User Guide

CellASIC® ONIX M04G-02 Microfluidic Gradient Plate

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

Introduction

The CellASIC® ONIX M04G-02 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 stable gradient formation and solution switching. This bio-inspired plate provides a controlled and dynamic microenvironment for culture of cells in standard planar (2D) and 3-dimensional formats. The easy-to-use format and superior technology redefine the standard for microfluidics-based experimentation.

Applications

- Time-lapse analysis of cells in a stable diffusion gradient (cell migration, chemotaxis, polarization)
- Gradient switching experiments (induction, inhibition, drug dosing)
- Comparison of up to four different cell types or exposure conditions (media components) in parallel
- Long-term continuous perfusion experiments (3 days typical)
- Temperature and gas atmospheric control (temperature shift, anoxic conditions)
- Automated immunostaining and "on-demand" fixation of live cells within the culture chamber

Plate Description

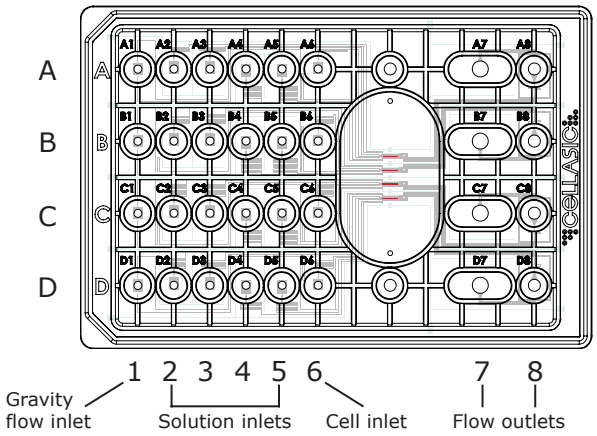

Figure 1. Plate configuration

The M04G microfluidic plate has four independent culture units (A–D), each with a gravity flow inlet (1), four solution inlets (2–5), a cell inlet (6), and two shared outlets (7 and 8). Flow channels are resistance matched for uniformity. Each row of wells (A–D) addresses the corresponding culture chamber. The plate is shipped preprimed with PBS (phosphate-buffered saline) solution, which can be replaced with a buffer of choice prior to experiment. The plate is for single use only.

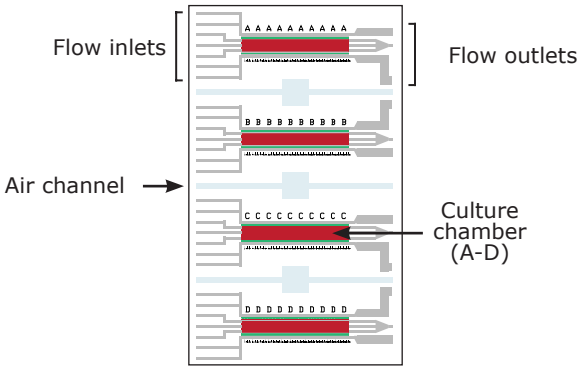

Figure 2. Chamber viewing window

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

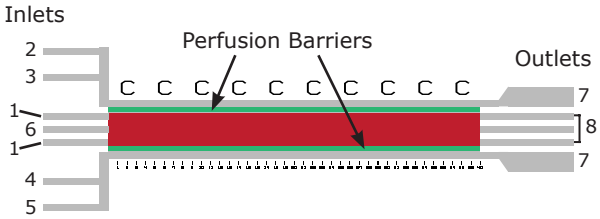

Figure 3. Culture chamber

Perfusion barriers bound the chamber on the top and bottom edges to separate the chamber from the flow channels. 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 gravity-driven perfusion	10	350
Inlet 2	Inlet for gradient establishment	50	350
Inlet 3	Inlet for gradient establishment	50	350
Inlet 4	Inlet for gradient establishment	50	350
Inlet 5	Inlet for gradient establishment	50	350
Inlet 6	Cell inlet for loading cells into culture chamber	10	350
Outlets 7 and 8	Accept flow-through from culture chamber	50	900*

* Outlets 7 and 8 combined

Manifold Description

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

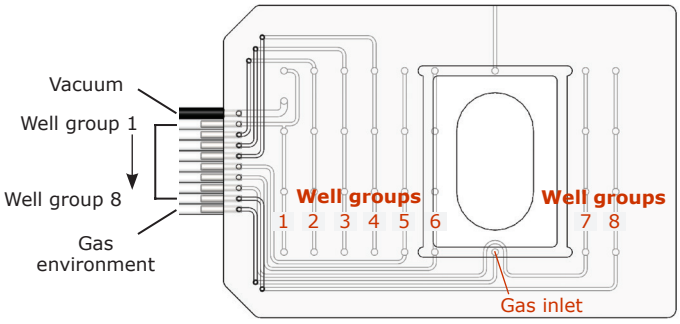

Figure 4. Lines to CellASIC® ONIX2 Microfluidic System

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 2–5 are shown in Figure 5 and those of well 6 are shown in Figure 6. Each figure shows the flow rate out of the well as a function of pressure. Solutions will diffuse through the perfusion barriers at the top and bottom of each cell chamber. If more than one channel is pressurized, multiply the well flow rate by the number of pressurized channels to derive the overall flow rate.

NOTE: The flow of liquid from well 6 is approximately 150 times faster than the flow from wells 2–5.

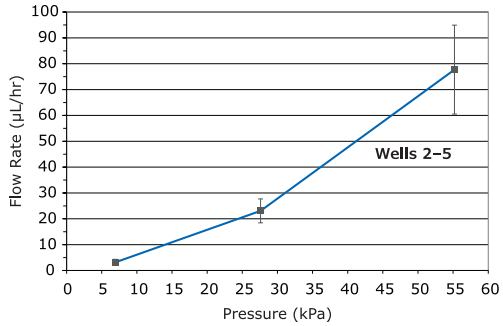

Figure 5. Flow rate for wells 2–5

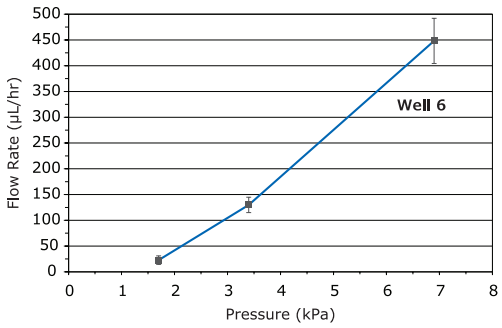

Figure 6. Flow rate for well 6

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 fewer than four chambers are going to be used, aspirate only the inlet/outlet wells of the chamber row(s) being used. All unused chamber wells should be kept filled with PBS or other medium. During and after an experiment, keep the unused wells filled with medium to prevent drying out. Unused chambers may be used at a later time.

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.

Precoating with ECM or Priming with Growth Medium

NOTE: ECM coating or priming with culture medium is recommended for either method of cell loading to ensure even distribution of cells in the culture chamber of the M04G plate.

Capillary Method

1. Prepare the ECM coating solution or medium according to desired procedure.
2. Aspirate PBS solution from the upper part of well 1 leaving PBS within the PTFE (polytetrafluoroethylene) ring at the bottom of the well.

   NOTE: The hydrophobic PTFE ring at the bottom of wells 1, 6, and 8 (Figure 7) provides sample containment, permitting loading of small volumes (≤ 10 µL) into these wells.

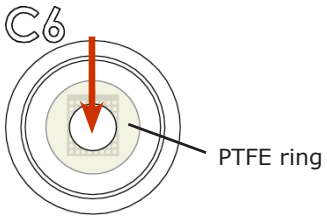

Figure 7. Well 6 cell inlet

3. Aspirate the PBS solution from well 6, including solution within the PTFE ring.
4. Pipette 10 µL of ECM coating solution (or culture medium) into the PTFE ring of well 6.
5. Immediately aspirate the PBS solution from outlet wells 7 and 8 including the hole and PTFE ring, respectively. This will cause capillary action to pull the coating solution or medium into the chamber.
6. Incubate according to desired procedure; we recommend 30 minutes to complete the chamber priming process.
7. If wash steps are required, aspirate medium completely from well 6 (including PTFE ring) and add 10 µL wash solution to well 6. Aspirate wells 7 and 8 including the hole and PTFE ring, respectively, to initiate capillary flow.

Pressure-Driven Method Using the CellASIC® ONIX2 Microfluidic System

1. Prepare the ECM coating solution or medium according to desired procedure.
2. Aspirate the PBS solution from wells 6 and 8, including the PTFE ring.
3. Aspirate PBS solution from wells 1 and 7 but leave solution in the inner holes (well 7 does not have a PTFE ring).
4. Pipette 10 µL of ECM coating solution (or culture medium) into the PTFE ring of well 6.
5. Seal the microfluidic plate to the ONIX2 manifold according to the CellASIC® ONIX2 Microfluidic System User Guide.
6. Open the CellASIC® ONIX2 Software, select one of the **New Experiment** options, and find the M04G plate on the drop down list. On the **Manual Mode** tab (Figure 10), click on the **Run liquid priming sequence** button. Alternatively, on the **Protocol Editor** tab (Figure 11) enter the desired steps and conditions. The recommended pressure and flow time for well group 6 are 1.7 kPa (0.25 psi) and 2 minutes. For information on creating a protocol, refer to the CellASIC® ONIX2 Microfluidic System User Guide.
7. If wash steps are required, aspirate medium completely from well 6 (including PTFE ring) and add 10 µL wash solution inside the PTFE ring of well 6.
8. Open the CellASIC® ONIX2 Software and set up the desired wash steps as in step 6.

Cell Loading

Capillary Method

1. Prepare a suspension of 1–4 × 10^6 cells/mL. Immediately prior to loading, mix the cell suspension thoroughly by repeated pipetting to minimize clumping.
2. Aspirate solution from well 6 including the PTFE ring.
3. Pipette 10 µL of cell suspension into the PTFE ring of well 6.
4. Immediately aspirate the PBS solution from outlet wells 7 and 8 including the base hole (well 7) and PTFE ring (well 8). This will cause capillary action to pull the coating solution or medium into the chamber.
5. Place the microfluidic plate on a microscope to monitor cell loading in real time. Allow cells to flow for up to 30 minutes.
6. Cells should be evenly dispersed throughout the center of the chamber between the inlet port for well 6 and the outlet port for well 7 (Figure 8). Arrows indicate the direction of cell flow through the chamber.

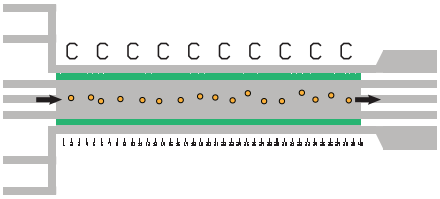

Figure 8. Cell loading profile

7. If more cells are desired, repeat steps 3-5.
8. If sufficient cell loading has occurred to fill the chamber, stop the loading process by adding 50 µL of culture medium to well 7.
9. Once sufficient cells have populated the chamber, proceed to the Cell Culture Section.

Pressure-Driven Method Using the CellASIC® ONIX2 Microfluidic System

NOTE: If loading cells using the pressure driven method after chamber priming, make sure to add medium to wells 7 and 8 (50 µL is sufficient) before adding cell suspension to the PTFE ring of well 6. This will prevent capillary action from drawing cells into the chamber prior to manifold attachment and protocol initiation.

1. Prepare a suspension of 1–4 × 10^6 cells/mL. Immediately prior to loading, mix the cell suspension thoroughly by repeated pipetting to minimize clumping.
2. Aspirate solution from well 1 including the PTFE ring. Load 10 µL of culture medium into the PTFE ring.
3. Aspirate solution from well 6 including the PTFE ring. Pipette 10 µL of cell suspension into the PTFE ring.
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 M04G plate on the drop down list. On the **Manual Mode** tab (Figure 10), click on the **Run cell loading sequence** button. The recommended pressure and flow time for well groups 1 and 6 are 2.8 kPa (0.4 psi) and 18 seconds but you may need to optimize these conditions depending on your cell type and/or concentration.
6. Proceed to the Cell Culture Section.

Matrigel™ Matrix-embedded Cell Loading Using the CellASIC® ONIX2 Microfluidic System

NOTES: All reagents should be kept on ice during experimental setup to minimize Matrigel™ polymerization. In addition, all pipette tips and the unopened CellASIC® ONIX M04G Microfluidic Plate should be chilled at 4 °C for approximately 1 hour prior to performing setup for cell loading.

These are general guidelines for experimental setup. Depending on the cell type, both cell concentration and Matrigel™ matrix input may need to be optimized.

1. Prepare a cell suspension of 8–32 × 10^6 cells/mL. Place on ice.
2. Thaw Matrigel™ matrix on ice. Mix thoroughly by pipetting immediately before use.
3. Dilute cells 1:8 with Matrigel™ matrix (final concentration of 1–4 × 10^6 cells/mL).
4. Unseal the CellASIC® ONIX M04G Microfluidic Plate and place on ice.
5. Aspirate PBS solution from wells 1, 7, and 8 including solution within the hole (well 7) or PTFE ring (well 8). Make sure the PTFE ring in well 1 is filled with PBS solution or replace with 10 µL of Matrigel™ matrix.
6. Add 50 µL of medium to wells 7 and 8.
7. Aspirate PBS solution from well 6 including the PTFE ring. Load 10 µL of cell/Matrigel™ matrix mixture into the PTFE ring.
8. Seal the microfluidic plate to the ONIX2 manifold according to the CellASIC® ONIX2 Microfluidic System User Guide.
9. Open the CellASIC® ONIX2 Software, select one of the **New Experiment** options, and find the M04G plate on the drop down list. On the **Manual Mode** tab (Figure 10), click on the **Run cell loading sequence** button. The recommended pressure and flow time for well groups 1 and 6 are 2.8 kPa (0.4 psi) and 30 seconds, but you may need to optimize these conditions depending on cell type and/or Matrigel™ matrix concentration.
10. Assess the loading density on a microscope. If insufficient loading has occurred, repeat the loading protocol.
11. Proceed to Cell Culture Section.

Cell Culture

Capillary-driven Preculture in an Incubator (optional)

1. Cells can be perfused in the culture chamber using gravity-driven media flow. Certain cell types which are slow to establish attachment may require preculturing in an incubator for several hours prior to using the CellASIC® ONIX2 Microfluidic System. Alternatively, this culture method can be used when environmental control is not available.
2. Aspirate solution from well 1 including the PTFE ring. Pipette 350 µL of growth medium into well 1 and 50 µL into well 7 to initiate the gravity-driven response.
3. Place the microfluidic plate in an incubator. For long-term culture, replace medium in well 1 and empty wells 7 and 8 every 2–3 days.

   NOTE: For long-term culture, wells 7 and 8 must be emptied periodically to prevent medium from overfilling the well and entering the manifold tubing and perfusion systems. The combined volume of wells 7 and 8 is approximately 900 µL.

Cell Culture with CellASIC® ONIX2 Microfluidic System

The CellASIC® ONIX M04G Microfluidic Plate has four flow inlet wells (2–5). Wells 2 and 3 will flow through the channel at the top of the culture chamber and wells 4 and 5 will flow through the channel at the bottom of the culture chamber. These wells can be used for media perfusion (short or long-term culture), gradient formation, and solution switching.

1. Aspirate PBS solution from wells that will be used for perfusion (flow inlet wells 2–5). Add 350 µL of desired medium to these wells. If less than four units (A–D) are to be used, fill the unused inlet wells with buffer to prevent dehydration.

   NOTES: If not all inlet wells are being used, leave the unused wells filled with buffer, but remove the PBS solution from well 1. This prevents the fluid in well 1 from interfering with flow from the other inlet wells.

   Perfusion will occur in all 4 units (A–D) whether cells have been seeded or not. Remember to monitor PBS solution levels in wells 2–5 of unused units. If wells become empty, dehydration and bubble formation can occur within the chamber preventing use of these units in subsequent experiments. Also, all wells 7 and 8 must be emptied periodically to prevent media (or PBS solution) overflow into the manifold tubing and microfluidic system.
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 M04G plate on the drop down list. Click on the **Protocol Editor** tab (Figure 11) and enter the desired steps and conditions. For wells 2–5, the recommended pressure of 3.4–6.9 kPa (0.5–1 psi) provides adequate nourishment with minimal stress. Once the experiment protocol is ready, it can be executed using the **Run** tab. For information on creating a protocol, refer to the CellASIC® ONIX2 Microfluidic System User Guide.

   NOTE: Medium should be simultaneously flowed from well(s) servicing both the top and bottom of culture chamber. Any of the following four combinations can be used: wells 2 + 4 (flow well groups 2 and 4), 2 + 5, 3 + 4, or 3 + 5.
4. To monitor cell growth, place the sealed plate/manifold assembly on an inverted microscope.
5. During extended perfusion experiments, empty wells 7 and 8 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 wells 7 and 8. Reseal the manifold to the plate, then on the **Run** tab, click **Resume** to restart the perfusion protocol.

   NOTE: For cell types that do not require preculture in an incubator, it is possible to prime, load cells, and perfuse in a single uninterrupted protocol, rather than in two separate protocols (using the **Manual Mode** and **Protocol Editor** tabs, respectively). Prepare wells as follows: add 10 µL of medium to the PTFE ring of well 1, add 10 µL of cell suspension to the PTFE ring of well 6, and add 350 µL of medium to wells 2–5. In **Protocol Editor**, set up the priming step for well 1 (flow well group 1 at 6.9 kPa (1 psi) for 1 minute), cell loading step for well 6 (flow well groups 1 and 6 at 2.8 kPa [0.4 psi] for 18 seconds), and media perfusion steps for wells 2–5. To maximize adherence of slow-attaching cell lines, a pause phase where no well group is selected (thus no solution will flow), can be included prior to the step that creates the media perfusion.

Gradient Formation

The CellASIC® ONIX M04G Microfluidic Gradient Plate is designed to maintain stable diffusion gradients in cell culture. Diffusion across the upper and lower perfusion barriers (2 µm pores) creates a stable spatial gradient within the culture chamber. Continuous flow between an infinite source (wells 2–5) and infinite sink (wells 7–8) acts to maintain the stable gradient profile for days (Figure 9). The flow properties of each inlet well are provided in Figures 5 and 6.

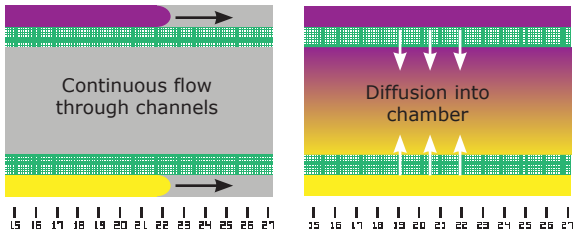

Figure 9. Creation of stable gradient

Spatial gradients are formed by flowing through the upper and lower inlet channels simultaneously. Inlet wells 2 and 3 flow solution across the barrier at the top of the chamber while wells 4 and 5 flow solution across the bottom barrier. While flowing through the channels, solutions will diffuse across the barriers and into the chamber. Once stable, the gradient profile will be linear in the y-axis with no variation in the x-axis.

1. Aspirate PBS solution from wells that will be used for gradient formation. Add 350 µL medium to the appropriate 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 one of the **New Experiment** options, and find the M04G plate on the drop down list. Click on the **Protocol Editor** tab (Figure 11). To establish a gradient, any of the following four combinations of wells can be used: wells 2 + 4 (flow well groups 2 and 4), 2 + 5, 3 + 4, or 3 + 5. The recommended pressure for linear gradient formation is 6.9–13.8 kPa (1–2 psi). If a sharper transition is required, a faster flow rate can be applied. In most cases, steady state is reached in approximately 20 minutes. A representative protocol is outlined in the Software Operation section.
4. For live-cell viewing, place the sealed plate/manifold assembly on an inverted microscope.

Solution Switching

Due to the short diffusive distance between the flow channels and culture chamber, and the small size of the chamber itself, the CellASIC® ONIX M04G Microfluidic Plate is capable of rapid solution switching. Each culture unit (A–D) has four switchable inlet wells for the manipulation of media conditions.

1. Aspirate PBS solution from the chosen inlet wells (2–5). Add 350 µL medium to the appropriate 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 one of the **New Experiment** options, and find the M04G plate on the drop down list. Click on the **Protocol Editor** tab and create the appropriate protocol. For information on creating a protocol, 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 for the initial transition (34.5 kPa [5 psi] for 1 minute), then reduce flow to standard pressure 3.4–6.9 kPa (0.5–1 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 details on software features.

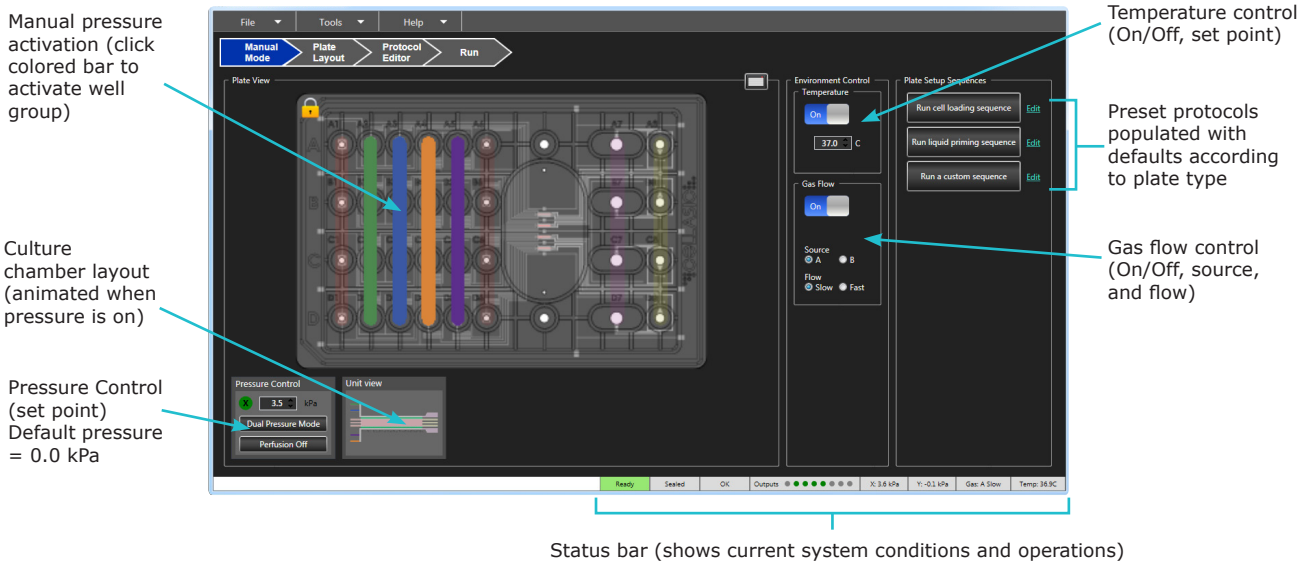

Figure 10. Manual Mode allows interactive operation of the ONIX2 System. Operating parameters can be set manually and this mode also provides the option to run short automated plate setup sequences that are prepopulated with plate-specific defaults. These setup sequences can be edited if desired.

In the culturing protocol example outlined below, cell chambers were primed with growth medium by gravity diffusion prior to attaching the CAX2-MXT20 manifold. In this experiment, cells were loaded into the culture chamber from well 6 by applying pressure (2.8 kPa [0.4 psi] for 18 seconds) to well groups 1 and 6. Following loading, a 1 hour pause step was included to allow for cell attachment. Next, cells were perfused with standard growth medium for 2 days (48 hours) from wells 2 and 4. At day 2, cells were synchronized by exposure to serum-free medium in wells 3 and 5 for 24 hours. Following serum starvation, cultures were exposed to a 0–20% FBS (fetal bovine serum) gradient using wells 2 (0% FBS medium) and 4 (20% FBS medium). At 24 hours, the gradient's orientation was flipped by switching to perfusion from wells 3 (20% FBS) and 5 (0% FBS). A 5-minute pause step was included to allow for media replacement in wells 2–5. Temperature was controlled with the CAX2-MXT20 manifold, using a setpoint of 37 °C.

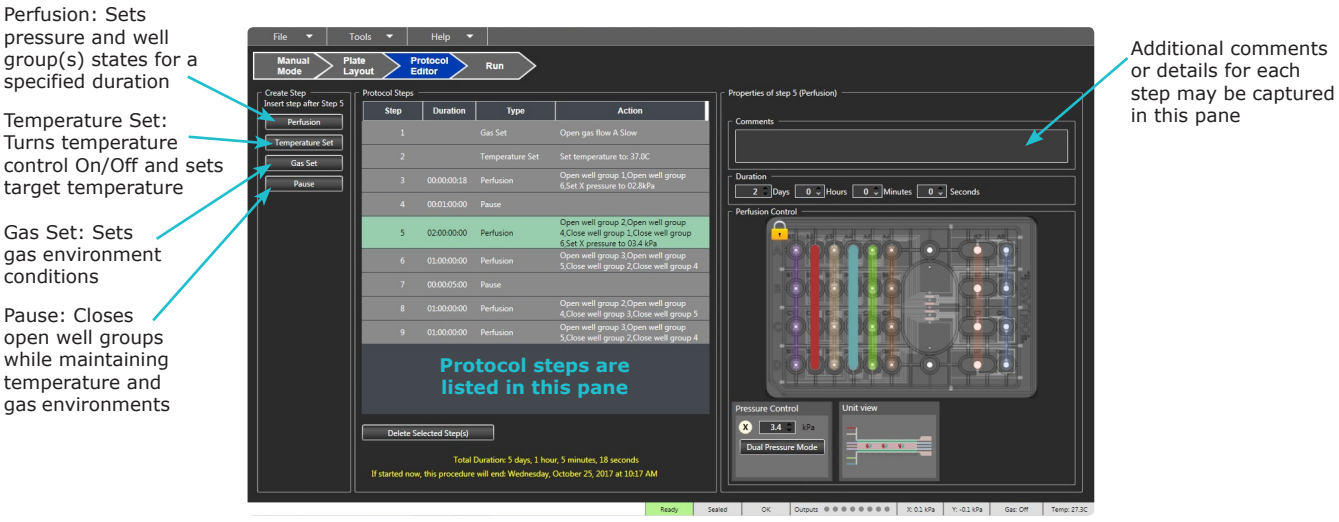

Figure 11. Protocol Editor mode allows the creation and editing of an experimental protocol. A protocol is comprised of a sequence of environmental control and/or perfusion steps. Steps can be added and altered as desired. When the protocol is ready, it can be executed using the **Run** tab.

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	4.0 mm (0.16 in.)
Width	0.5 mm (0.02 in.)
Trap heights	50 µm
Culture chamber sample volume	0.1 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

Notice

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Technical Assistance

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Standard Warranty

The applicable warranty for the products listed in this publication may be found at www.sigmaaldrich.com/terms.

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