

User Guide

CellASIC® ONIX B04F Microfluidic Bacteria Plate

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

Introduction

The CellASIC® ONIX B04F Microfluidic Plate is a 4-chamber cell culture plate designed for use with the CellASIC® ONIX Microfluidic System and CellASIC® ONIX F84 Manifolds for enabling perfusion-based, long-term, live-cell analysis with solution switching that monitors trapped cells growing into biofilms. 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
- 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
- Cell division tracking (follow cell division over generations)

Plate Description

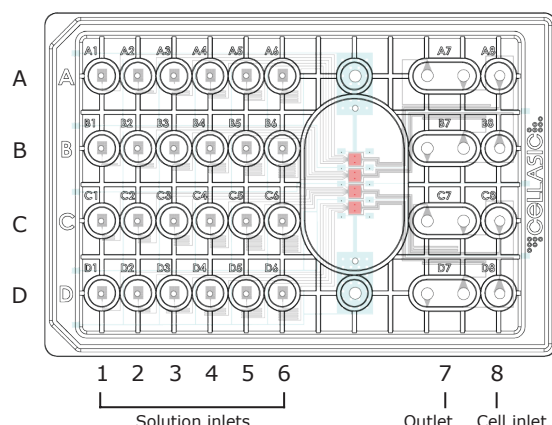


Figure 1. Plate configuration

The B04F plate has 4 independent units (A–D), each with 6 inlet wells (1–6), a cell inlet well (8), 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.

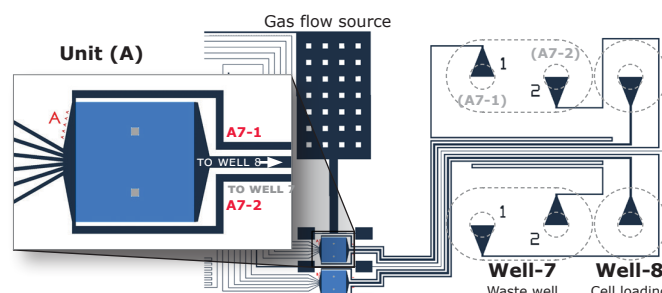


Figure 2. Chamber viewing window

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

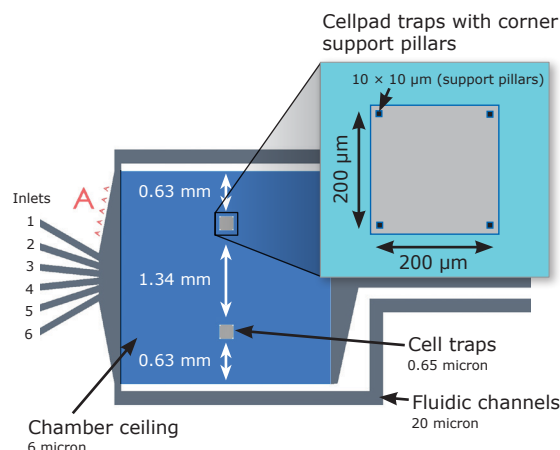


Figure 3. Culture chamber

The culture chamber is 3.0 × 3.0 mm in area with trap heights of 0.65 µm. Two elastic PDMS pads gently hold biofilms against the glass in each chamber. 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	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
Inlet 7	Accepts flow-through from culture chamber	50	700
Inlet 8	Cell inlet for loading cells into culture chamber/ Accepts flow-through from culture chamber	50	300

Cell Trapping Mechanism

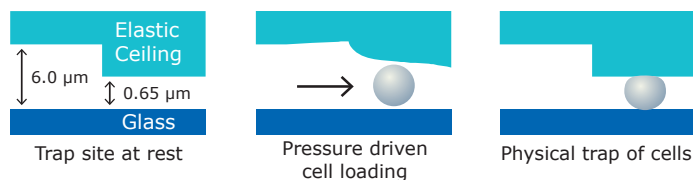


Figure 4. Cell trapping mechanism

The microfabricated chamber and the PDMS barrier gently hold biofilms against the glass viewing surface to maintain a single focal plane during perfusion analysis experiments. The B04F plate has trap heights of 0.65 µm.

Manifold Description

The CellASIC® ONIX F84 Manifold connects the microfluidic plate to the CellASIC® ONIX Microfluidic System.

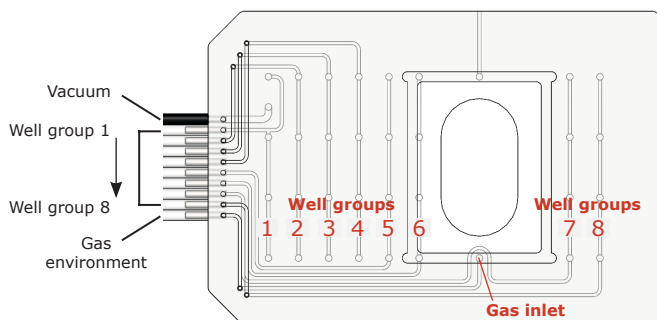


Figure 5. Lines to CellASIC® ONIX Microfluidic System

The manifold has 8 pressure channels with corresponding control valves (V1-V8) to control flow rates through the microfluidic channels. 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.

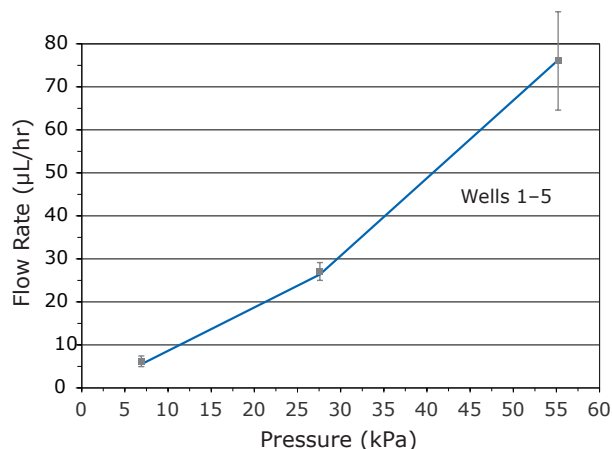


Figure 6. Flow rate for wells 1–6

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 environmental control is needed, use the CellASIC® ONIX Microincubator Controller and Manifold. Refer to the CellASIC® ONIX Microfluidic Platform User Guide for setup instructions.

Plate Preparation

Plate Purging (Optional)

1. We recommend to implement the purging step on the plates that may be on the shelf for a long period of time to ensure the integrity of the traps and plates.
2. Right after opening the package, 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 B04F 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 1–8 are 55.2 kPa (8 psi) and 2 minutes, respectively.

Plate Priming (Optional)

1. If your experiment requires complete removal of PBS, replace the PBS in the solution (1–6) and cell inlet (8) wells with 150–300 µL of your desired priming solution or media.
2. Aspirate the PBS solution from wells 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 B04F 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 1–6, and 8 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 $0.5\text{--}3.0 \times 10^6$ cells/mL. This concentration may need optimization depending on the bacterial strain and desired trapping density.
2. Aspirate solution from cell inlet well 1 through 8 without disturbing the cutouts.
3. Pipette 50 μL of cell suspension into cell inlet well 8 and 300 μL of culture broth into solution inlet well 1 through 6, making sure to cover the hole at the bottom of the well.
4. Seal the microfluidic plate to the F84 manifold according to the CellASIC® ONIX Microfluidic Platform User Guide.
5. Open the CellASIC® ONIX FG Software, select the B04F plate (Use Bacteria tab) on the user interface, and click on the **Load Cells** tab. The recommended pressure and flow time for V1 through V6, and V8, at 8 psi for 10 seconds, but you may need to optimize these conditions 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 up to 5 times.
7. To wash out untrapped cells, click on **Run Liquid Priming Sequence**. The recommended pressure and flow time for V1 through V6 at 4 psi for 5 minutes. For more information on creating protocols, refer to the CellASIC® ONIX Microfluidic Platform 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 1–6) and for the waste reservoir (well 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 F84 manifold according to the CellASIC® ONIX Microfluidic Platform User Guide.
3. Open the CellASIC® ONIX FG Software, select the B04F plate on the user interface and click on the **Create Protocol** tab. Click on **Gas** to set the gas content. Click on **Temperature** to set the culture temperature.
4. Click on **Perfusion** to set the perfusion protocols. For wells 1–6, the recommended pressure of 1–2 psi provides adequate nourishment with minimal stress. For information on creating a protocol, refer to the CellASIC® ONIX Microfluidic Platform User Guide.
5. To monitor cell growth, place the sealed plate/manifold assembly on the inverted microscope.
6. During extended perfusion experiments, empty well 7 and 8 periodically to avoid outlet overflow into the manifold tubing and perfusion system. To do this, click on **Pause** button on the CellASIC® ONIX FG Software window, remove the manifold from the plate, and aspirate well 7 and 8. Reseal the manifold to the plate and click **Resume** to restart the perfusion protocol.

Note: The protocol is designed for cell culture that lasts for less than 24 hours. For experiments that last for over 24 hours (up to 48 hours), the following technique can be applied:

1. Repeat Step 1 and 2 as described in the above.
2. Open the CellASIC® ONIX FG Software, select the B04F plate on the user interface and click on the **Create Protocol** tab.
3. Click on **Gas** to set the gas content. Click on Temperature to set the culture temperature.
4. Click on **Perfusion** to set the perfusion protocols that are incorporated a flush protocol as follows:
 - A. Perfusion from well 1, 3, and 5 at 1 psi for 12 hours.
 - B. Perfusion from well 2, 4, and 6 at 1 psi for 12 hours.

Note: if chambers get overcrowded, it is recommended to perfuse from well 1–6 at 8 psi for 5 seconds and repeating this flushing step three times periodically during the culture protocol is recommended. And then resume back to Step A and B. The protocol has been validated to accommodate up to 24 hours of cell culture. No outlet overflow has been observed.

Solution Switching

1. Aspirate solution from the chosen inlet wells (1–6). Add up to 300 μL of the desired solution to the wells. If less than 4 units (A–D) are to be used, fill the unused inlet wells with buffer to prevent dehydration.
2. Seal the microfluidic plate to the F84 manifold according to the CellASIC® ONIX Microfluidic Platform User Guide.
3. Open the CellASIC® ONIX FG Software, select the B04F plate on the user interface, and create the appropriate protocol using the **Create Protocol** tab. For more information on creating a protocol, refer to the CellASIC® ONIX Microfluidic Platform User Guide.

NOTE: For experiments requiring rapid solution exchange, the following technique can be applied: Flow at high pressure for the initial transition, then reduce flow to standard pressure (1–2 psi) for long-term exposure.

NOTE: If the formation of biofilm matrix is desired, it is recommended to introduce some environmental perturbation to the cell culture by perfusing cells with media from well 1 and 6 at 1 psi at 37 °C for 6 hours, followed by perfusing from well 1 and 6 at 1 psi at 30 °C for 18 hours or until film formation can be observed. To date, the protocol has been proven using *Bacillus subtilis* (NCIB 3610 strain).

Software Operation

The culturing protocol example shown in the figures below uses the **Create Protocol** tab in the software interface window, as well as the **Run Protocol** tab. Refer to the CellASIC® ONIX Microfluidic Platform User Guide for details on the user interface software.

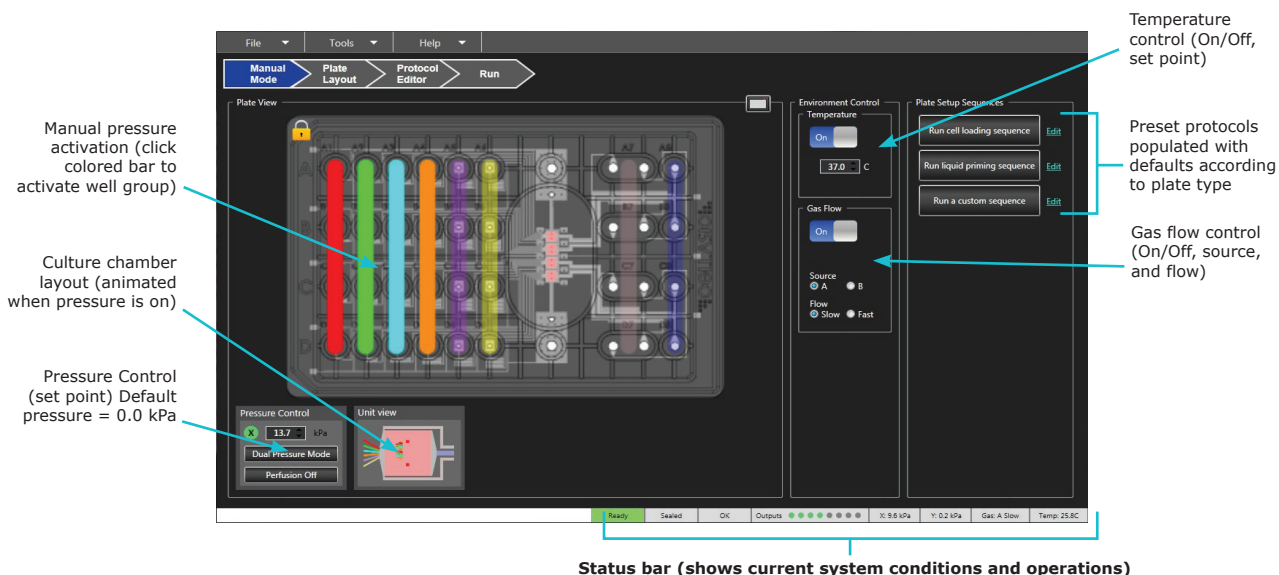


Figure 7. Manual Mode allows interactive operation of the ONIX2 System. Operating parameters can be set manually and this mode also provides the option to run short automated plate setup sequences that are prepopulated with plate-specific defaults. These setup sequences can be edited if desired.

In this experiment, cells were perfused with baseline wash or growth solution for 30 minutes from well 1. Next, cells were exposed to inducer from well 2 for 1 hour. The latter two steps are repeated and finished with a second inducer exposure from well 3 for 6 hours. Steps are outlined in protocol editor list below.

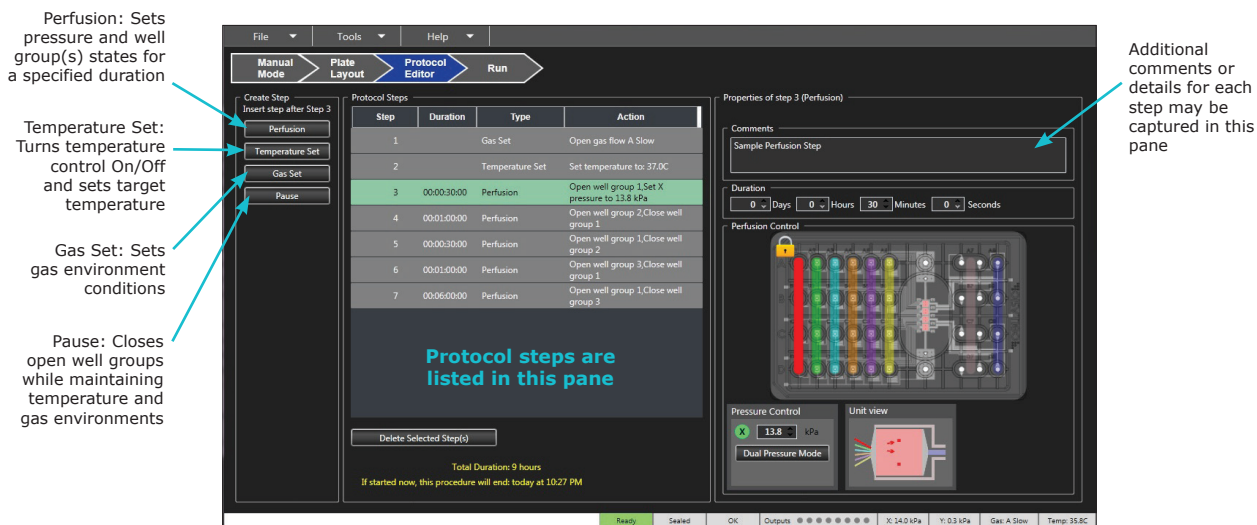


Figure 8. Protocol Editor Mode allows the creation and editing of an experimental protocol. A protocol is comprised of a sequence of environmental control and/or perfusion steps. Steps can be added and altered as desired. When the protocol is ready, it can be executed using the **Run** tab.

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 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 Biofilm Plate for Bacteria Cells (4-chamber, trap height of 0.65 µm)	5	B04F-01-5PK
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 for Mammalian Cells (4-chamber, trap height of 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 for Yeast Cells (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

Description	Qty/ pk	Catalogue Number
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

Notice

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