Millipore®

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User Guide

CellASIC® ONIX M04L-03 Microfluidic Plate

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

Introduction

The CellASIC® ONIX M04L-03 Microfluidic Plate is a 4-chamber cell culture plate with an open-top format permitting direct pipet access to the culture chamber. The plate is well-suited for loading multicellular aggregates such as spheroids. It can be used with the CellASIC® ONIX2 Microfluidic System and CellASIC® ONIX2 basic manifold for perfusion-based, short-term (3–6 hours), live-cell analysis with solution switching. The open chambers are subject to rapid evaporative loss of fluid; for this reason, the M04L plate is not compatible with CellASIC® ONIX2-driven long term culture applications.

Applications

- Short-term time-lapse analysis of adherent cells and multicellular aggregates (3–6 hours)
- Long-term gravity-based perfusion experiments (3 days typical)
- Solution exchange experiments (induction, inhibition, drug dosing etc.)
- Automated immunostaining and "on-demand" fixation of live cells within the culture chamber
- Comparison of up to 4 different cell types or exposure conditions (media components) in parallel

Plate Description

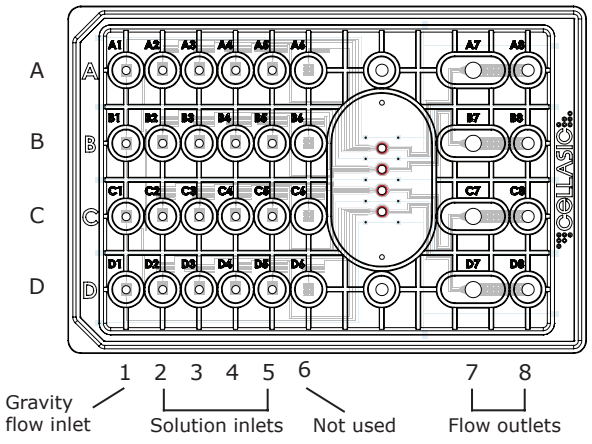

Figure 1. Plate configuration

The M04L microfluidic plate has four independent culture units (A–D), each with a gravity flow inlet (1), four solution inlets (2–5), and two shared outlets (7 and 8). Well 6 is not used. 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. A strip of tape is used to seal the open chambers during shipping. This should be removed prior to use. Do not remove the ring around the chambers. The plate is for single use only.

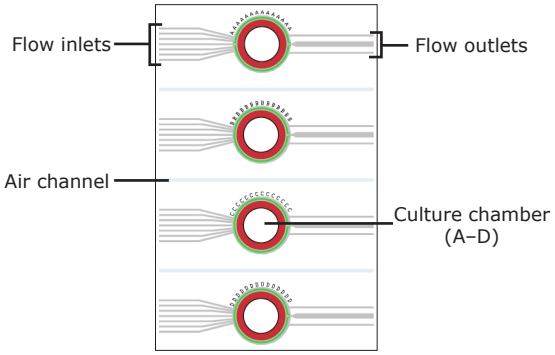

Figure 2. Chamber viewing window

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

Figure 3. Culture chamber

A perfusion barrier surrounds the chamber to separate it from the flow channels, while the open-top design uses surface tension above the chamber to prevent liquid from flowing out into the viewing well. A hydrophobic ring around each chamber enhances the surface tension forces. 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 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 6	Not used	N/A	N/A
Outlets 7 and 8	Outlet from culture chamber	50	900*

* Outlets 7 and 8 combined

Manifold Description

The CellASIC® ONIX2 basic manifold (CAX2-MBC20) connects the microfluidic plate to the CellASIC® ONIX2 Microfluidic System providing media perfusion and switching, but without gas or temperature control.

NOTE: Due to issues with media evaporation, the M04L plate is not compatible with the CellASIC® ONIX2 heated manifold (CAX2-MXT20) and thus cannot be used for long-term culture with complete environmental control.

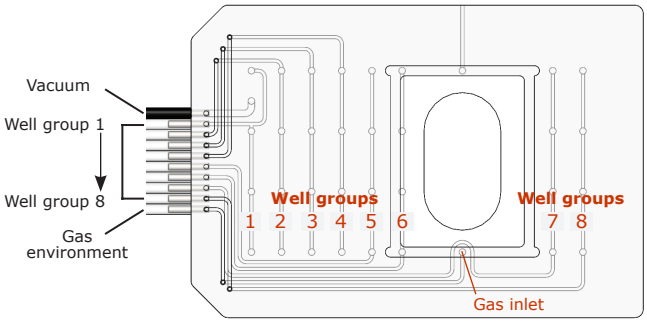

Figure 4. Lines to CellASIC® ONIX2 Microfluidic System

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

The flow properties of wells 2–5 are shown in Figure 5. 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.

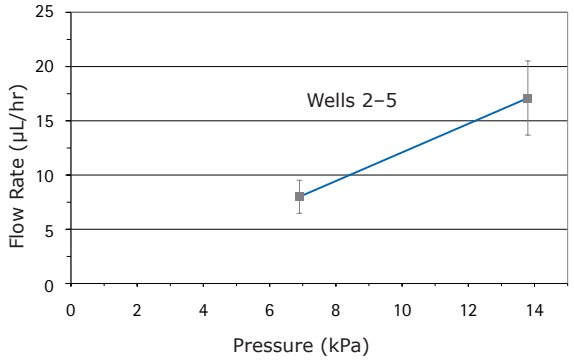

Figure 5. Flow rate for wells 2–5

Plate Storage

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

Limitations

This plate cannot be used with the CellASIC® ONIX2 heated manifold (CAX2-MXT20) for long-term CellASIC® ONIX2-driven automated cell culturing because the open chambers are subject to evaporative loss. For long term culture applications, refer to the Cell Culture with the CellASIC® ONIX2 Microfluidic System section for recommendations.

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

NOTE: Avoid overfilling or underfilling the open chamber. A volume of 5 µL is recommended. If more is used, there will be flow out of the culture chamber. If less is used, there will be flow into the culture chamber.

When manipulating the volumes of the open chamber, make sure that well 7 is empty, but leave liquid in the hole at the bottom of the well.

When not manipulating the open chamber volume, make sure that the hole at the bottom of well 7 is covered with liquid (~50 µL) to ensure proper flow.

Do not use pressures higher than 13.8 kPa (2 psi), as this will cause the chamber volume to increase and the culture chamber will overflow.

Precoating with ECM (Optional)

NOTE: For some cell types, pretreating the chambers with ECM (extracellular matrix) coating solutions may be necessary.

1. Prepare the ECM coating solution according to desired procedure.
2. Aspirate the PBS solution from wells 7 and 8, but leave solution in the hole at the bottom of both wells.
3. Replace the open chamber volume with 5 µL of coating solution.
4. Incubate according to desired coating protocol.
5. If wash step(s) are required, aspirate the coating solution from the open chamber and add 5 µL of wash solution.
6. If longer coating times (>2 hours) are required, add 300 µL of coating solution to well 1 and 50 µL to well 7 for gravity perfusion coating.

Cell Loading

1. Prepare a cell suspension of 0.25–1 × 10^6 cells/mL.
2. Aspirate wells 7 and 8, but leave solution in the hole at the bottom of both wells.
3. Replace the open chamber volume with 5 µL of cell suspension.
4. Proceed to Cell Culture section.

Cell Culture

Culture in an Incubator

1. Aspirate PBS solution from the top of well 1. Do not aspirate the bottom hole. Pipette 350 µL of growth medium into well 1 and 50 µL into well 7 to initiate gravity-driven perfusion.
2. Place plate in incubator. Replace the medium in well 1 and empty wells 7 and 8 every 2–3 days for long-term cell culture.

Cell Culture with the CellASIC® ONIX2 Microfluidic System

NOTE: This requires a microscope equipped with a heated enclosure (refer to manufacturer instructions for setup) and the CellASIC® ONIX2 Premixed Gas Regulator (CAX2-ABR00) or similar gas mixer. Connect the manifold to the premixed gas mixture. A mix of 5% CO_2 balanced with air is recommended, but this may vary depending on the cell type.

1. Aspirate PBS solution from wells that will be used for perfusion (wells 2–5). Add 350 µL medium to these wells.
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 M04L plate on the drop down list. Click on the **Protocol Editor** tab (Figure 7) and enter the desired steps and conditions. For wells 2–5, 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.
4. To monitor cell growth, place the sealed plate/manifold assembly on an inverted microscope.
5. During extended pe rfusion 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.

Solution Switching

1. Fill the four sets of solution inlet wells (2–5) with up to 350 µL of solution.

   NOTE: If not all solution inlet wells are being used, leave the unused wells (2–5) filled with buffer, but remove the PBS from inlet well 1. This prevents the fluid in well 1 from interfering with flow in the other inlet wells.

2. We do not recommend using well 1 for solution switching experiments. Fully aspirate the liquid from this well.
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 the M04L plate on the drop down list, and click on the **Protocol Editor** tab (Figure 7) to create and initiate custom protocols. To manually control flow, use the **Manual Mode** tab to select the desired wells and pressure. For information on automated protocols or manual perfusion, refer to the CellASIC® ONIX2 Microfluidic System User Guide.

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.

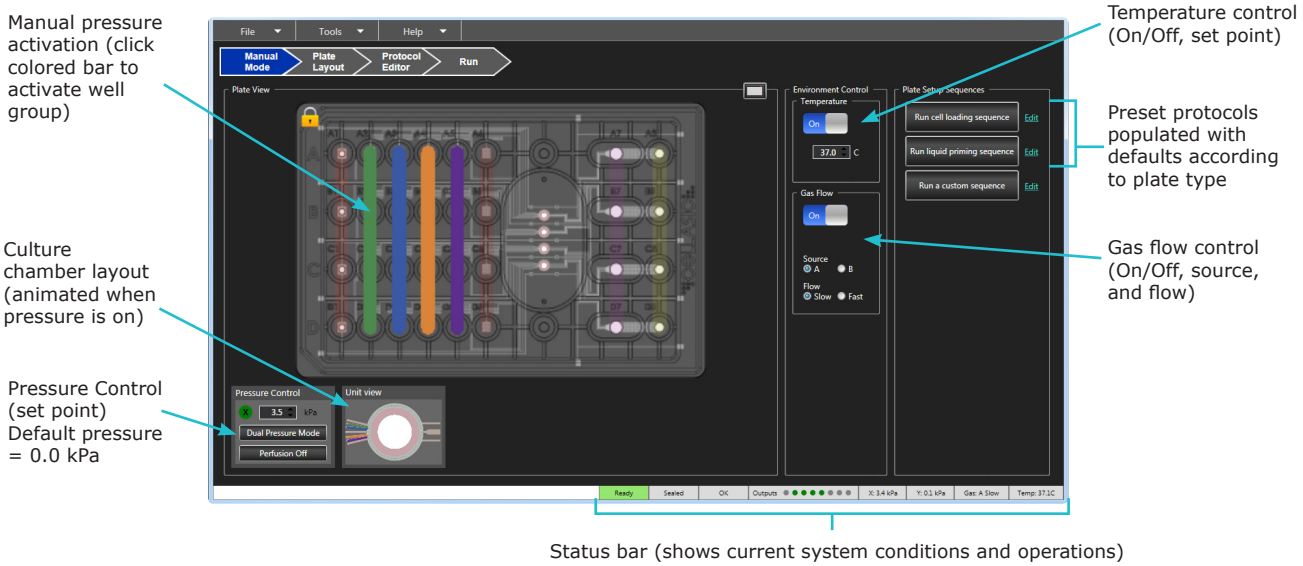

Figure 6. Manual Mode allows interactive operation of the CellASIC ONIX2 System. Operating parameters can be set manually and this mode also provides the option to run short automated plate setup sequences that are prepopulated with plate-specific defaults. These setup sequences can be edited if desired.

In the culturing protocol example outlined below, cells were perfused with reagent 1 from wells 2 and 4 for 3 hours, followed by perfusion with reagent 2 from wells 3 and 5 for 3 hours.

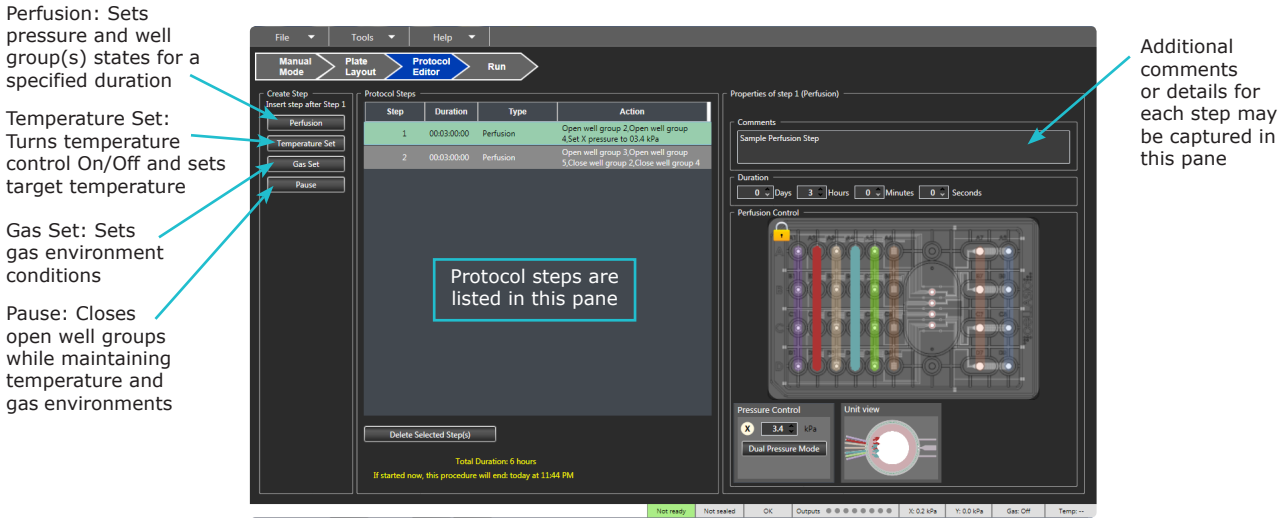

Figure 7. Protocol Editor mode allows the creation and editing of an experimental protocol. A protocol is comprised of a sequence of environmental control and/or perfusion steps. Steps can be added and altered as desired. When the protocol is ready, it can be executed using the **Run** tab.

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	
Diameter	2.0 mm (0.08 in.)
Height	1.4 mm (0.06 in.)
Culture chamber sample volume	5.0 µL
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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Standard Warranty

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