User Guide

CellASIC® ONIX M04T-01 Microfluidic Gradient Plate

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

Introduction

The CellASIC® ONIX M04T Microfluidic Plate is a 4-chamber cell culture plate designed for use with the CellASIC® ONIX2 Microfluidic System and CellASIC® ONIX2 Manifolds, enabling real-time imaging of 12 µm-sized suspension cells[1]. This bio-inspired plate provides a controlled and dynamic microenvironment for cells, which when used in conjunction with the ONIX2 system, permits perfusion-based, long-term, live-cell analysis with automated solution switching. The easy-to-use format and novel microfluidics-based technology redefine the standard for live cell imaging experimentation.

Applications

- Trapping and monitoring of mammalian suspension of 5–12 µm-sized cells[1]
- Time-lapse analysis of suspension 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 type and reagent concentration) in parallel

Plate Description

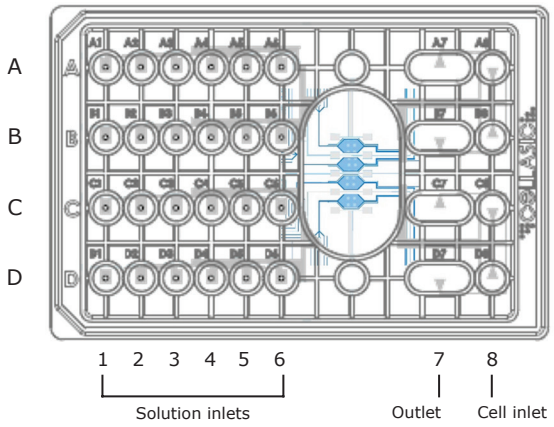

Figure 1. Plate configuration

The M04T 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 pre-primed with a PBS (phosphate-buffered saline) solution, which can be replaced with a buffer of choice prior to experiment. Each chamber has an array of 104 barrier trap pads 12.0 µm in height to hold cells in a single focal plane during long-term analysis. The plate is for single use only.

1. The trap has height of 12 µm with vertical barrier gap spacing of 3 µm (See "Cell Trapping Mechanism" for more detail), suitable to use with cells that are 5–12 µm in size. Depending on cell morphology, it is also possible to capture cells of larger sizes.

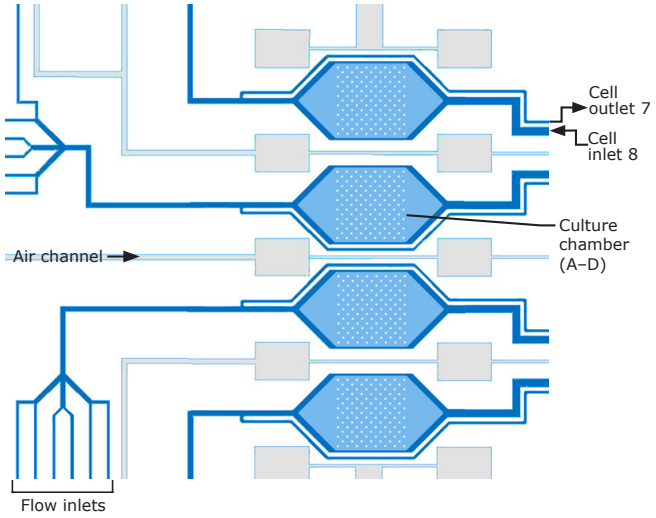

Figure 2. Chamber viewing window

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

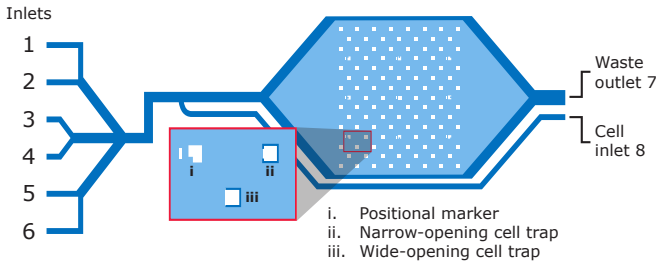

Figure 3. Culture chamber

Schematic indicating the dimensional features of tissue culture chamber with cell trap array. Notice that the colors represent different heights. White: 12 µm; light blue: 25 µm; dark blue: 40 µm. Zoomed-in area (red rectangle) shows the barrier surrounding each trap marked by royal blue with vertical gap height of 3 µm. Additional description on trap design can be found in Figure 4. The culture chamber hexagon marquee is 3.0×6.0 mm with a ceiling height of 25 µm (area with light blue color). Within each chamber, the culture array area is 3.0×3.0 mm with 104 interdigitating individual traps with two different openings (wide and narrow) and heights of 12 µm. Wide and narrow openings enable a balance between initial trapping efficiency and retention of cells over time, allowing more experimental flexibility. For example, narrow opening traps will have lower number of suspension cells initially trapped, while retaining these cells within the trap for a longer period, compared to the wide-opening traps. Nine position markers indicate unit number and relative position.

The inlet/outlet functions and minimum/maximum recommended 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
Outlet 7	Outlet from culture chamber	50	795
Inlet 8	Inlet for cell loading into culture chamber / Additional outlet from culture chamber	50	265

Cell Trapping Mechanism

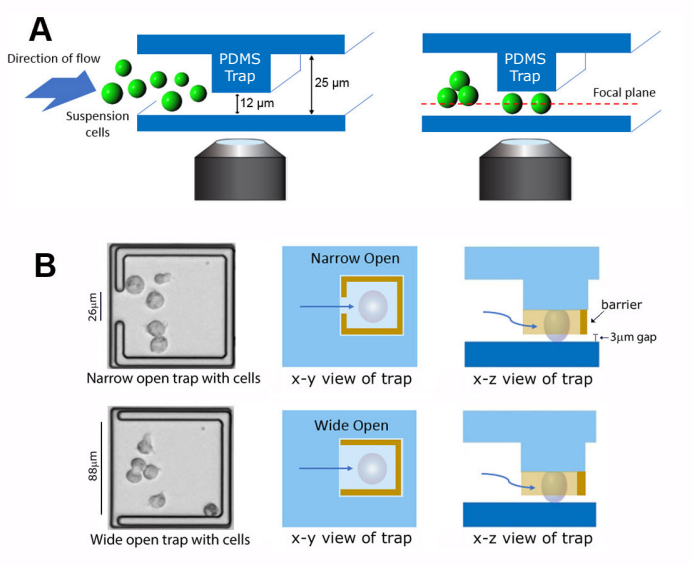

Figure 4. Cell trapping mechanism

A. Cross-sectional view of an individual trap. The microfabricated polydimethylsiloxane (PDMS) trap has x-y dimension of 100 µm and height of 12 µm. Due to its height, the traps can hold suspension cells gently against the glass viewing surface and maintain these cells within single focal plane during live cell imaging, facilitating more detailed imaging of suspension cells.

B. Image of traps with retained cells. Shown are narrow (top) and wide (bottom) opening traps with captured Jurkat cells. Notice the barriers bracketing each trap pad's perimeter on three sides, which act to retain motile cells within FOV (field of view) during imaging. Narrow and wide traps have openings of 26 µm, and 88 µm, respectively, with vertical gap height of 3 µm. Each trap is "open" on the side facing the direction of flow during cell loading.

Manifold Description

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

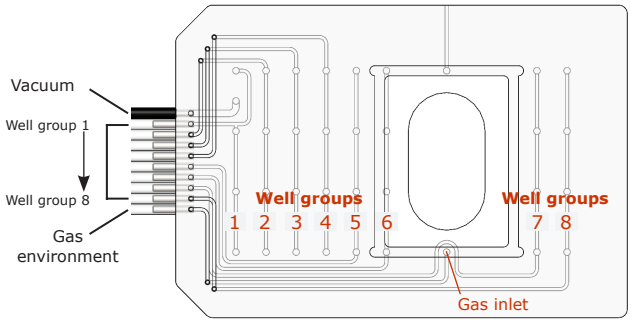

Figure 5. Lines to CellASIC® ONIX2 Microfluidic System

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.

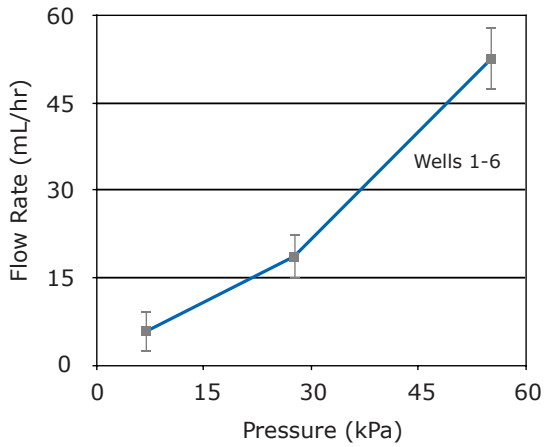

Figure 6. Flow rate for wells 1–6

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 Priming

1. For units to be used (A–D, see Figure 1), replace the PBS in the solution inlet (wells 1–6) and cell inlet (well 8) wells with 100–300 µL of your desired media, e.g., RPMI with 10% FBS. Aspirate and empty well 7 (waste well). Make sure to leave all the wells of any unused units filled PBS solution while emptying well 7.

   NOTE: It is best to perform the priming and cell loading (see next section) steps at room temperature as certain cell types can become sticky at 37 °C.
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 M04T plate on the drop down list. On the **Manual Mode** tab (Figure 7), click on the **Run liquid priming sequence** button.

   NOTE: The preloaded priming sequence should contain the following steps for well group 1–6 and 8:

   i) 7 kPa (1 psi) for 3 minutes.

   ii) 50 kPa (7.3 psi) for 20 seconds.

   iii) 7 kPa (1 psi) for 20 seconds.

   iv) 50 kPa (7.3 psi) for 20 seconds.

   For more information on creating custom protocols, refer to the CellASIC® ONIX2 Microfluidic System user Guide.
4. Unseal the plate by pressing the **Seal** button on the instrument or in the **Tools** drop-down menu, click **Unseal Plate**. Remove the manifold from the plate. Proceed to cell loading step.

Cell Loading

1. Prepare cell suspension of 3~10 x 10^6 cells/mL for loading. This concentration can be optimized depending on the desired trapping efficiency.

   NOTE: It is critical that cells are in good health. Only use cells in their logarithmic growth phase.

   NOTE: Since trap height is 12 µm, some large suspension cells, such as RAW264.7 may be difficult to trap. However, in most cases, cells with size larger than 12 µm can be captured with the trap. To date, we have done live cell imaging using the following commonly used cell lines: Jurkat, Raji, THP-1, U937, K562, and HL-60.
2. Aspirate media from cell inlet well 8.
3. Pipette 80 µL of prepared cell suspension into cell inlet well 8. Make sure cell solution covers 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 M04T plate on the drop-down list. On the **Manual Mode** tab (Figure 7), click on the **Run cell loading sequence** button.

   NOTE: for initial experiment, we recommend pressurizing well groups 1–6 and 8 at 3 kPa (0.4 psi) for 20 seconds. This loading protocol must be repeated minimum of 4 times.
6. Assess the overall trapping level by microscope. To increase trapping, repeat the loading protocol.

   NOTE: Increasing the number of loading will also increase trapping of cellular debris and dead cells. Do not increase the number of loading beyond the minimum required.

   NOTE: Alternatively, it is also possible to capture more cells by increasing the loading pressure to 4~5 kPa. However, as before, it is important to strike the balance between the trapping of healthy cells vs. cellular debris and dead cells. Do not increase the pressure beyond the minimum required.
7. Once desired level of cell trapping has been achieved, leave the plate at room temperature for 30 minutes before proceeding to setting up imaging experiment.
8. Unseal the plate by pressing the **Seal** button on the instrument or in the **Tools** drop-down menu, click on **Unseal Plate**. Remove the manifold from the plate. Proceed to setting up the experiment.

Setting up the dynamic live cell imaging experiment: Cell Culture and Solution Switching

1. Aspirate and replace media from desired wells with appropriate solution (any wells 1–6). Add up to 300 µL of desired solution to the wells. Optional: cells can be removed from well 8 at this point.
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 M04T plate on the drop-down list. Click on the **Protocol Editor** tab and enter the desired steps and conditions. For an example, see Figure 8.

   NOTE: It is important to keep perfusing cells during culturing to provide adequate nourishment and removal of potential waste products. We recommend minimum pressure of 14 KPa (2 psi) from at least one inlet during culturing to keep cells healthy.

   NOTE: When designing the duration of experiment, flow rate data from Figure 6 should be used as reference. For example, when perfusion setting of 14 kPa (2 psi) is used, 300 µL of solution from a given well will easily be able to perfuse cells for an overnight 16-hour experiment without emptying. However, if 50 kPa (7.3 psi) is used, then 300 µL of solution will run out within 10 hours.

   For information on creating a protocol, refer to the CellASIC® ONIX2 Microfluidic System User Guide.
4. To start imaging, place the sealed plate/manifold assembly on an inverted microscope. Survey the trap array and identify the traps to be used for live cell imaging.
5. During extended perfusion experiments, empty well 7 periodically to avoid outlet overflow into the manifold tubing and perfusion system, since the capacity of the waste well is about 800 µL. 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 **Unseal Plate**. Remove the manifold from the plate, and aspirate well 7.
6. Reseal the manifold to the plate, then on the **Run** tab, click **Resume** to restart the perfusion protocol.

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.

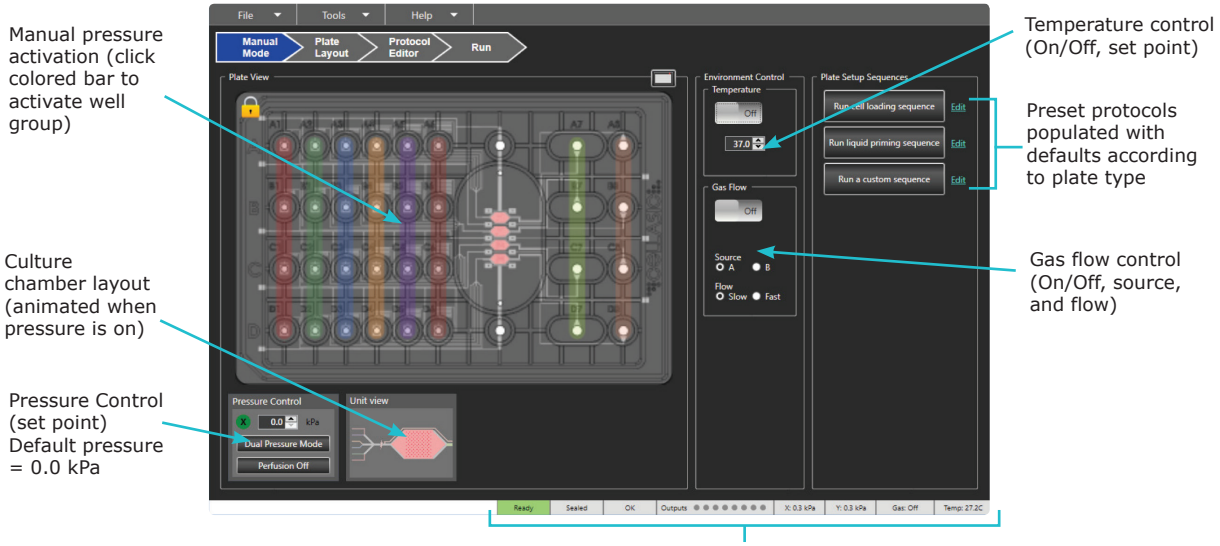

Figure 7. Manual Mode allows interactive operation of the ONIX2 System. Operating parameters can be set manually and this mode also provides the option to run short automated setup sequences that are prepopulated with plate-specific defaults. These setup sequences can be edited if desired.

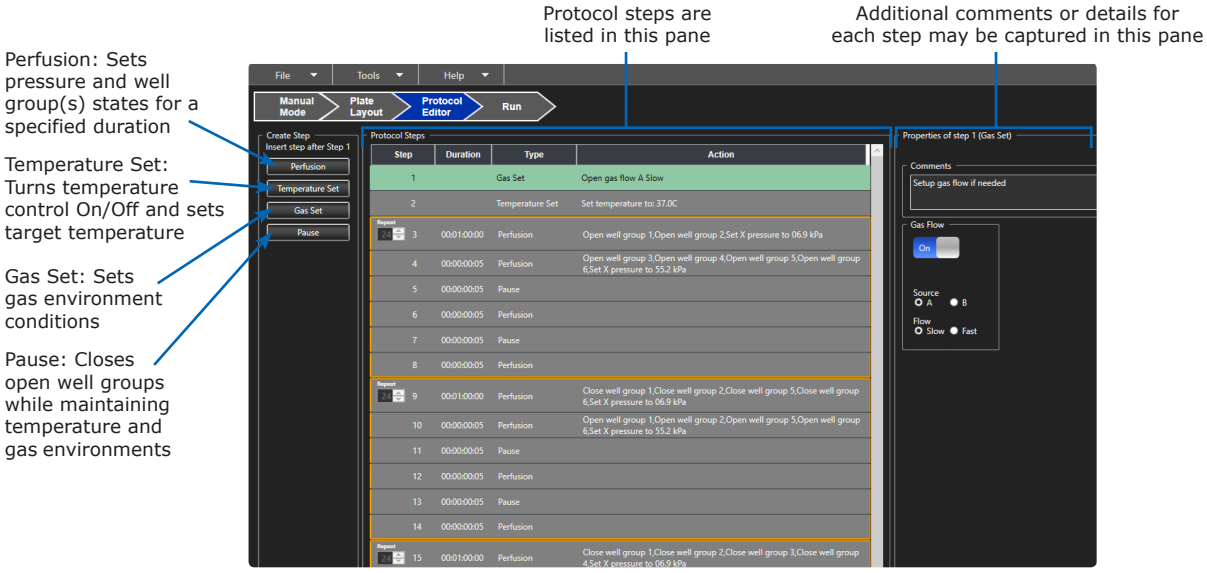

Figure 11. Protocol Editor mode allows the creation and editing of an experimental protocol. A protocol is comprised of a sequence of environmental control and/or perfusion steps. Steps can be added and altered as desired. When the protocol is ready, it can be executed using the **Run** tab.

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.)
Number of Traps	104
Wide opening	64
Narrow opening	40
Culture Chamber Dimensions	
Length	6.0 mm (0.24 in.)
Width	3.0 mm (0.12 in.)
Trap height	12.0 µm
Chamber height	25.0 µm
Culture array length	3.0 mm
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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Rev. 04/19