

User Guide

CellASIC® ONIX Y04C-02 Microfluidic Yeast Plate

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

Introduction

The CellASIC® ONIX Y04C-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 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 yeast 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 mother/daughter cells over generations)
- Temperature and gas atmospheric control (temperature shift, anoxic conditions etc.)

Plate Description

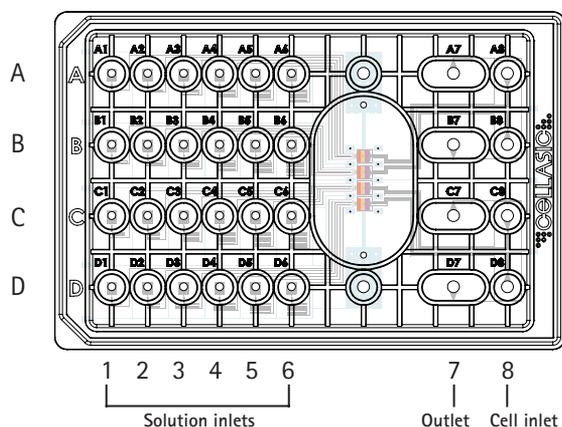


Figure 1. Plate configuration

The Y04C plate has 4 independent units (A-D), each with 6 inlet wells (1-6), a cell inlet (8), and a large outlet well (7). 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. Each chamber has trap regions of 3.5, 4.0, and 4.5 μm in height to hold cells in a single focal plane during long-term analysis. The plate is for single use only.

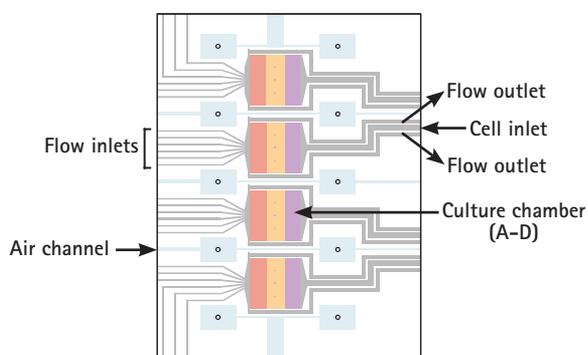


Figure 2. Chamber viewing window

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

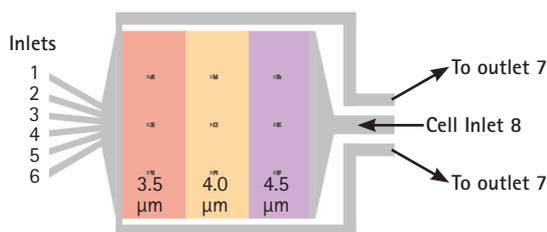


Figure 3. Culture chamber

The culture chamber is 3.0 \times 3.0 mm in area with trap heights of 3.5, 4.0, and 4.5 μm . Nine position markers indicate unit number and relative position. 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 6 | Inlet for solution switching | 50 | 350 |
| Inlet 8 | Cell inlet for loading cells into culture chamber | 50 | 350 |
| Outlet 7 | Accepts flow-through from culture chamber | 50 | 600 |

Cell Trapping Mechanism

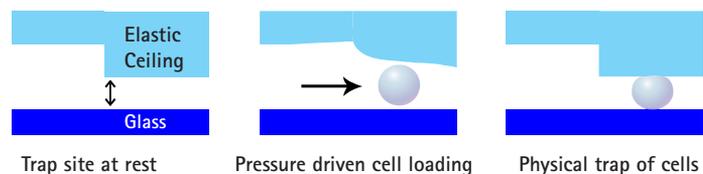


Figure 4. Cell trapping mechanism

The microfabricated chamber gently holds cells against the glass viewing surface to maintain a single focal plane during perfusion analysis experiments. The Y04C plate has trap heights of 3.5, 4.0, 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.

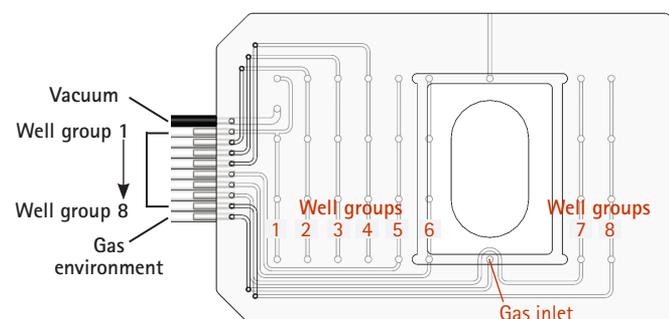


Figure 5. Lines to CellASIC® ONIX2 Microfluidic System

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

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.

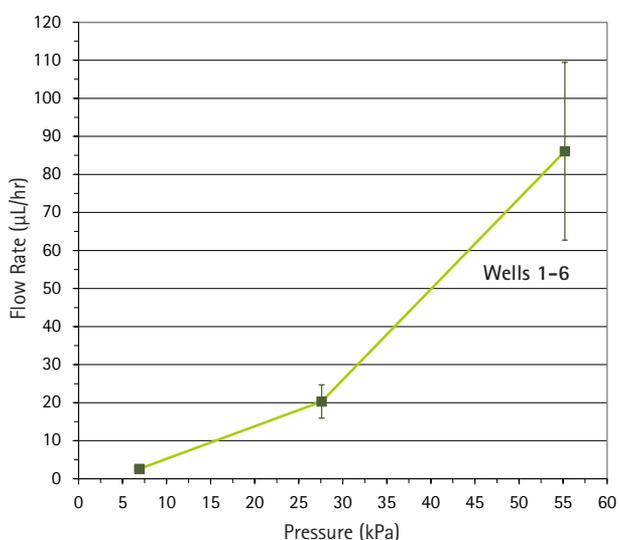


Figure 6. Flow rate for wells 1–6

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 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

1. Aspirate the PBS solution from wells that will be used for the experiment and add 350 μL of your solution/medium to these wells. Make sure that the unused solution inlet wells are filled with buffer.
2. **Steps 2–4 are optional:** If your experiment requires complete removal of PBS, replace the PBS in the solution (1–6) and cell inlet (8) wells with 150 μL of your desired priming solution.
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 Y04C 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 are 34.5 kPa [5 psi] and 5 minutes, respectively. For information on creating a protocol, refer to the CellASIC® ONIX2 Microfluidic System User Guide.

Cell Loading

Pressure-Driven Method Using the CellASIC® ONIX2 Microfluidic System

1. Prepare a yeast/cell suspension of $1.5\text{--}6 \times 10^6$ cells/mL. This concentration may need optimization depending on the yeast strain and desired trapping density.
2. Aspirate solution from cell inlet well 8 and waste outlet well 7.
3. Pipette 50 μL of cell suspension into cell inlet well 8, making sure to cover the hole at the bottom of the well.
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 Y04C 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 55.2 kPa [8 psi] and 5 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.
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 1–6). Add 350 μL medium to these wells. Make sure that the unused solution inlet wells are filled with buffer.
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 Y04C plate on the drop down list. Click on the **Protocol Editor** tab and enter the desired steps and conditions. For wells 1–6, 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 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 (1–6). 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 Y04C 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.

For symmetric flow switching between 2 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 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.

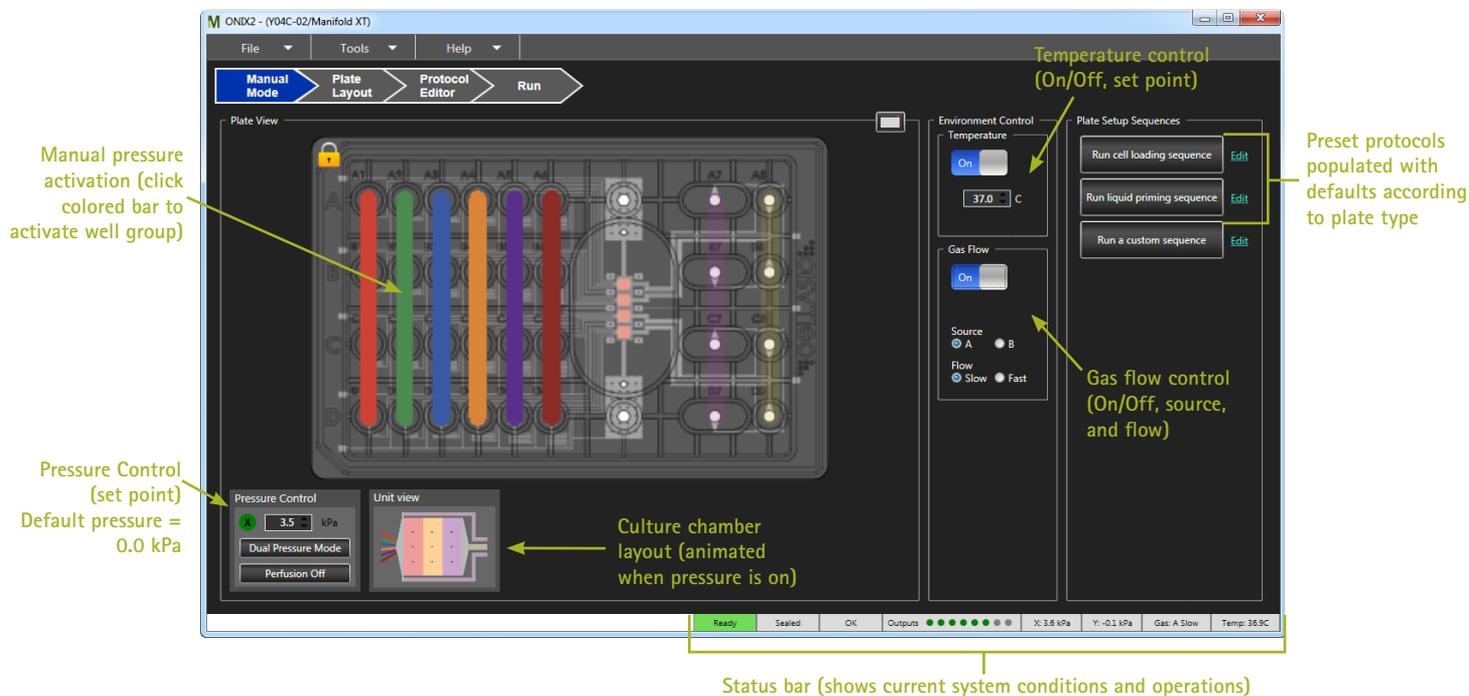
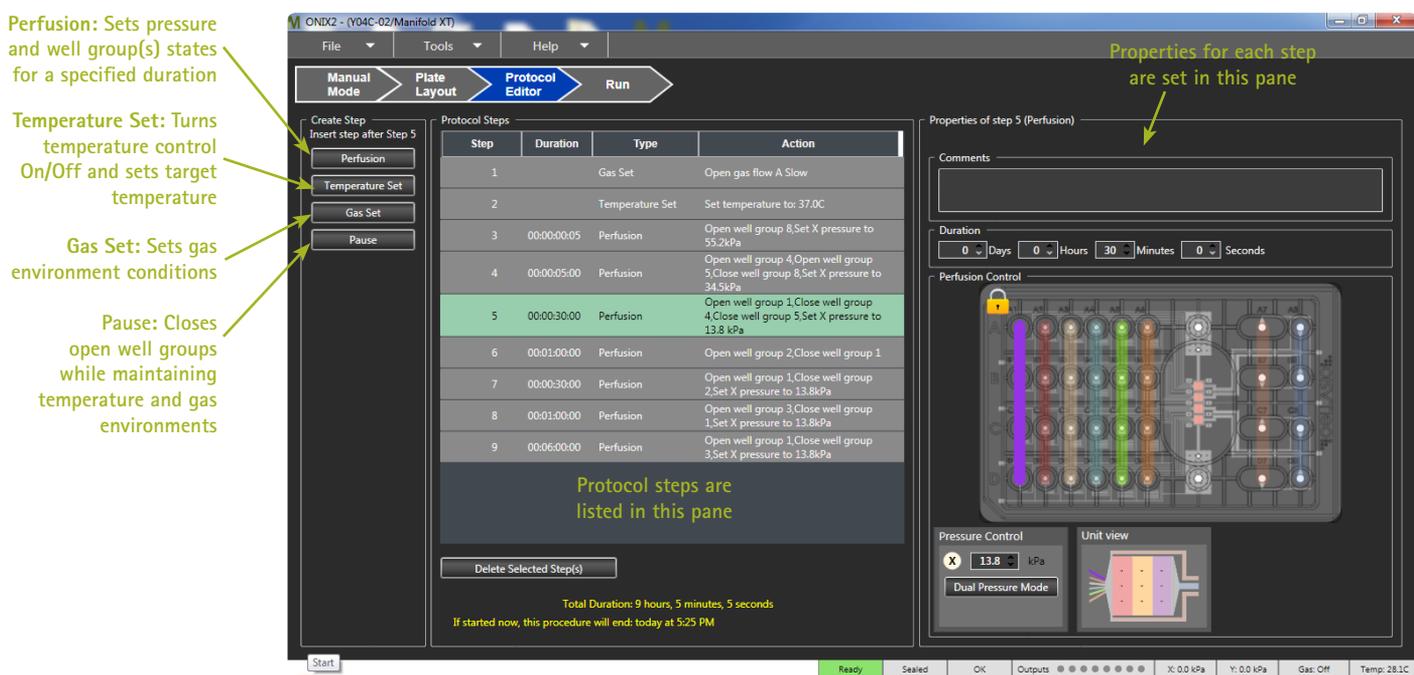


Figure 8. Protocol Editor mode allows the creation and editing of an experimental protocol. A protocol is comprised of a sequence of environmental control and/or perfusion steps. Steps can be added and altered as desired. When the protocol is ready, it can be executed using the **Run** tab. In the culturing protocol example outlined below, cells were loaded from well 8 by applying pressure (55.2 kPa [8 psi] for 5 seconds) to well group 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.



Specifications

| | |
|-------------------------------------|---|
| Culture Plate Dimensions | |
| Length x width | 127.3 x 85.2 mm (5.0 x 3.4 in.) |
| Height without lid | 14.3 mm (0.6 in.) |
| Culture Chamber Dimensions | |
| Length | 3.0 mm (0.1 in.) |
| Width | 3.0 mm (0.1 in.) |
| Trap heights | 3.5, 4.0, and 4.5 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. 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 x 4 mm and 1 x 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.

00003673, Rev. 11/17