ImageXpress Pico Automated Cell Imaging System

Environmental Control System Gas Requirements

Welcome to the family of ImageXpress system users. The ImageXpress® Pico Automated Cell Imaging System is available with the optional Environmental Control System, which enables you to perform multi-day, live-cell, time-lapse experiments and hypoxia experiments. This document provides the information you need to prepare for the Environmental Control (EC) System.

It is important to consider the proper connections and fittings to connect your ImageXpress Pico instrument to a gas supply. Unlike other imaging systems, the ImageXpress Pico system allows independent control of both carbon dioxide (CO_2) and oxygen (O_2) levels. As a result, you may need to connect up to three separate gas supplies to the system.

The regulators and connections required to connect the instrument are dependent on how the gas is supplied—from a compressed gas cylinder, a lab gas line, or an air compressor.

If your ImageXpress Pico system includes the EC option, you must provide the following:

- **Pressurized compressed air** from a gas cylinder, lab gas line, or oil-free air compressor. A compressed air supply is required for all environmental control experiments.
- **Pressurized, medical-grade CO_2** from a gas cylinder. CO_2 is used to regulate the pH of cell culture media for mammalian cells. If you are using an organic buffer solution (for example, HEPES) to regulate the pH of your media, then pressurized CO_2 may not be required.
- **Pressurized, medical-grade N_2** from a gas cylinder or lab gas line. N_2 is only required for hypoxia experiments.

- **Pressure regulators** to deliver gases at between 0.8 bar to 1.2 bar (11.6 psi to 17.4 psi). The ideal setting is 1.0 bar (14.5 psi). This guide provides details on gas regulator requirements.
- **Teflon tape and hose clamps** to secure the tubing and fittings.
- **Ultrapure water (18 Mohm•cm)** to maintain humidity inside the environmental control cassette. The humidifying column can hold up to 130 ml (4.4 oz) of ultrapure water and must be refilled before the level drops to 50 ml (1.7 oz).

See below for details on supported and unsupported gas supplies.

See the ImageXpress Pico User Guide for specific warning and caution statements for the EC system.

Supported Gas Supplies

The ImageXpress Pico EC system requires that the gas supply be oil-free and medical grade. CO_2 and N_2 are typically supplied from a compressed gas cylinder. Compressed air is often supplied from one of the following:

- Gas cylinder
- Lab gas line
- Oil-free air compressor

The connections required to connect the instrument to the gas source vary based on the gas supply you use. This document describes each connection.

Unsupported Gas Supplies

To avoid damage to the instrument and the EC System, DO NOT use the following:

- Pre-mixed gas supplies
- N_2 or CO_2 boil-off from a Dewar flask
- N_2 from an N_2 generator
- Any gas supply that is not oil-free
- Any gas supply that is not medical grade
- Any gas supply that cannot be set to supply gas at between 0.8 bar and 1.2 bar (11.6 psi and 17.4 psi)

NEVER connect pure O_2 or any other unspecified gas supply to the instrument.

Using a Gas Cylinder

Gas pressure to the ImageXpress Pico EC system must be regulated within the range of 0.8 bar to 1.2 bar (11.6 psi to 17.4 psi). The ideal setting is 1.0 bar (14.5 psi). A two-stage regulator is required to step-down and regulate the pressure from the gas cylinder.

Regulators type designations for gas cylinders vary based on the region. The following table lists examples of region-specific regulator types:

Region	CO₂ Regulator Type	N₂ Regulator Type	Compressed Air Regulator Type
North America	CGA320	CGA580	CGA590
Germany	DIN477-1 Nr.6	DIN 477-1 No.10	DIN477-1 Nr.13
Great Britain	BS 341 No.8	BS 341 No.3 or BS 341 No.30	BS 341 No.3 or BS 341 No.31
Italy	UNI 4406 /UNI2	UNI 4409 / UNI5	UNI 4410 / UNI6
France	ANFOR NF E 29-650/C	ANFOR NF E 29-650/C	ANFOR NF E 29-650/D or B

Note that the list above is not exhaustive and may change without notice.

The regulator type is often stamped on the end of the regulator on the side that will connect to the cylinder.

Select a cylinder size that meets your needs. Gas within the ImageXpress Pico EC system flows at up to 20 l/hr (0.7 ft³/hr). So, for example, for typical CO_2 regulation at 5% volume, a 10 l (0.35 ft³) liquid CO_2 cylinder can last a year.

Several vendors offer gas regulators, and the one you use is not critical as long as the following conditions are met:

- The regulator type is appropriate for the tank as indicated in the table above.
- The regulator is a two-stage, gas pressure regulator, not a flow control valve.
- The maximum delivery pressure of the regulator is 10 bar (145 psi) or less. We recommend a regulator with a maximum delivery pressure of 4.14 bar (60 psi), which makes it easy to set the required pressure.

Some examples of regulator vendors in North America are listed in the following table:

| Vendor (Website)	Gas	Type	Part Number
McMaster-Carr (www.mcmaster.com)	CO₂	CGA320	7951A67
	N₂	CGA580	7951A62
Airgas (www.airgas.com)	Air	CGA590	Y12244B590-AG
	CO₂	CGA320	Y12244B320-AG
	N₂	CGA580	Y12N245B580-AG
Matheson (store.mathesongas.com)	Air	CGA320	SEQ3121A320
	CO₂	CGA580	SEQ3121A580
	N₂	CGA590	SEQ3121A590

Note that the list above is not exhaustive and may change without notice.

You can also purchase an acceptable regulator from one of the following vendors:

- Air Products (www.airproducts.com)
- Fisher Scientific (www.fishersci.com)
- Linde (www.linde-gas.com)
- VWR (www.vwr.com)

Connecting a Gas Cylinder to the Instrument

Many gas regulators can accommodate a ¼" NPT male fitting. To connect this type of gas cylinder to the instrument, Molecular Devices provides three (3) ¼" NPT male to 6 mm O.D. Push-to-Connect fittings. Attach the fitting to the regulator to easily connect the hose; no other connectors are required.
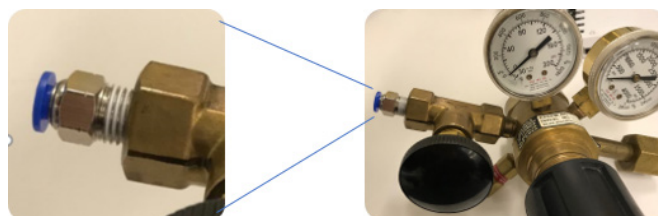
Push-to-Connect Fitting on the Two-Stage Regulator

If your regulator terminates with a ¼" NPT male fitting, you will need to provide a ¼" NPT female to ¼" NPT female connector. You can purchase this connector from many vendors, including Anderson Metals (p/n 56103-04).

Female to Female Connector   *Push-to-Connect Fitting*

Using a Lab Gas Line

Some laboratories are equipped with a gas line to supply compressed air and N_2. The line output is typically greater than 1.2 bar (17.4 psi), which is the maximum allowed pressure for the ImageXpress Pico EC system. In this case, a single-stage line pressure regulator is required.

Several vendors offer single-stage line regulators, and the one you use is not critical as long as maximum delivery pressure of the regulator is 10 bar (145 psi) or less. We recommend that you use a regulator with a maximum delivery pressure of 4.14 bar (60 psi), which makes it easy to set the required pressure. One example of an acceptable line regulator is the Matheson Model 3470A General Purpose Line Regulator (part number SEQ3473A), which is shown below.

Single-Stage Regulator for a Lab Gas Line

Connecting a Lab Gas Line to the Instrument

Many lab gas lines terminate with a hose barb connector, like the one shown below.

Hose Barb Connector

For a hose barb connector, connect a short piece of tubing and secure it with a hose clamp. Then attach a second hose barb connector and, again, secure it with a hose clamp. Finally attach the supplied ¼" NPT male to 6 mm O.D. Push-to-Connect fitting. The finished assembly should look like the following:

It is also possible that your lab gas line terminates with a line regulator. In this case, you can easily attach the ¼" NPT male to 6 mm O.D. Push-to-Connect fitting directly to the line regulator.

To connect a lab gas line, you may need the following:

Manufacturer	Part Number	Description
FasParts	FP126-8B	Hose ID / Hose Barb to ¼" Female NPT FIP FPT Straight Brass Fitting
EDGE INDUSTRIAL	E.I. BARB 53	¼" Hose ID to ¼" Female NPT FNPT Straight Brass Fitting
EDGE INDUSTRIAL	E.I. BARB 58	⅜" Hose ID to ¼" Female NPT FNPT Straight Brass Fitting
Various	---	Hose Clamp

Note that the list above is not exhaustive and may change without notice.

Using an Oil-Free Air Compressor

If a lab gas line or a gas cylinder is not available, you can connect an oil-free air compressor to the instrument. This is the least preferred option since air compressors tend to be noisy and a source of vibration. If you must use an air compressor, it is critical that the compressor be oil-free because hydrocarbons can contaminate the EC system and the instrument.

Most oil-free air compressors have an internal regulator. The one you use must be adjustable to between 0.8 bar and 1.2 bar (11.6 psi and 17.4 psi). Otherwise, you will need to connect a line regulator as described above in the "Using a Lab Gas Line" section.

Ibidi (ibidi.com) is one example of a vendor of a supported oil-free air compressor.

Connecting an Oil-Free Air Compressor to the Instrument

Similar to a lab gas line, most laboratory air compressors terminate with a hose barb connection or a ¼" NPT-style connection. Use the instructions above in the "Connecting a Lab Gas Line to the Instrument" section to connect to the instrument.

Attaching a Push-to-Connect Fitting to a Hose Barb Connector

EC System Accessories

The ImageXpress Pico EC system includes the following accessory parts:

Item	Molecular Devices Part Number
EC Cassette	5070105
Humidifying Column	5070110
Humidifying Column Tubing/Wiring	5070108
Two (2) Humidifying Column Stoppers	5070107
Gas Supply Tubing, 10 m (32.8 ft)	5070103
Push-to-Connect Fitting 6 mm O.D. - ¼" NPT Male	5075610
Two (2) Blind Plugs	5070106
Three (3) Plate Skirt Height Adapters	5077006

Replacement parts are available from Molecular Devices.
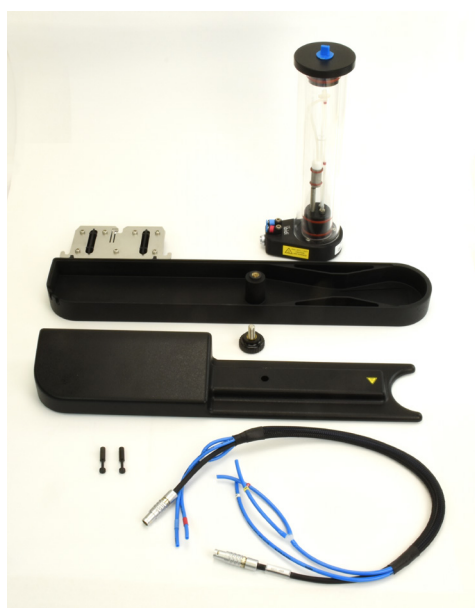

Humidifying Column with Tray, Wiring, and Blind Plugs

Documentation

Go to the Molecular Devices Knowledge Base at support.moleculardevices.com for complete documentation for the ImageXpress Pico system and the CellReporterXpress software, including:

- ImageXpress Pico Pre-Installation Guide
- ImageXpress Pico EC Gas Requirements Pre-Installation Guide
- ImageXpress Pico Installation Guide
- ImageXpress Pico User Guide
- ImageXpress Pico Calibration Kit Guide
- ImageXpress Pico Product Safety Sheet
- CellReporterXpress Installation & IT Guide
- CellReporterXpress User Guide
- CellReporterXpress Release Notes

The Knowledge Base also contains technical notes, software upgrades, safety data sheets, and many other resources.
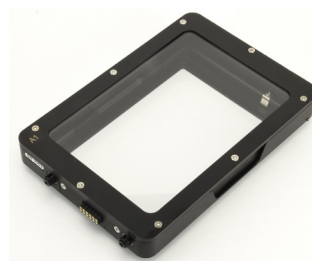
EC Cassette

Support and Service

Molecular Devices provides comprehensive support and service solutions for the ImageXpress Pico system supported by a global network of factory-trained engineers. To contact us, go to:

Web: www.moleculardevices.com/service-support
       www.moldev.com/support
Email: support@moldev.com