User Guide

CellASIC® ONIX M04S-03 Microfluidic Plate

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

Introduction

The CellASIC® ONIX M04S-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 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 adherent cells
- Long-term continuous 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 four different cell types or exposure conditions (media components) in parallel
- Temperature and gas atmospheric control (temperature shift, anoxic conditions, etc.)

Plate Description

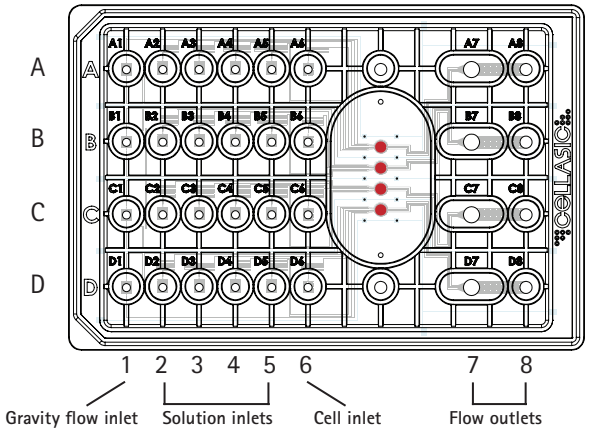


Figure 1. Plate configuration

The M04S 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 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.

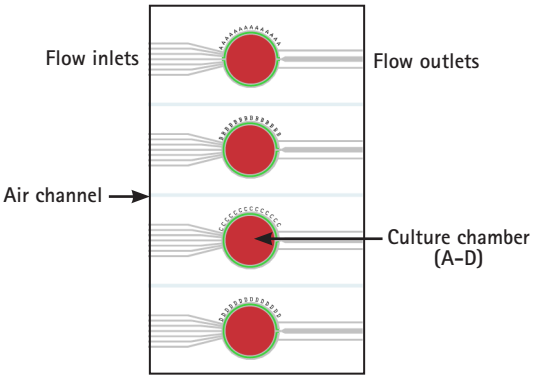


Figure 2. Chamber viewing window

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

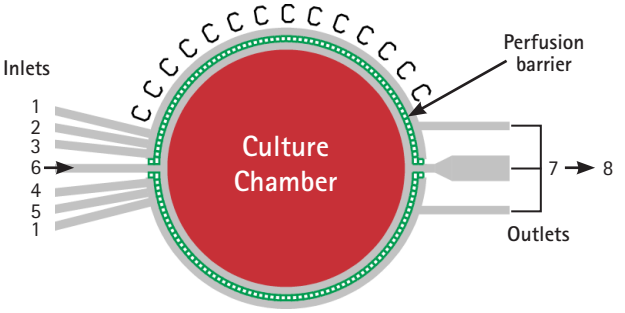


Figure 3. Culture chamber

A perfusion barrier surrounds the chamber to separate it 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 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	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.

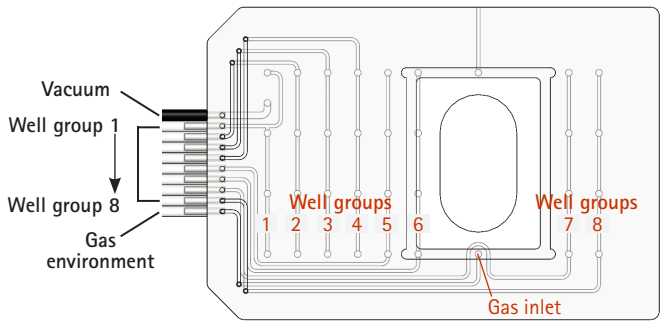


Figure 4. Lines to CellASIC® ONIX2 Microfluidic System

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

The flow properties of wells 2–5 are shown in Figure 5 and those of wells 1 and 6 are shown in Figure 6. Each 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.

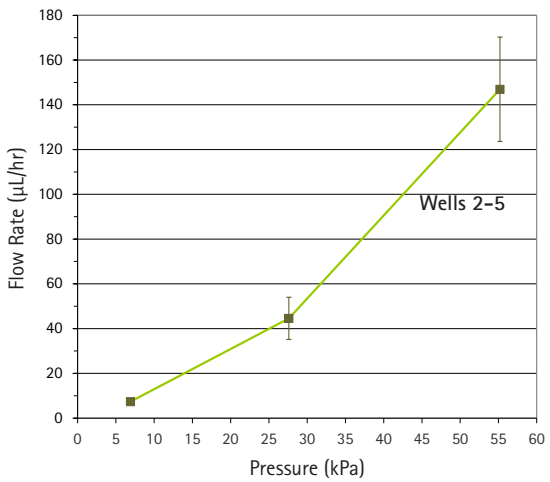


Figure 5. Flow rate for wells 2–5

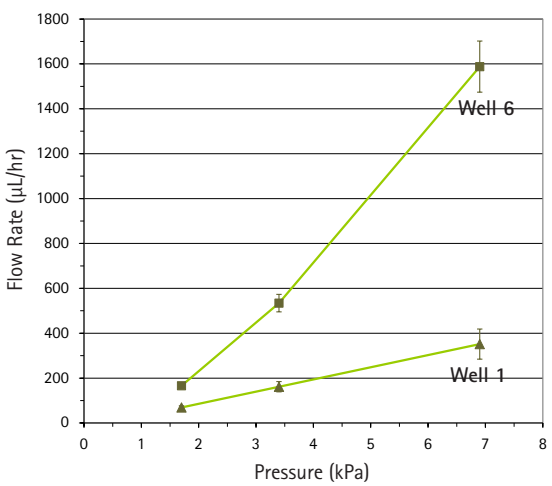


Figure 6. Flow rate for wells 1 and 6

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 wells 1, 6, 7, and 8, but not the bottom holes, of the unused chamber row(s). This will prevent liquid from overflowing during the experiment. After the experiment, add PBS back into these wells 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: For some cell types, pretreating the chambers with medium or ECM (extracellular matrix) coating solutions may be necessary. This step is recommended when using capillary loading to ensure even distribution of cells in the cell chamber.

1. Prepare the ECM coating solution or medium according to desired procedure.
2. Aspirate the PBS solution from the upper part of wells 1 and 8, leaving PBS in the bottom holes.
3. Aspirate the PBS solution from well 6, including the bottom hole with the PTFE (polytetrafluoroethylene) ring around it (Figure 7).
4. Aspirate the PBS solution from well 7, including the bottom hole.

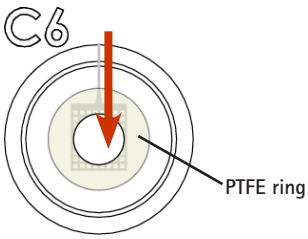


Figure 7. Well 6 cell inlet

5. Pipette 10 µL of ECM coating solution (or medium) into the bottom hole of well 6. This initiates capillary flow from well 6 to the culture chamber. The empty well 7 causes capillary action to pull the ECM coating solution or medium into the chamber.
6. Incubate according to desired coating protocol.
7. If wash step(s) are required, aspirate ECM coating solution (or medium) from well 6 (including bottom hole) and add 10 µL wash solution. Aspirate wells 7 and 8 (including bottom holes) to induce capillary flow.

Cell Loading

Capillary Method

NOTE: When choosing capillary action for loading, ECM coating or priming is recommended to ensure even distribution of cells in the cell chamber.

1. Prepare a cell suspension of 1–5 × 10^6 cells/mL.
2. Aspirate solution from cell inlet well 6 including the bottom hole with the PTFE ring around it. (Figure 7).
3. Aspirate well 7, including the bottom hole.
4. Pipette 10 µL of cell suspension into the bottom hole of well 6. This initiates capillary action to pull the cells into the chamber.
5. Place the plate on a microscope to monitor loading progression in real time.

Cell Loading, continued

6. Allow the cells to flow for up to 30 minutes. If more cells are desired, repeat steps 4 and 5.
7. Proceed to Cell Culture section.

Pressure-Driven Method Using the CellASIC® ONIX2 Microfluidic System

1. Prepare a cell suspension of 1–5 × 10^6 cells/mL.
2. Aspirate solution from cell inlet well 6 including the bottom hole with the PTFE ring around it at the bottom of the well (Figure 7).
3. Aspirate PBS from upper part of wells 7 and 8 but leave PBS in bottom holes.

   NOTE: If loading cells using the pressure-driven method after ECM coating, or priming with the capillary method, make sure to add medium or PBS to the bottom hole of well 7 (10 µL or enough volume to fill the bottom hole) before adding cell suspension to the bottom hole of well 6. This prevents capillary action from pulling unwanted cells into the cell chamber.
4. Pipette 10 µL of cell suspension into the bottom hole 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 M04S plate on the drop down list. On the **Manual Mode** tab (Figure 8), click on the **Run cell loading sequence** button. The recommended pressure and flow time for well group 6 are 1.7 kPa (0.25 psi) and 6 seconds but you may need to optimize these conditions depending on your cell type and/or concentration.
7. Check loading density on a microscope. If more cells are desired, repeat steps 4–7.
8. Proceed to Cell Culture section.

Cell Culture

Preculture in incubator (optional)

1. Cells can be perfused in the cell chamber using gravity-driven flow. Some cell types may require a preculture step in the incubator for several hours or this culture method can be used when environmental control is not available.
2. 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.
3. 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 CellASIC® ONIX2 Microfluidic System

1. Aspirate PBS solution from wells that will be used for perfusion (wells 2–5). Add 350 µL medium to these wells.

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

   For any experiment, even if not all culture units (A, B, C, or D) on a microfluidic plate are to be used, remember to monitor wells 2–5 for solution level. If wells become empty, dehydration and bubble formation can occur within the cell chamber, preventing use of these units in subsequent experiments. Also, wells 7 and 8 must be emptied periodically to prevent solution overflow into the manifold tubing and microfluidic system. The combined volume of wells 7 and 8 is approximately 900 µL.
2. Seal the microfluidic plate to the ONIX2 manifold according to the CellASIC® ONIX2 Microfluidic System User Guide.

Cell Culture, continued

3. Open the CellASIC® ONIX2 Software, select one of the **New Experiment** options, and find the M04S plate on the drop down list. Click on the **Protocol Editor** tab (Figure 9) 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.
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 load cells and perfuse in a single uninterrupted protocol, rather than in two separate protocols. Set up the loading cell step for well group 6, followed by the perfusion steps for well groups 2–5 when creating the protocol. Refer to basic protocol example in Figure 9. Load 10 µL of cell suspension into well 6, and 350 µL of medium into the wells that will be used for perfusion (2–5).

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 wells 1 and 6 for solution switching experiments when using the CellASIC® ONIX2 Microfluidic System. Fully aspirate the liquid from these wells.

   NOTE: If the experiment requires more than four inlets, there is an option to use well 1. If well 1 is used in experiments and contains a solution volume, the solution inlet wells (2–5) need to flow at 48.3 kPa (7 psi).
3. Seal the microfluidic plate to the manifold according to the CellASIC® ONIX2 Microfluidic System User Guide.
4. Open the CellASIC® ONIX2 Software, select the M04S plate on the drop down list, and click on the **Protocol Editor** tab (Figure 9) 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 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.

For symmetric flow switching between two solutions, use inlets 2 and 5 for the first solution and 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 8. 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.

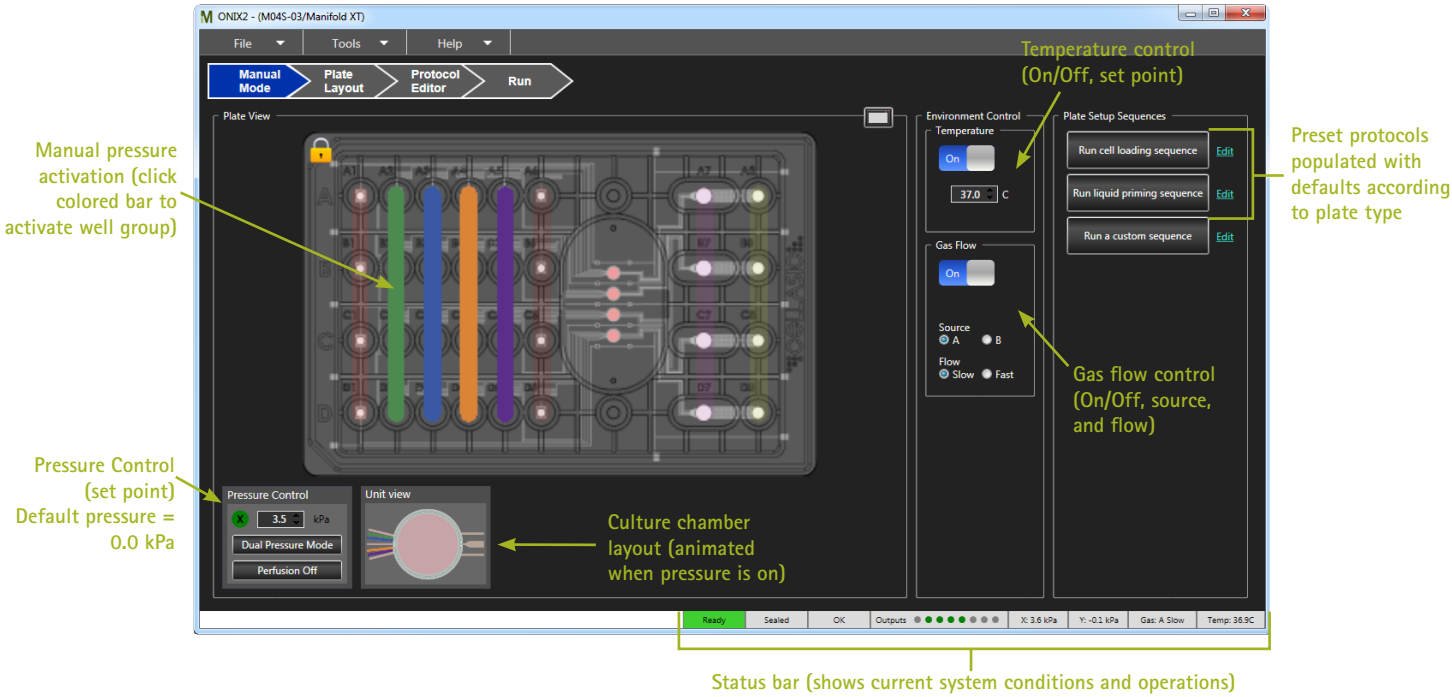
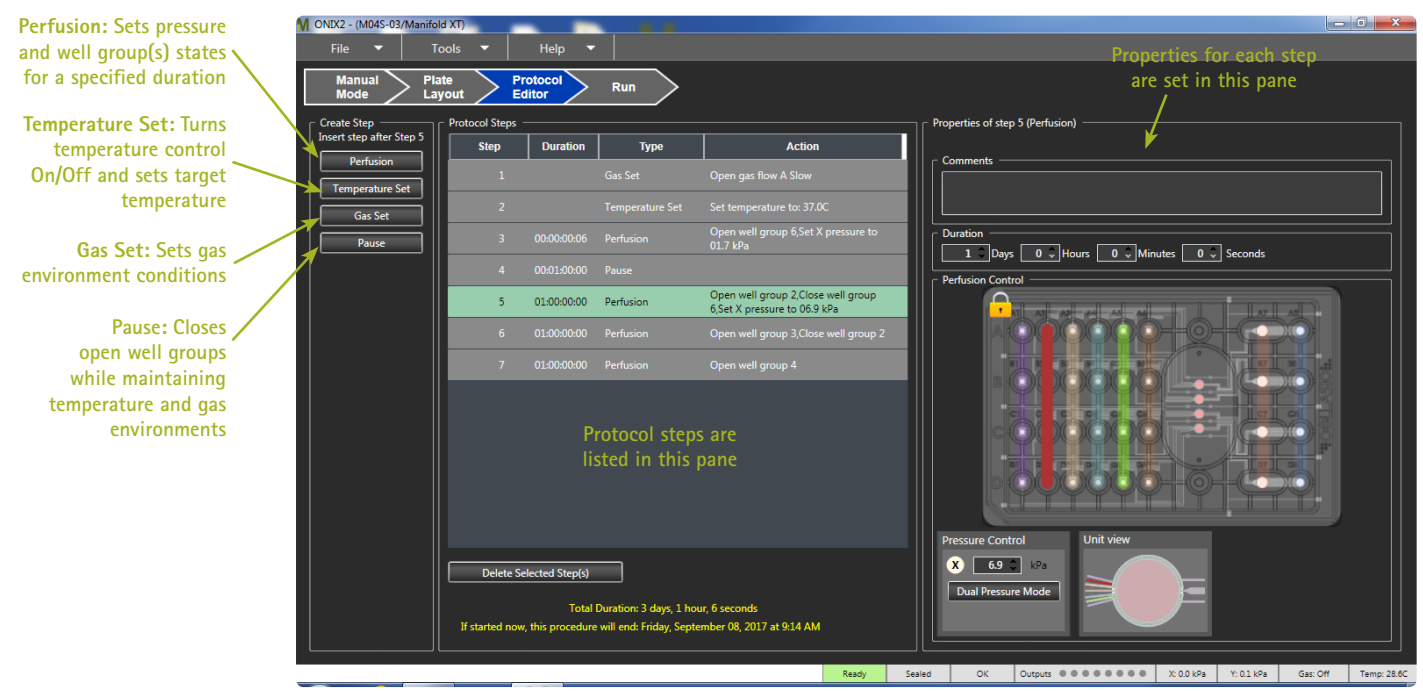


Figure 9. Protocol Editor mode allows the creation and editing of an experimental protocol. A protocol is comprised of a sequence of environmental control and/or perfusion steps. Steps can be added and altered as desired. When the protocol is ready, it can be executed using the **Run** tab.

In the culturing protocol example outlined below, cells were loaded into the culture chamber from well 6 by applying pressure (1.7 kPa [0.25 psi] for 6 seconds) to well group 6. Next, cells were perfused with standard growth medium for one day (24 hours) from well 2, one day from well 3, and one day from well 4. Temperature was controlled with the CAX2-MXT20 manifold, using a setpoint of 37 °C.



Specifications

Culture Plate Dimensions	
Length × width	127.3 × 85.2 mm (5.0 × 3.4 in.)
Height without lid	14.3 mm (0.6 in.)
Culture Chamber Dimensions	
Diameter	2.8 mm (0.1 in.)
Height	120 µm
Culture chamber sample volume	0.9 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. See Technical Assistance section for contact information. You can purchase these products and find the most up-to-date software, plate maps, and user guides at www.millipore.com/cellasic.

Description	Catalogue Number	Qty/pk
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)	B04A-03-5PK	5
CellASIC® ONIX Gradient Plate for Mammalian Cells (4-chamber)	M04G-02-5PK	5
CellASIC® ONIX Open-top Plate for Mammalian Cells (4-chamber)	M04L-03-5PK	5
CellASIC® ONIX Switching Plate for Mammalian Cells (4-chamber)	M04S-03-5PK	5
CellASIC® ONIX Plate for Haploid Yeast Cells (4-chamber, trap heights of 3.5, 4.0, and 4.5 µm)	Y04C-02-5PK	5
CellASIC® ONIX Plate for Diploid Yeast Cells (4-chamber, trap heights of 5.0, 6.0, and 7.0 µm)	Y04D-02-5PK	5
CellASIC® ONIX Pad Trap Plate (4-chamber, trap height of 4.0 µm)	Y04T-04-5PK	5
CellASIC® ONIX2 Microfluidic System and Manifolds		
CellASIC® ONIX2 Microfluidic System	CAX2-S0000	1
CellASIC® ONIX2 Manifold XT (temperature controlled)	CAX2-MXT20	1
CellASIC® ONIX2 Manifold Basic (no temperature control)	CAX2-MBC20	1
Replacement Parts/Accessories		
CellASIC® ONIX2 Filter Multiconnector (includes filters)	CAX2-AMC00	1
CellASIC® ONIX2 Software USB Drive	CAX2-SSW01	1
CellASIC® ONIX2 Gasket	CAX2-AGK20	1
CellASIC® ONIX2 Self Check Plate	CAX2-ASP20	1
CellASIC® ONIX2 Cleaning Plate	CAX2-ACP20	1
CellASIC® ONIX2 Replacement Filter Pack (9 × 4 mm and 1 × 13 mm Millex® 0.45 µm PTFE filters)	CAX2-AFP00	1
CellASIC® ONIX2 Accessory Fittings (quick-connect gas fitting, 2/pk)	CAX2-ABF00	1
CellASIC® ONIX2 Temperature Calibration Plate	CAX2-ACT20	1

Product Ordering Information, continued

Description	Catalogue Number	Qty/pk
Replacement Parts/Accessories		
CellASIC® ONIX2 Premixed Gas Regulator (for use with 103 L or 112 L gas cylinders with a C10 connection)	CAX2-ABR00	1
CellASIC® ONIX2 Microfluidic Services		
CellASIC® ONIX2 Essential Service Plan	CAX2-ESVC	1
CellASIC® ONIX2 Total Service Plan	CAX2-TSVC	1
CellASIC® ONIX2 Installation	CAX2-INST	1

Notice

The information in this document is subject to change without notice and should not be construed as a commitment by EMD Millipore Corporation ("Millipore") or an affiliate. Neither EMD Millipore Corporation nor any of its affiliates assumes responsibility for any errors that may appear in this document.

Technical Assistance

For more information, contact the office nearest you. In the U.S., call 1-800-645-5476. Outside the U.S., go to our web site at www.millipore.com/offices for up-to-date worldwide contact information. You can also visit the tech service page on our web site at www.millipore.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at www.millipore.com/terms ("Conditions of Sale").

The M logo, Millipore, Millex, and CellASIC are trademarks of Merck KGaA, Darmstadt, Germany. Detailed information on trademarks is available via publicly accessible resources.

© 2017 Merck KGaA, Darmstadt, Germany and/or its affiliates. All rights reserved.

00003670, Rev. 11/17