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User Guide

CellASIC® ONIX B04A-03 Microfluidic Bacteria Plate

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

Introduction

The CellASIC® ONIX B04A-03 Microfluidic Plate is a 4-chamber cell culture plate designed for use with the CellASIC® ONIX2 Microfluidic System and ONIX2 Manifolds for enabling perfusion-based, long-term, live-cell analysis with solution switching. This bio-inspired plate provides a controlled and dynamic microenvironment for cells. The easy-to-use format and superior technology redefine the standard for microfluidics-based experimentation.

Applications

- Time-lapse analysis of bacteria cells
- 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
- Cell division tracking (follow cell division over generations)
- Temperature and gas atmospheric control (temperature shift, anoxic conditions, etc.)

Plate Description

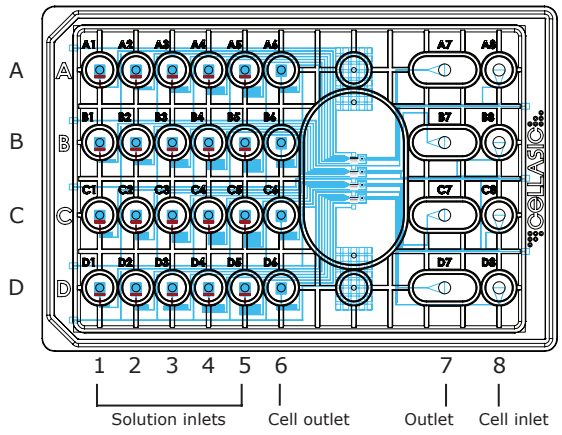

Figure 1. Plate configuration

The B04A plate has 4 independent units (A–D), each with 5 inlet wells (1–5), a cell inlet (8), a cell outlet (6), and a large outlet well (7). Flow channels are resistance matched for uniformity. Each row of wells (A–D) addresses the corresponding culture chamber. The plate is shipped preprimed with a PBS (phosphate-buffered saline) solution, which can be replaced with a buffer of choice prior to experiment. The plate is for single use only.

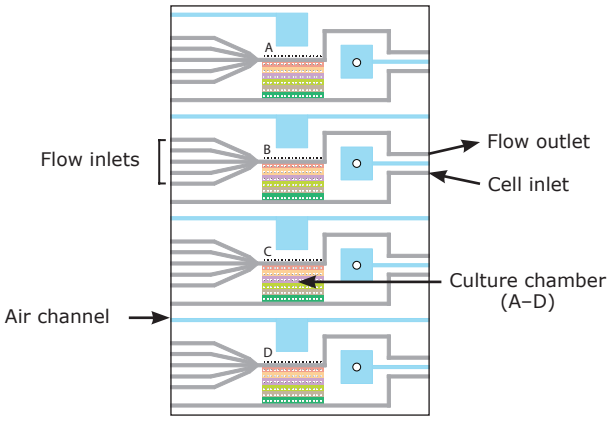

Figure 2. Chamber viewing window

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

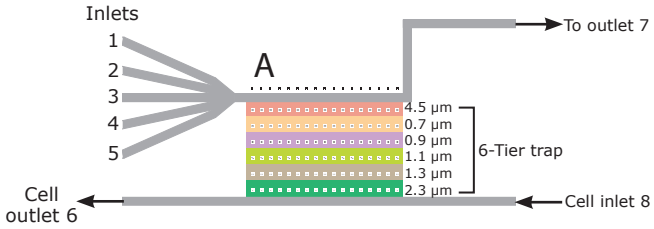

Figure 3. Culture chamber

The culture chamber is 2.0 × 1.2 mm in area with trap heights of 0.7, 0.9, 1.1, 1.3, 2.3 and 4.5 µm. Support posts with position markers maintain a uniform ceiling height. The inlet/outlet functions and minimum/maximum volumes for each culture unit are listed below.

	Function	Minimum Volume (µL)	Maximum Volume (µL)
Inlet 1	Inlet for solution switching	50	350
Inlet 2	Inlet for solution switching	50	350
Inlet 3	Inlet for solution switching	50	350
Inlet 4	Inlet for solution switching	50	350
Inlet 5	Inlet for solution switching	50	350
Inlet 8	Cell inlet for loading cells into culture chamber	50	350
Outlet 6	Cell outlet	10	350
Outlet 7	Accepts flow-through from culture chamber	50	600

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Cell Trapping Mechanism

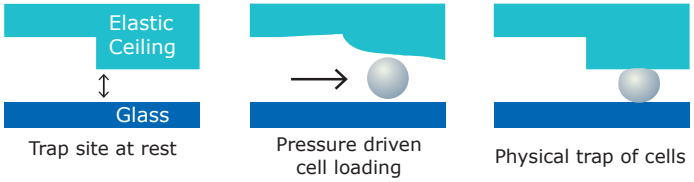

Figure 4. Cell trapping mechanism

The microfabricated chamber gently holds cells against the glass viewing surface to maintain a single focal plane during perfusion-based analysis experiments. The B04A plate has trap heights of 0.7, 0.9, 1.1, 1.3, 2.3 and 4.5 µm.

Manifold Description

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

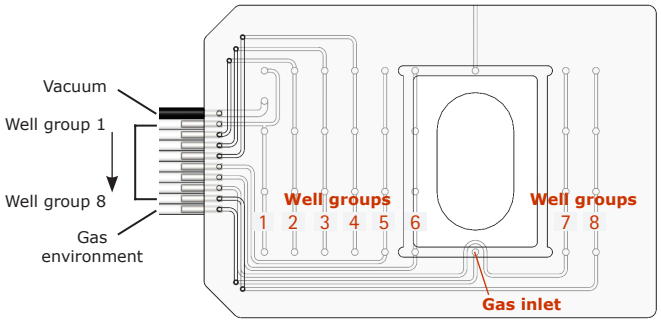

Figure 5. Lines to CellASIC® ONIX2 Microfluidic System

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

The flow properties of wells 1–5 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.

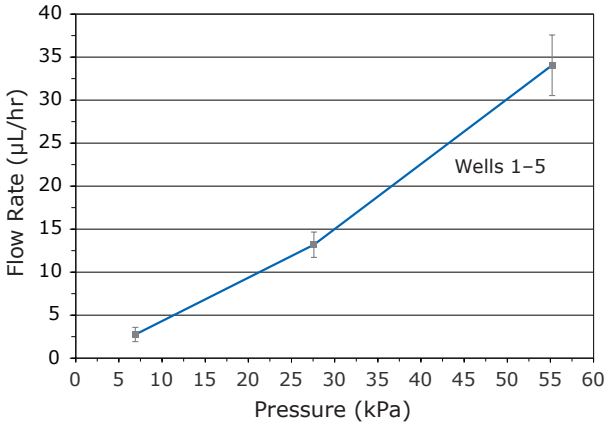

Figure 6. Flow rate for wells 1–6

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 Preparation

Plate Priming (Optional)

1. If your experiment requires complete removal of PBS, replace the PBS in the solution (1–5) and cell inlet (8) wells with 150 µL of your desired priming solution.
2. Aspirate the PBS solution from wells 6 and 7.
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 B04A plate on the drop down list. On the **Manual Mode** tab (Figure 7), click on the **Run liquid priming sequence** button. Alternatively, on the **Protocol Editor** tab (Figure 8) enter the desired steps and conditions. The recommended pressure and flow time for well groups 1–5 are 34.5 kPa (5 psi) and 5 minutes, respectively.

NOTE: For cells that tend to adhere or aggregate, priming well group 8 in addition to well groups 1–5 will minimize the incidence of cell attachment to the channels during cell loading.

For more information on creating protocols, refer to the CellASIC® ONIX2 Microfluidic System User Guide.

Cell Loading

Pressure-Driven Method Using the CellASIC® ONIX2 Microfluidic System

1. Prepare a bacteria/cell suspension of 1–20 × 10^6 cells/mL. This concentration may need optimization depending on the type of cell and desired trapping density.
2. Aspirate solution from cell outlet well 6 and flow outlet well 7.
3. Aspirate solution from cell inlet well 8 and pipette 50 µL of cell suspension into this well. Pipette 50 µL of PBS into cell outlet well 6. Make sure to cover the hole at the bottom of the wells with fluid.
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 B04A plate on the drop down list. On the **Manual Mode** tab (Figure 7), click on the **Run cell loading sequence** button. The recommended pressure and flow time for well group 8 are 13.8 kPa (2 psi) and 15 seconds to prime the cells for loading, followed by flowing well groups 8 and 6 at 27.6 kPa (4 psi) for 15 seconds to trap the cells. Then flow well group 6 at 6.9 kPa (1 psi) for 30 seconds to rinse the loading channel. 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 one or more inlet well solutions at 34.5 kPa (5 psi) for 5 minutes. On the **Manual Mode** tab, click on the **Run a custom sequence** button or go to **Protocol Editor** to enter the desired parameters. For more information on creating protocols, refer to the CellASIC® ONIX2 Microfluidic System User Guide.
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 1–5). Add 350 µL medium to these wells. Make sure that the unused solution inlet wells are filled with buffer.
2. Empty wells 6 and 8 to ensure proper solution exchange during perfusion.
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 B04A plate on the drop down list. Click on the **Protocol Editor** tab and enter the desired steps and conditions. For wells 1–5, 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.
5. To monitor cell growth, place the sealed plate/manifold assembly on an inverted microscope.
6. 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.

Solution Switching

1. Aspirate solution from the chosen inlet wells (1–5). Add up to 350 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 B04A 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 high pressure (55.2 kPa [8 psi]) for the initial transition, then reduce flow to standard pressure 6.9–13.8 kPa (1–2 psi) for long-term exposure.

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 detail on software features.

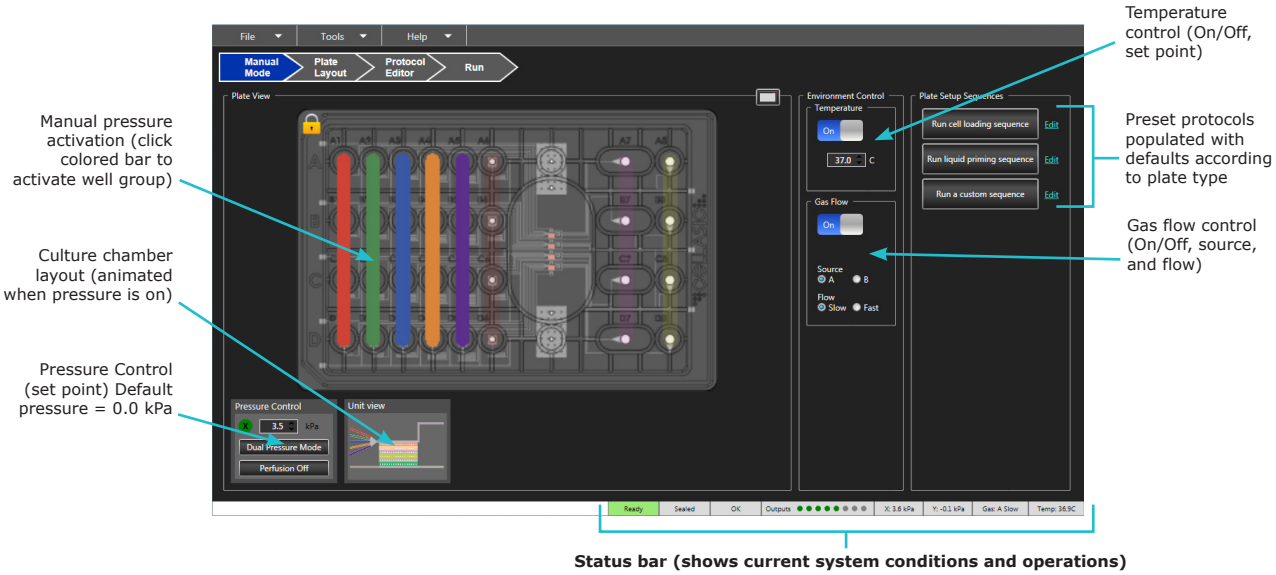

Figure 7. Manual Mode allows interactive operation of the ONIX2 System. Operating parameters can be set manually and this mode also provides the option to run short automated plate setup sequences that are prepopulated with plate-specific defaults. These setup sequences can be edited if desired.

In the culturing protocol example outlined below, cells were loaded from well 8 by applying pressure (27.6 kPa [4 psi] for 15 seconds) to well groups 6 and 8. Untrapped cells were flushed from the chambers by flowing wells 4 and 5 at 34.5 kPa (5 psi) for 5 minutes. Next, wells were perfused with baseline wash or growth solution for 30 minutes from well 1. Cells were exposed to inducer from well 2 for 1 hour, then inducer was washed away with wash or growth solution from well 1 for 30 minutes. The latter two steps were repeated for a second inducer in well 3. Temperature was controlled with the CAX2-MXT20 manifold, using a setpoint of 37 °C.

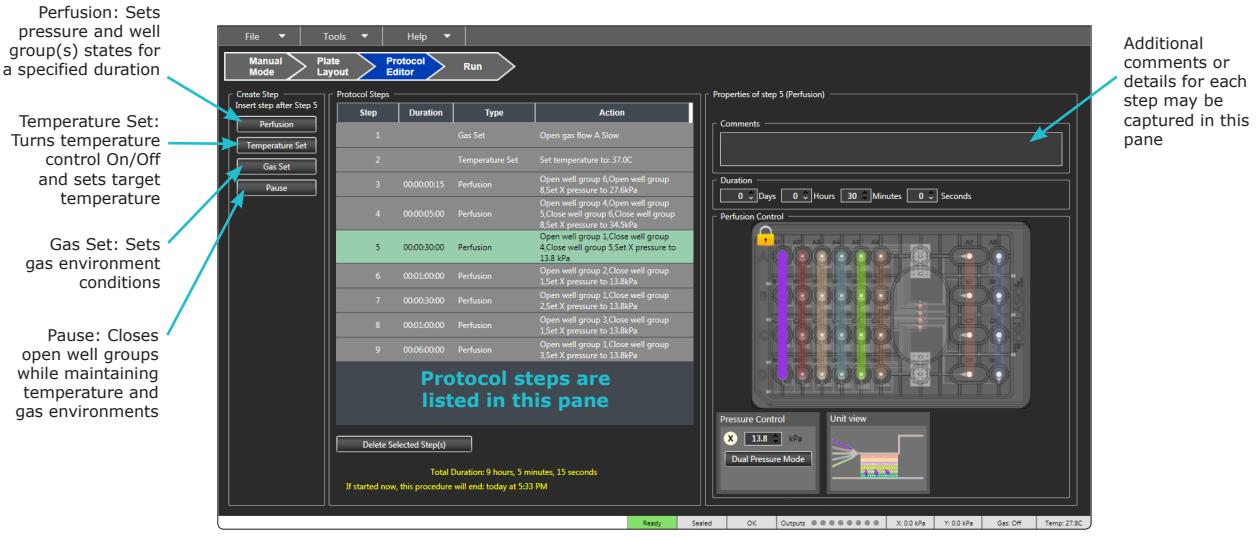

Figure 8. Protocol Editor Mode allows the creation and editing of an experimental protocol. A protocol is comprised of a sequence of environmental control and/or perfusion steps. Steps can be added and altered as desired. When the protocol is ready, it can be executed using the **Run** tab.

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	2.0 mm (0.08 in.)
Width	1.2 mm (0.05 in.)
Trap heights	0.7, 0.9, 1.1, 1.3, 2.3, and 4.5 µm
Glass bottom thickness (#1.5 slide)	170.0 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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Standard Warranty

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