires complete removal of PBS, replace the PBS in the solution (1–6) and cell inlet (8) wells with 150 µL of your desired priming solution.
3. Seal the microfluidic plate to the ONIX2 manifold according to the CellASIC® ONIX2 Microfluidic System User Guide.
4. Open the CellASIC® ONIX2 Software, select one of the **New Experiment** options, and find the Y04T plate on the drop down list. On the **Manual Mode** tab (Figure 7), click on the **Run liquid priming sequence** button. The recommended pressure and flow times for well groups 1–6 are 55.2 kPa (8 psi) and 15 seconds, respectively. For more information on creating protocols, refer to the CellASIC® ONIX2 Microfluidic System User Guide.

Cell Loading

Pressure-Driven Method Using the CellASIC® ONIX Microfluidic System

1. Prepare a yeast/cell suspension of 0.1–2.0 × 10^6 cells/mL. This concentration may need optimization depending on the yeast strain and desired trapping density.
2. Aspirate solution from cell inlet wells 1–8 without disturbing the cutouts.

3. Pipette 100 µL of cell suspension into cell inlet well 8 and 300 µL of culture broth into solution inlet wells 1–6, making sure to cover the hole at the bottom of the well.
4. Seal the microfluidic plate to the ONIX2 manifold according to the CellASIC® ONIX2 Microfluidic System User Guide.
5. Open the CellASIC® ONIX2 Software, select one of the **New Experiment** options, and find the Y04T plate on the drop down list. On the **Manual Mode** tab (Figure 7), click on the **Run cell loading sequence** button. To load cells, the recommended pressure and flow time for well groups 1–6 and 8 are 55.2 kPa (8 psi) and 15 seconds, respectively. These conditions may need to be optimized depending on your cell type/strain and desired trapping density.
6. Assess the loading density on a microscope. If insufficient loading has occurred, repeat the loading protocol.
7. To clear the chamber of untrapped cells, flow from well groups 1–6 at 55.2 kPa (8 psi) for 15 seconds.
8. Proceed to Cell Culture or Solution Switching sections.

Cell Culture

Cell Culture with CellASIC® ONIX2 Microfluidic System

1. Aspirate solution from wells that will be used for perfusion (wells 1–6) and for the waste reservoir (wells 7–8). Add 300 µL medium to these wells. Make sure that the unused solution inlet wells are filled with buffer.
2. Seal the microfluidic plate to the ONIX2 manifold according to the CellASIC® ONIX2 Microfluidic System User Guide.
3. Open the CellASIC® ONIX2 Software, select one of the **New Experiment** options, and find the Y04T plate on the drop down list. Click on the **Protocol Editor** tab and enter the desired steps and conditions. For an example, see Figure 8. For wells 1–6, the recommended pressure of 6.9–13.8 kPa (1–2 psi) provides adequate nourishment with minimal stress. For information on creating a protocol, refer to the CellASIC® ONIX2 Microfluidic System User Guide.

   NOTE: Due to cell division, the traps will fill with cells, and over time, there is an increased likelihood of cells escaping. These cells will continue to divide outside the traps, potentially impacting overall cell growth and the ability to visualize the traps. To overcome this issue, a routine flushing protocol is recommended. Outlined below (and in Figure 8) is a standard culture protocol with media perfusion (for cell growth) and flushing steps.

Cell Growth	• Pressurize wells 1 and 2 at 6.9 kPa (1 psi) for 1 hour
Chamber Flush	• Pressurize wells 1–6 at 55.2 kPa (8 psi) for 5 seconds • Pause for 5 seconds • Pressurize wells 1–6 at 55.2 kPa (8 psi) for 5 seconds • Pause for 5 seconds • Pressurize wells 1–6 at 55.2 kPa (8 psi) for 5 seconds

Repeat above steps for 24 hours.

This protocol is designed for a cell culture experiment lasting up to 24 hours. For experiments > 24 hours (up to 72 hours), the culturing protocol can be extended by changing the media source for the Cell Growth step to wells 3 and 4 (for 24–48 hours) and then wells 5 and 6 (for 48–72 hours). The flushing protocol remains unchanged.

4. To monitor cell growth, place the sealed plate/manifold assembly on an inverted microscope.
5. During extended perfusion experiments, empty well 7 periodically to avoid outlet overflow into the manifold tubing and perfusion system. On the **Run** tab in the CellASIC® ONIX2 Software, click the **Pause** button. Press the **Seal** button on the instrument or in the **Tools** drop down menu, click on **Unseal Plate**. Remove the manifold from the plate, and aspirate well 7. Reseal the manifold to the plate, then on the **Run** tab, click **Resume** to restart the perfusion protocol.

NOTE: This protocol has been validated to accommodate up to 72 hours of cell culture without the need to add media or empty outlet well 7. No outlet overflow was observed.

Solution Switching

1. Aspirate solution from the chosen inlet wells (1–6). Add up to 250 µL of the desired solution to the wells. If less than four units (A–D) are to be used, fill the unused inlet wells with buffer to prevent dehydration.
2. Seal the microfluidic plate to the ONIX2 manifold according to the CellASIC® ONIX2 Microfluidic System User Guide.
3. Open the CellASIC® ONIX2 Software, select the Y04T plate on the drop down list, and click on the **Protocol Editor** tab (Figure 8) to create and initiate custom protocols. To manually control flow, use the **Manual Mode** tab to select the desired wells, pressure, and temperature (if using heated manifold). For information on automated protocols or manual perfusion, refer to the CellASIC® ONIX2 Microfluidic System User Guide.

NOTE: For experiments requiring rapid solution exchange, the following technique can be applied: Flow at 55.2 kPa (8 psi) for 5 seconds, then reduce flow to standard pressure 6.9–13.8 kPa (1–2 psi) for long-term exposure.

For symmetric flow switching between 2 solutions, use inlets 2 and 5 for the first solution, 3 and 4 for the second solution.

Software Operation

The figures below show two modes for running experiments using the CellASIC® ONIX2 software. Refer to the CellASIC® ONIX2 Microfluidic System User Guide for details on software features.

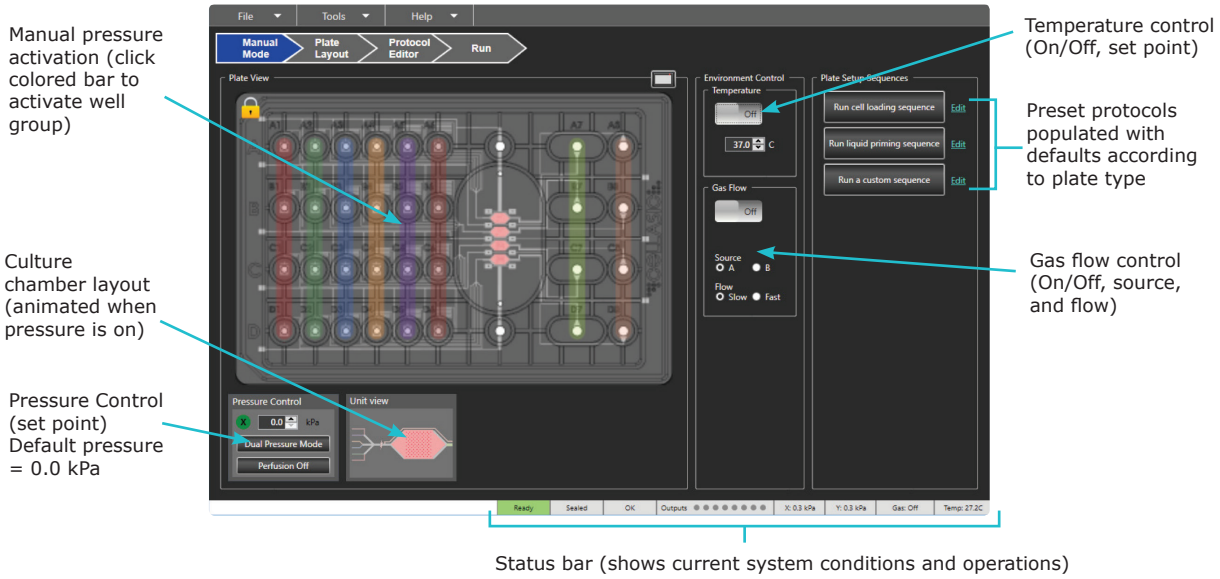


Figure 7. Manual Mode allows interactive operation of the ONIX2 System. Operating parameters can be set manually and this mode also provides the option to run short automated setup sequences that are prepopulated with plate-specific defaults. These setup sequences can be edited if desired.

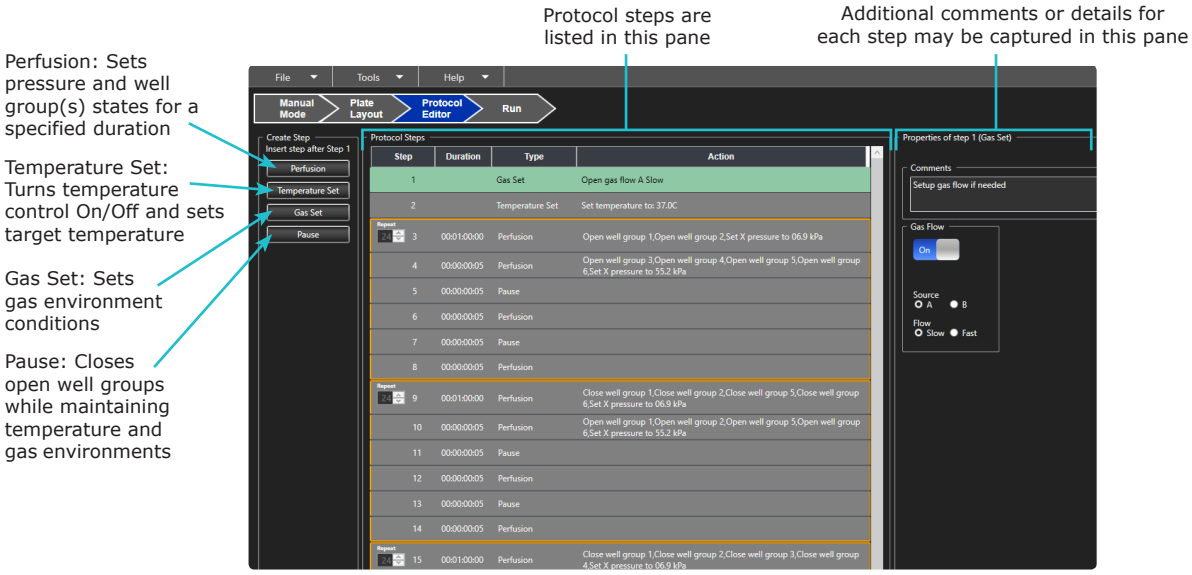


Figure 8. Protocol Editor mode allows the creation and editing of an experimental protocol. A protocol is comprised of a sequence of environmental control and/or perfusion steps. Steps can be added and altered as desired. When the protocol is ready, it can be executed using the **Run** tab.

Specifications

Culture Plate Dimensions	
Length × width	127.3 mm (5.0 in.) × 85.2 mm (3.4 in.)
Height without lid	14.3 mm (0.6 in.)
Culture Chamber Dimensions	
Length	6.0 mm (0.24 in.)
Width	3.0 mm (0.12 in.)
Trap height	4.0 µm
Glass bottom thickness (#1.5 slide)	170 µm
Plate materials of construction	Polycarbonate, silicone, acrylic, glass

Product Ordering Information

This section lists catalogue numbers for the CellASIC® ONIX products. You can purchase these products and find the most up-to-date software, plate maps, and user guides at www.sigmaaldrich.com/cellasic.

Description	Qty/pk	Catalogue Number
Microfluidic Plates		
CellASIC® ONIX Plate for Bacteria Cells (4-chamber, trap heights of 0.7, 0.9, 1.1, 1.3, 2.3, and 4.5 µm)	5	B04A-03-5PK
CellASIC® ONIX Gradient Plate for Mammalian Cells (4-chamber)	5	M04G-02-5PK
CellASIC® ONIX Open-top Plate for Mammalian Cells (4-chamber)	5	M04L-03-5PK
CellASIC® ONIX Switching Plate for Mammalian Cells (4-chamber)	5	M04S-03-5PK
CellASIC® ONIX Pad Trap Plate (4-chamber, trap heights 12.0 µm)	5	M04T-01-5PK
CellASIC® ONIX Plate for Haploid Yeast Cells (4-chamber, trap heights of 3.5, 4.0, and 4.5 µm)	5	Y04C-02-5PK
CellASIC® ONIX Plate for Diploid Yeast Cells (4-chamber, trap heights of 5.0, 6.0, and 7.0 µm)	5	Y04E-01-5PK
CellASIC® ONIX Pad Trap Plate (4-chamber, trap height of 4.0 µm)	5	Y04T-04-5PK
CellASIC® ONIX2 Microfluidic System and Manifolds		
CellASIC® ONIX2 Microfluidic System	1	CAX2-S0000
CellASIC® ONIX2 Manifold XT (temperature controlled)	1	CAX2-MXT20
CellASIC® ONIX2 Manifold Basic (no temperature control)	1	CAX2-MBC20
Replacement Parts/Accessories		
CellASIC® ONIX2 Filter Multiconnector (includes filters)	1	CAX2-AMC00
CellASIC® ONIX2 Software USB Drive	1	CAX2-SSW01
CellASIC® ONIX2 Gasket	1	CAX2-AGK20
CellASIC® ONIX2 Self Check Plate	1	CAX2-ASP20
CellASIC® ONIX2 Cleaning Plate	1	CAX2-ACP20
CellASIC® ONIX2 Replacement Filter Pack (9 × 4 mm and 1 × 13 mm Millex® 0.45 µm PTFE filters)	1	CAX2-AFP00
CellASIC® ONIX2 Accessory Fittings (quick-connect gas fitting, 2/pk)	1	CAX2-ABF00
CellASIC® ONIX2 Temperature Calibration Plate	1	CAX2-ACT20
CellASIC® ONIX2 Premixed Gas Regulator (for use with 103 L or 112 L gas cylinders with a C10 connection)	1	CAX2-ABR00
CellASIC® ONIX2 Microfluidic Services		
CellASIC® ONIX2 Essential Service Plan	1	CAX2-ESVC
CellASIC® ONIX2 Total Service Plan	1	CAX2-TSVC
CellASIC® ONIX2 Installation	1	CAX2-INST

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