invitrogen

EVOS™ Onstage Incubator (OSI-2)

USER GUIDE

for use with:
EVOS™ M5000 Imaging System
EVOS™ M7000 Imaging System

Catalog Number AMC2000
Publication Number MAN0027987
Revision A.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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Revision history: MAN0027987 A.0 (English)

Revision	Date	Description
A.0	6 March 2023	Initial release.

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1 Product information

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Product description

EVOS™ Onstage Incubator (OSI-2)

The EVOS™ Onstage Incubator (OSI-2) is an optional accessory for the EVOS™ M5000 and EVOS™ M7000 Imaging Systems that enables the incubation of cells at user-defined temperature, humidity, and gas concentration (O_2, CO_2), allowing the capture of images from the same sample over long periods of time and recording of time lapse movies.

The EVOS™ Onstage Incubator (OSI-2) consists of a stagetop environmental chamber that is placed on the X-Y stage of the imaging system and a separate control unit that supplies the power and humidified gas (air or air-CO_2 premix, CO_2-only, and N_2-only). The second generation EVOS™ Onstage Incubator contains an on-board air compressor that enables the use of on-board air instead of a separate air tank. The Onstage Incubator (OSI-2) is controlled by the same software and user interface that controls the imaging system.

Standard items included

- Stagetop Environmental Chamber
- Control Unit
- Master Stage Plate (also available separately as EVOS™ Onstage Master Plate, Cat. No. AMEPVH106)
- Vessel holder for multiwell plates (also available separately as EVOS™ Onstage Vessel Holder, Multiwell Plates, Cat. No. AMEPVH028)
- Light shield and light shield cover
- Sensor data cable with 6-pin connector
- Cable, USB 2.0 A-to-B, 180 cm (6 ft)
- Heated hose with temperature control, 180 cm (6 ft) (also available separately as EVOS™ Onstage Incubator Hose, Cat. No. AMEP4728)
- Gas line, 1/8 in. ID, ¼ in. OD (also available separately as EVOS™ Onstage Incubator Gas Line, Cat. No. AMEP4732, for an extra 25 ft)
- Push-to-connect gas line adaptor, ¼ in. NPT male (3 each)
- Standard-head open-end wrench
- Hex screw driver (2 mm)

- Power Cord, Type A (North America)

Note: A country-specific power cord must be ordered separately in regions not using the Type A power plug.

Required materials not provided

- CO_2 gas or CO_2/air premix for CO_2 regulation
- N_2 gas for O_2 regulation (if performing hypoxia experiments)
- Multistage regulators capable of providing continuous 40–50 psi output pressure for all gas sources used with the OSI-2. The regulators must have a ¼ in. NPT female output; if the regulator has a ¼ in. NPT male output, a male-to-female adaptor is needed.

Incubator components

Control unit and Environmental chamber

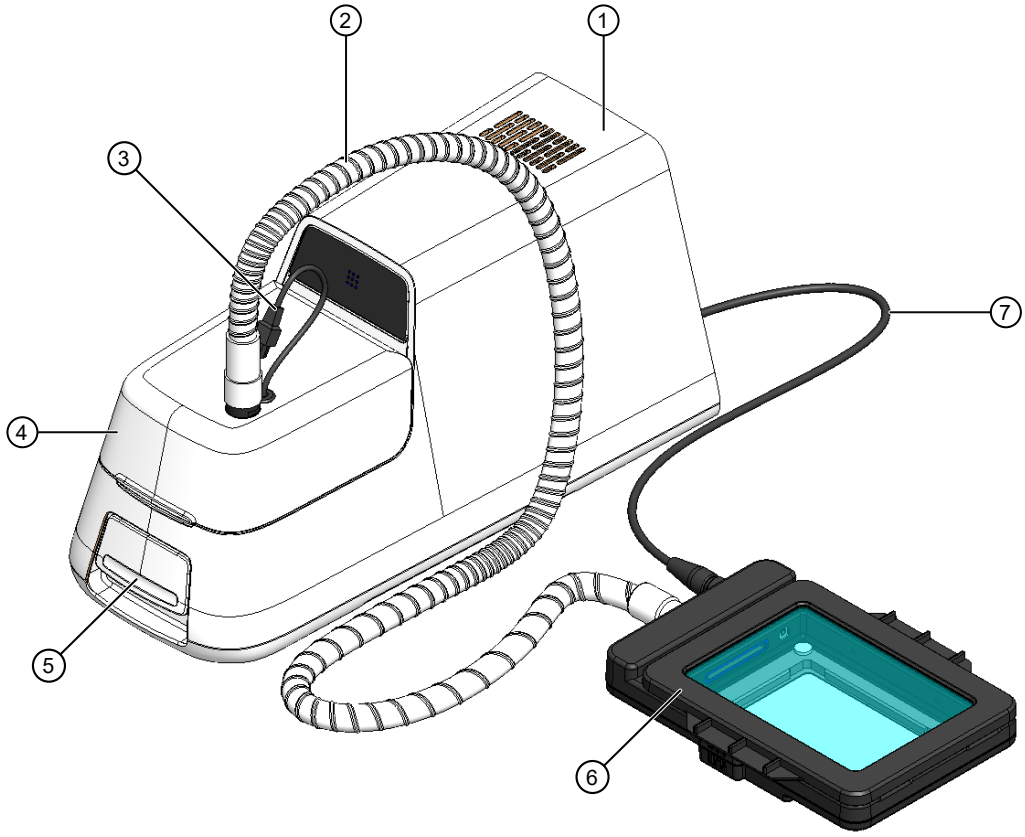


Figure 1 EVOS™ Onstage Incubator (OSI-2) Control unit and Environmental chamber

1. Control unit
2. Heated hose
3. Hose heater connector
4. Water reservoir door
5. Water reservoir door release
6. Environmental chamber
7. Sensor data cable with 6-pin connector

Control unit (back view)

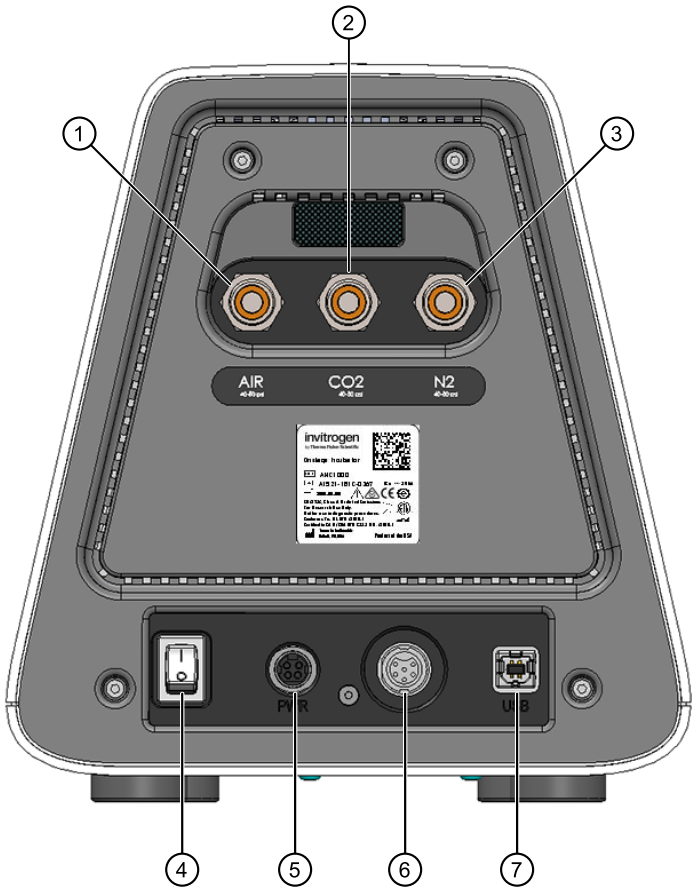


Figure 2 Back view of the OSI-2 Control unit showing the locations of the gas ports, input jacks, and the power switch

① Port 1: Air or air-CO_2 premix
② Port 2: CO_2
③ Port 3: N_2
④ Power switch
⑤ Power input jack
⑥ 6-pin sensor data cable jack
⑦ USB control cable jack

Status LED modes

The status indicator LED on the OSI-2 control unit identifies the status of the incubator.

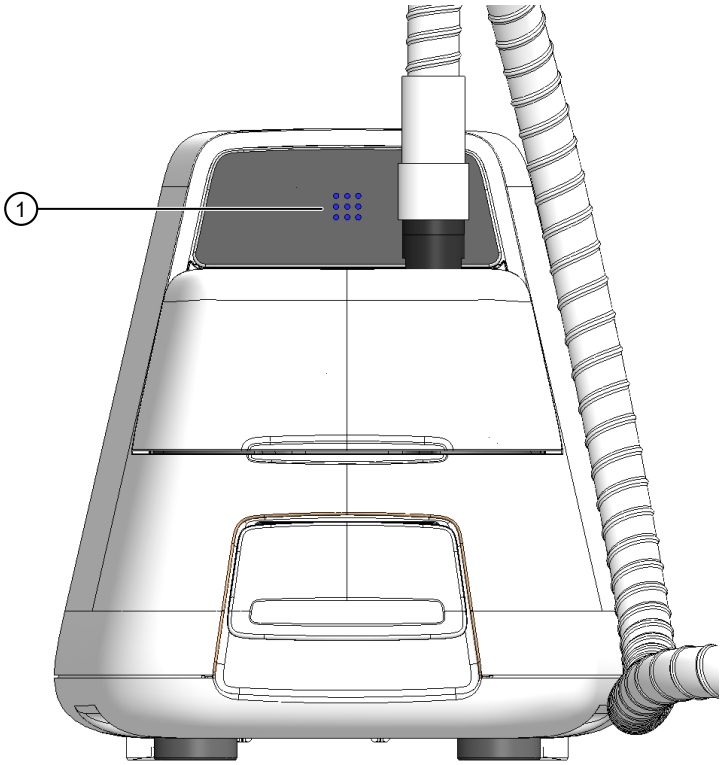


Figure 3 OSI-2 status indicator LED on the control unit

① Status indicator LED

LED color	LED mode	Incubator status
Blue	Solid	The OSI-2 is powered on and connected to the computer, but no functions have been enabled.
Blue	Blinking	The temperature/humidity/gas concentration features have been enabled and the system is warming up.
Blue	Slow blink – fade in/fade out	All temperature/humidity/gas concentration targets are met and being maintained.
Amber	Blinking	The OSI-2 has deviated from the temperature/humidity/gas concentration target.
Red	Blinking	The USB cable is connected, but the power switch is off or no power supply is connected.

Enviromental chamber

The environmental chamber of the EVOS™ Onstage Incubator (OSI-2) consists of the incubator chamber, the vessel holder/adaptor, the heated glass lid, the light shield, and the light shield cover.

The environmental chamber sits on the onstage incubator master stage plate that is attached to the X-Y stage of the imaging system.

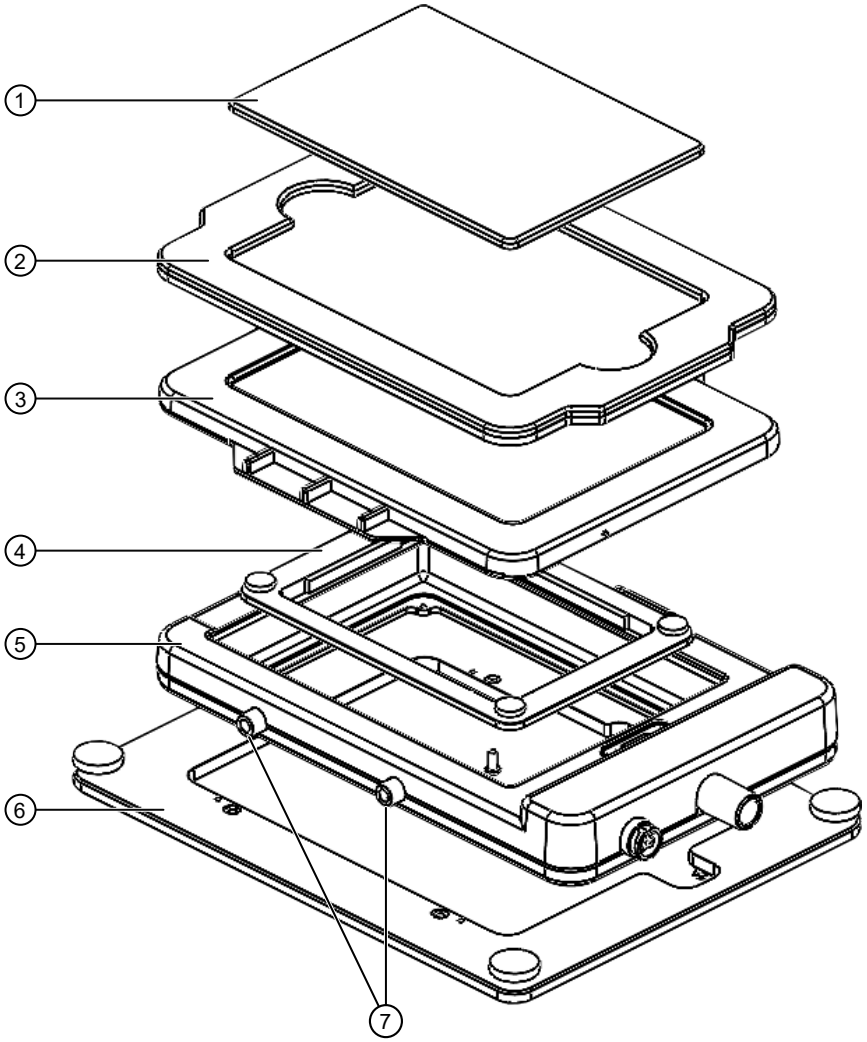


Figure 4 Components of the Environmental chamber

1. Light shield cover
2. Light shield
3. Heated glass lid
4. Vessel holder/adaptor
5. Incubator chamber
6. Master stage plate
7. Perfusion ports

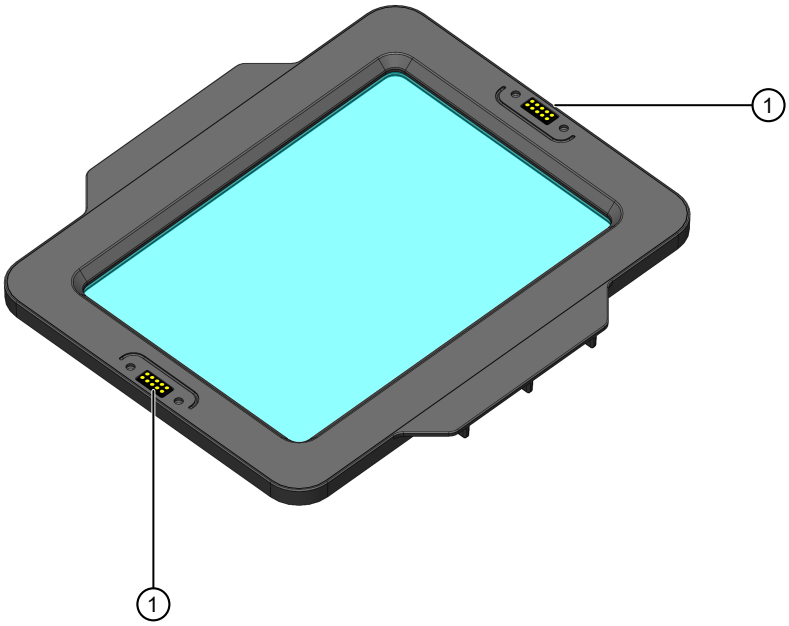


Figure 5 Bottom view of the Environmental chamber lid showing the lid contacts

① Environmental chamber lid contacts

2 Methods

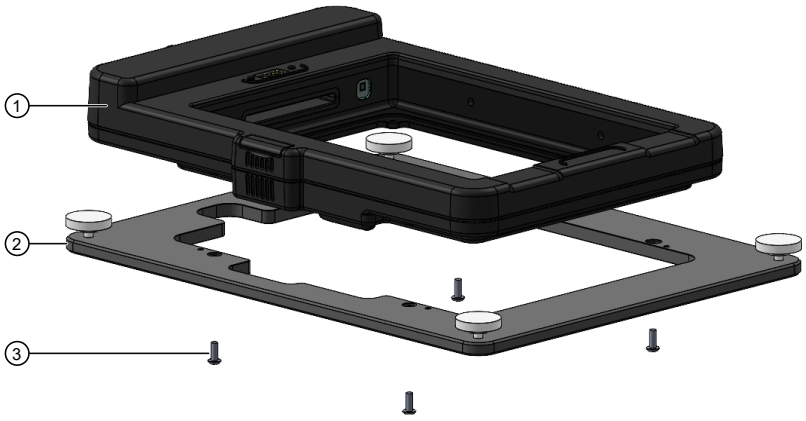
Set up the EVOS™ Onstage Incubator (OSI-2)

IMPORTANT! Before using this product, read and understand the information in the “Safety” appendix in this document.

Install the EVOS™ Onstage Incubator (OSI-2)

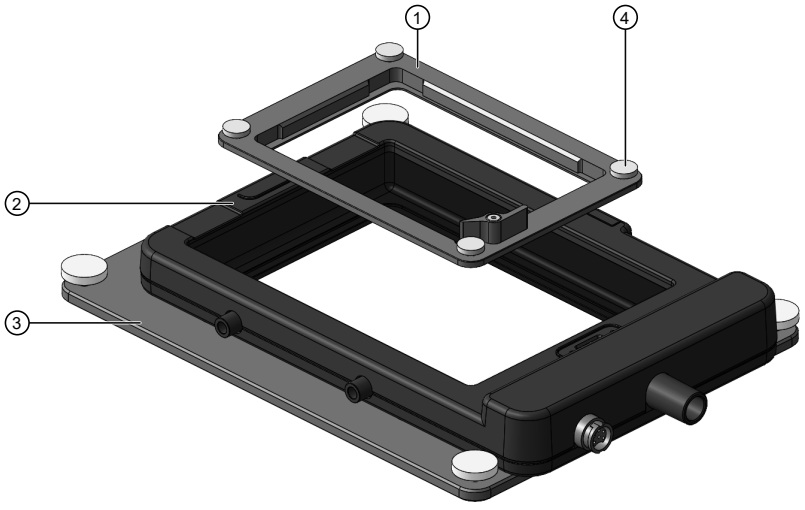
Assemble the Environmental chamber

1. Place the incubator chamber on the onstage incubator master plate, then secure it in place with the four 2.0-mm hex screws.



1. Incubator chamber
2. Onstage incubator master plate
3. 2.0-mm hex screws

2. Attach the vessel holder/adaptor to the incubator chamber using the four thumb screws.

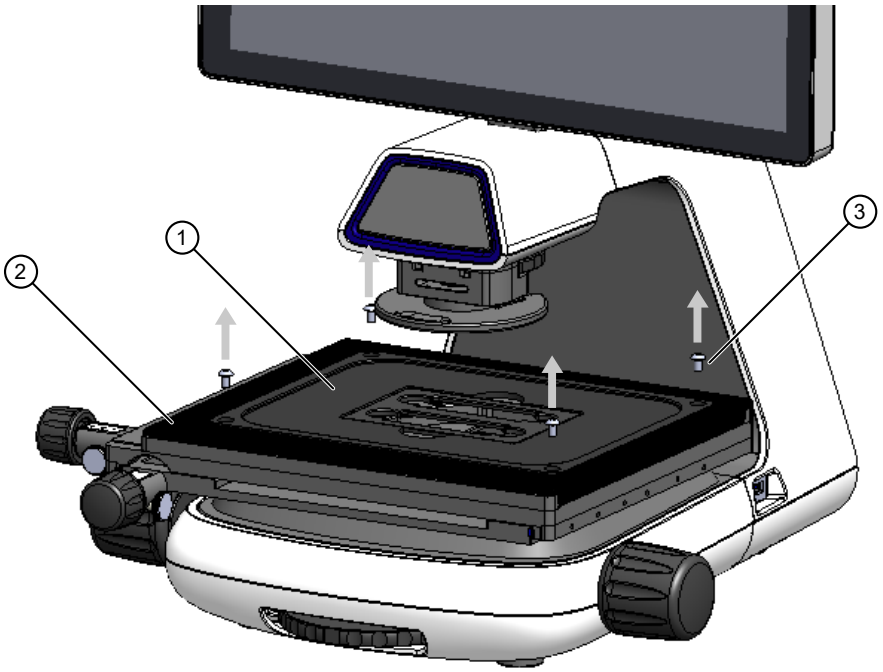


1. Vessel holder/adaptor
2. Incubator chamber
3. Onstage incubator master plate
4. Thumb screws

Install the Onstage Incubator Master Plate

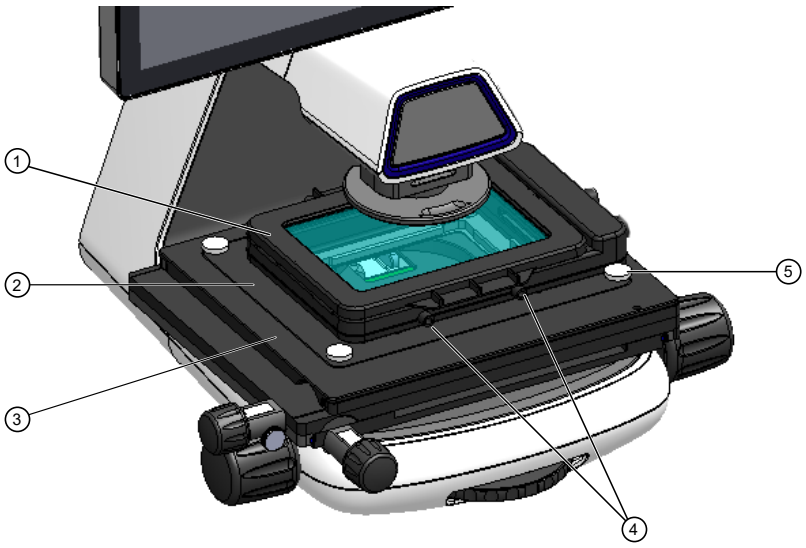
Note: The images in the following steps show the installation of the master plate on the EVOS™ M5000 Imaging System X-Y stage. However, the steps to install the master plate on the EVOS™ M7000 Imaging System are identical to the steps for the EVOS™ M5000 Imaging System.

1. Unscrew the four 3.0-mm screws on the base plate, then remove the base plate from the X-Y stage. If necessary, unscrew and remove the vessel holder/adaptor before removing the base plate.



① Base plate
② X-Y stage
③ 3.0-mm screw

2. Secure the onstage incubator master plate with the environmental chamber to the X-Y stage with the four thumb screws.



1. Environmental chamber
2. Onstage incubator master plate
3. X-Y stage
4. Perfusion ports
5. Thumb screw

IMPORTANT! If you plan to use the perfusion ports on the environmental chamber, install the master plate such that the perfusion ports face towards the front of the system (as shown in the image above). Otherwise, install the master plate such that the perfusion ports face the back of the system.

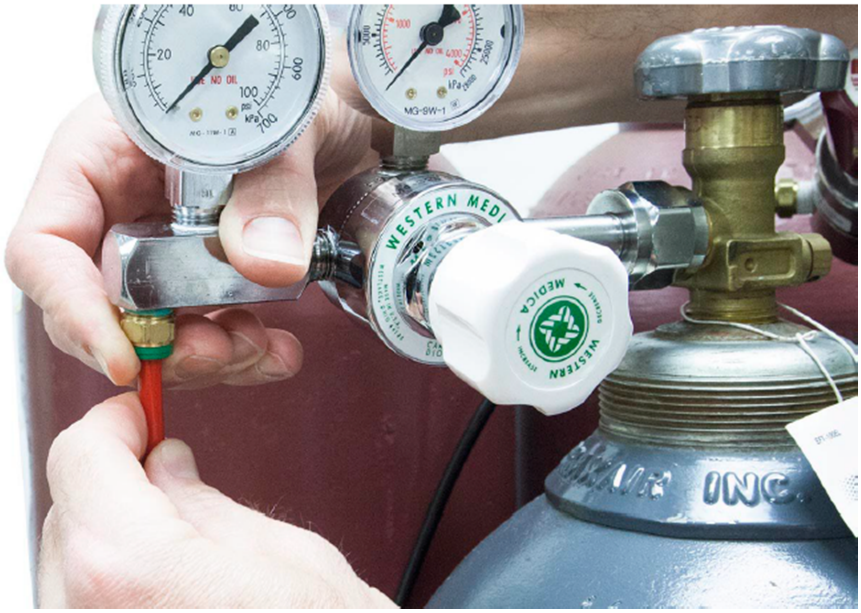
Set up the EVOS™ Onstage Incubator (OSI-2) for operation

Follow the procedures below to set up the EVOS™ Onstage Incubator (OSI-2) for operation. For the locations of various input jacks and gas ports, refer to “Control unit (back view)” on page 8.

IMPORTANT! Do not position the control unit so that it is difficult to turn off the main power switch (see page 8 for the location of the power switch). In case of an instrument malfunction, turn the main power switch to the OFF position and disconnect the power cord from the outlet.

Connect the gas lines

1. Connect each gas line to the appropriate gas tank via the PTC (push-to-connect) connectors threaded into the regulator. To do this, push the tubing into the PTC connector until it clicks into place. Pull on the tubing slightly to ensure a tight connection; the tubing should not come out.



2. Attach the gas lines to the control unit via the PTC connectors for the appropriate gas intake port.
   - If using external air or premixed air, attach to **Port 1: Air**.
   - If using CO_2, attach to **Port 2: CO_2**.
   - For nitrogen or oxygen displacement, attach to **Port 3: N_2**.

Note: A combination of air and CO_2 gases is needed to perform normoxic live cell imaging with the OSI-2, which can be accomplished with one of these configurations:

- OSI-2 on-board air compressor + CO_2 tank
- Premix gas tank containing air + CO_2
- Compressed air tank + CO_2 tank

The use of a pure O_2 tank is **not** recommended.

To perform hypoxic cell imaging, the following tank configuration is recommended:

- N_2 tank + CO_2 tank

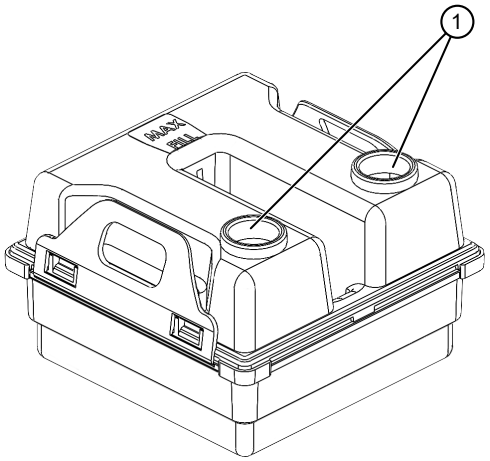
Connect the power and system control lines

1. Plug the power cord into the power input jack on the control unit and the outlet.
2. Plug the USB cable into the USB control cable jack on the control unit and the USB port on the imaging system computer.
3. Plug the 6-pin sensor data cable to the stagetop environmental chamber and the appropriate input jack on the control unit.

Set up the control unit

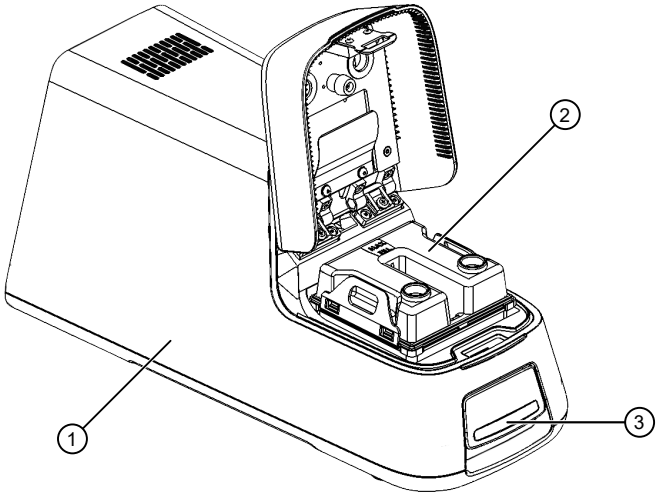
1. Assemble the water reservoir and add warm water (approximately 50°C) to the max fill line through the fill hole. Distilled water is recommended to avoid contaminants.

IMPORTANT! Do not overfill the water reservoir.



① Reservoir fill holes

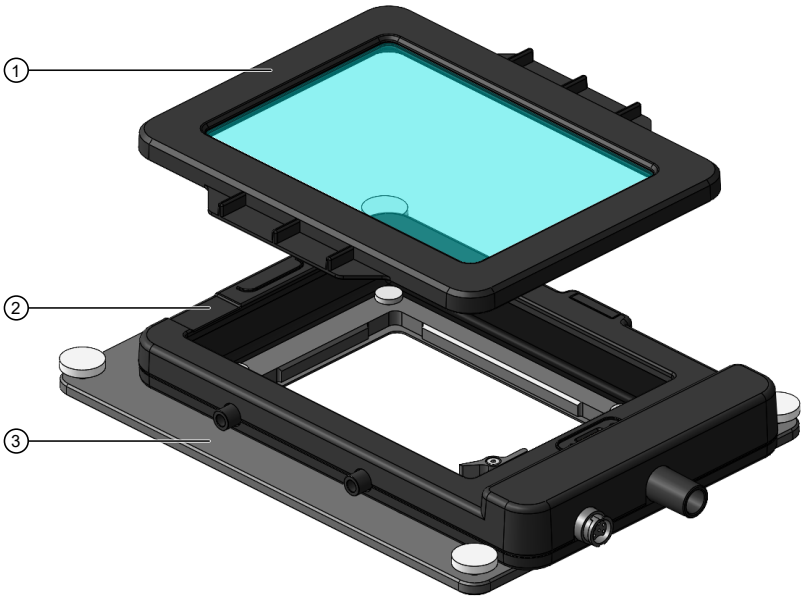
2. Place the water reservoir into the control unit **with the fill holes to the front** and close the lid.



① EVOS™ Onstage Incubator (OSI-2) control unit
② Water reservoir
③ Water reservoir door release

Set up the Environmental chamber for operation

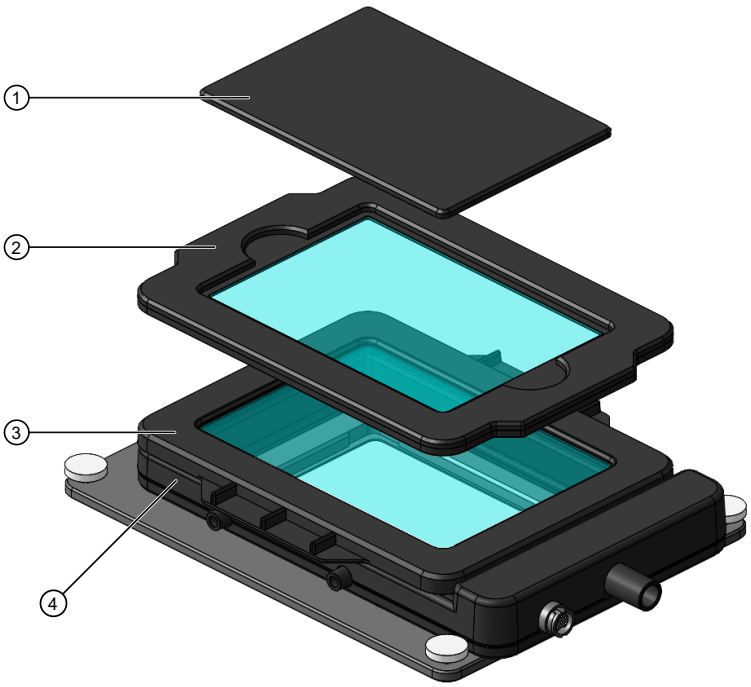
1. Place the heated glass lid with the no-fog glass window on the incubator chamber. The heated glass lid is guided and attached in its place by the two magnets on its rim.



1. Heated glass lid
2. Incubator chamber
3. Onstage incubator master plate

2. Place the light shield with tinted plastic window on top of the heated glass lid.

Note: Use of the light shield is strongly recommended for fluorescence imaging applications.



1. Light shield cover
2. Light shield
3. Heated glass lid
4. Incubator chamber

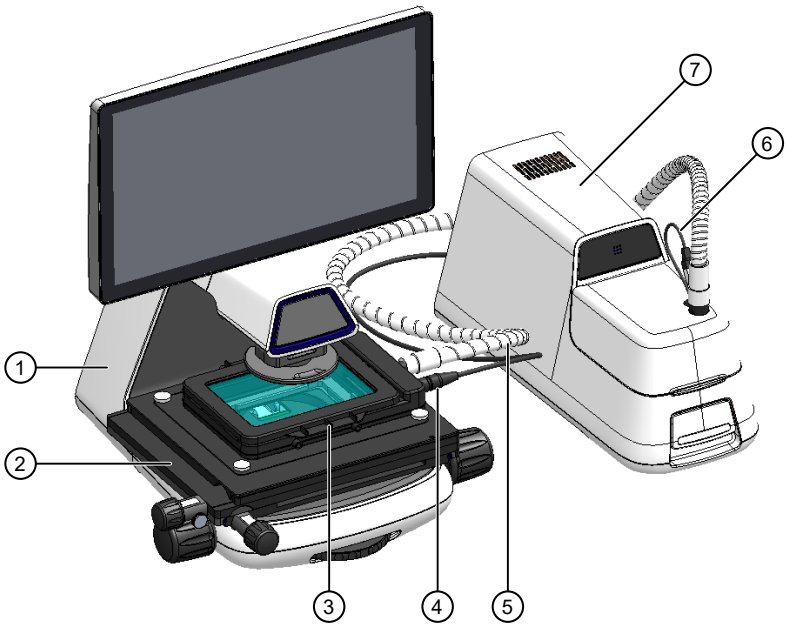
3. If desired, place the light shield cover on the light shield for fluorescence imaging applications.

Note: The light shield cover completely blocks any ambient light from entering the environmental chamber and improves image quality in fluorescence imaging applications.

4. Attach the heated hose between the stagetop environmental chamber and the control unit.

5. Plug the hose heater cable from the control unit into the connector on the heated hose.

Note: The following image shows the environmental chamber set up for operation on the EVOS™ M5000 Imaging System. However, the steps to set up the environmental chamber on the EVOS™ M7000 Imaging System are identical to the steps for setup on the EVOS™ M5000 Imaging System.



1. Imaging system
2. X-Y stage
3. Stagetop environmental chamber
4. Sensor data cable
5. Heated hose
6. Hose heater cable
7. Control unit

Use the Onstage Incubator with the EVOS™ M5000 Imaging System

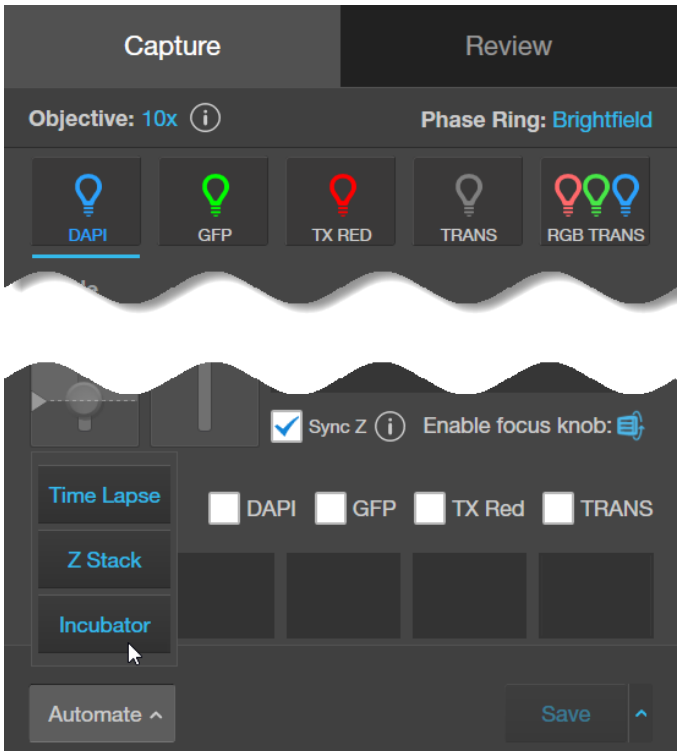
Note: For EVOS™ M7000 Imaging System instructions, see "Use the Onstage Incubator with the EVOS™ M7000 Imaging System" on page 27.

IMPORTANT! Before using the EVOS™ Onstage Incubator (OSI-2) in experiments, ensure that:

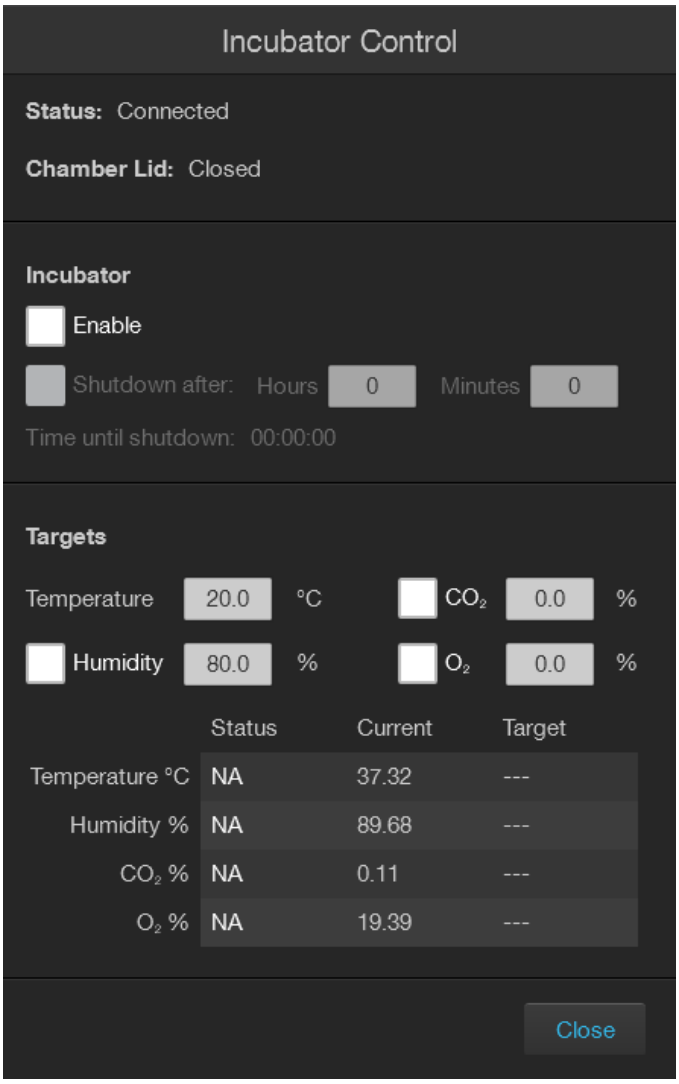
- The gas inputs have been configured (see "Configure gas inputs" on page 23).
- The oxygen sensor has been calibrated (see "Calibrate incubator oxygen sensor" on page 24).

Power on the EVOS™ Onstage Incubator (OSI-2)

1. Power on the EVOS™ M5000 Imaging System as described in the *EVOS™ M5000 Imaging System User Guide* (Pub. No. MAN0017563).
2. Power on the EVOS™ Onstage Incubator (OSI-2) control unit.
3. Start the EVOS™ M5000 Software. The software recognizes the Onstage Incubator on connection.
4. On the **Capture** tab, click **Automate**, then select **Incubator** to open the **Incubator Control** window.



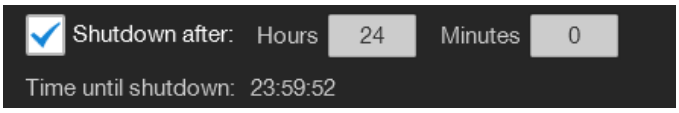
Note: Alternatively, go to the **Settings** tab, click **Incubator** to expand the **Incubator panel**, then click **Incubator Control** to open the **Incubator Control** window.



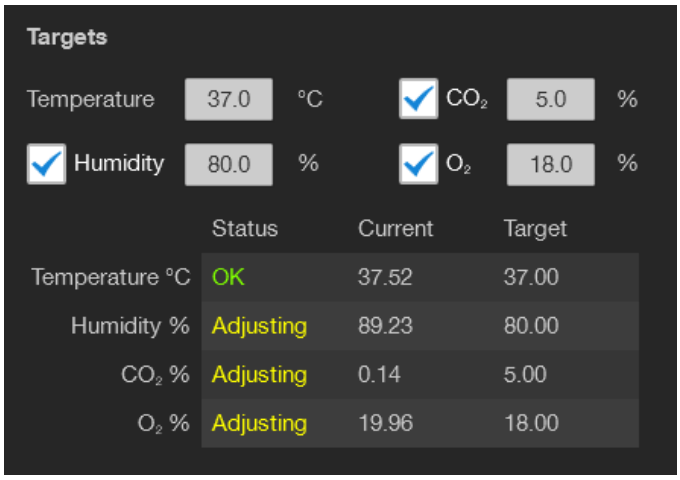
5. Select **Enable** to initiate the control of the incubator.

6. If you are using the incubator for the first time or if the configuration of the gas inputs has changed, configure the gas inputs (see “Configure gas inputs” on page 23).

7. Select the desired **Shutdown** option:
   - **Manual shutdown**: The incubator remains on until you manually de-select the **Enable** option, then click **Close**.
   - **Automatic shutdown**: Select **Shutdown after**, then enter the time in **Hours** and **Minutes** that must elapse before the incubator is shut down automatically.



8. Enter the target values for **Temperature**, **CO_2**, and **Oxygen**. Select **Humidity** to use a humidified atmosphere in the incubator chamber, then enter the target value for **Humidity**.
   Incubator status changes to **Running** and the control panel displays the **Target** and **Actual** values for **Temperature**, **Humidity**, **CO_2**, and **Oxygen**.



Note: If the setting exceeds the range of the OSI-2, the OSI-2 reflects the closest value in range. For example, if you try to set humidity at 50%, the system sets the value to 70%.

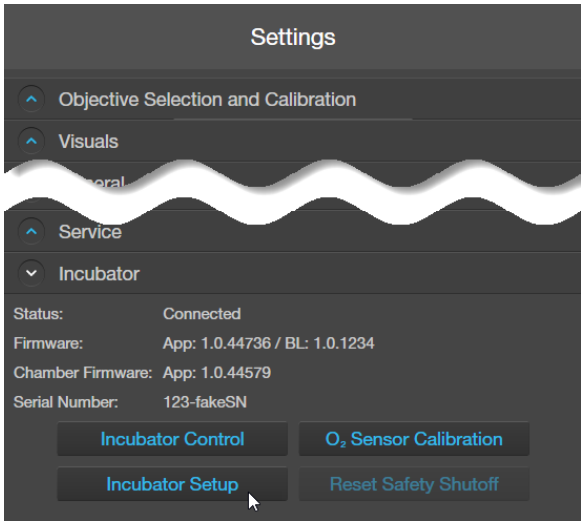
9. Click **Close** to return to the **Capture tab**.

IMPORTANT! Place an empty "dummy" culture plate into the vessel holder/adaptor for the first warm up and equilibration to prevent build-up of condensation on the optical components and the inside of the imaging system. See Appendix B, “Compatible vessel holders and stage plates” for a list of compatible vessels and vessel holders.

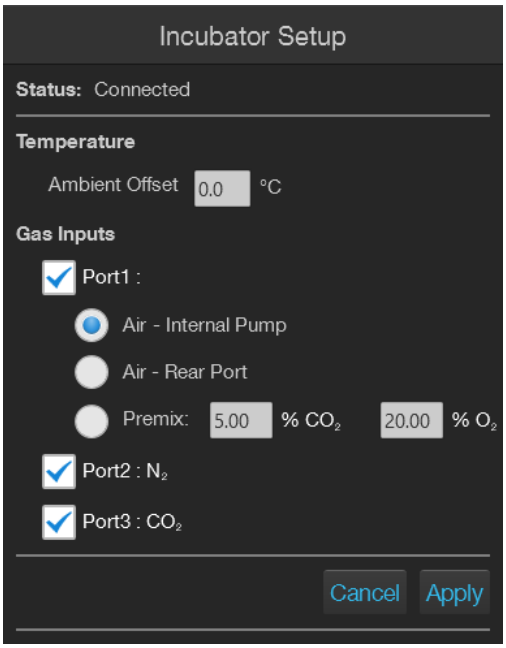
Optional: Set Ambient Temperature Offset

Ambient Offset enables you to adjust the ambient room temperature setting, which may be needed if condensation is observed on the glass lid. Review "Troubleshooting" on page 33 before you adjust the ambient temperature offset.

1. Go to the **Settings tab**, then click **Incubator** to expand the **Incubator panel**.



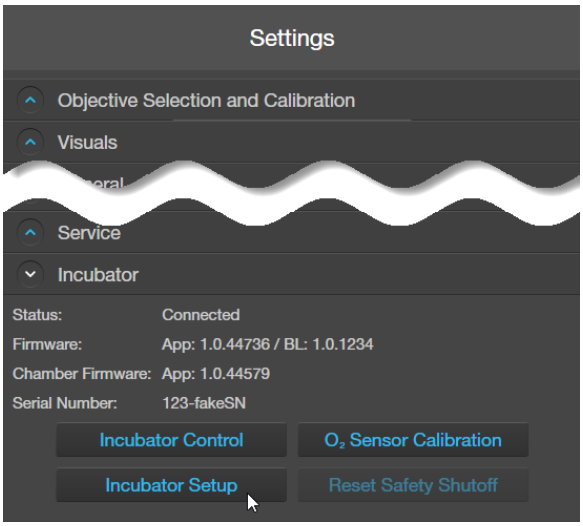
2. Click **Incubator Setup** to open the **Incubator Setup window**.



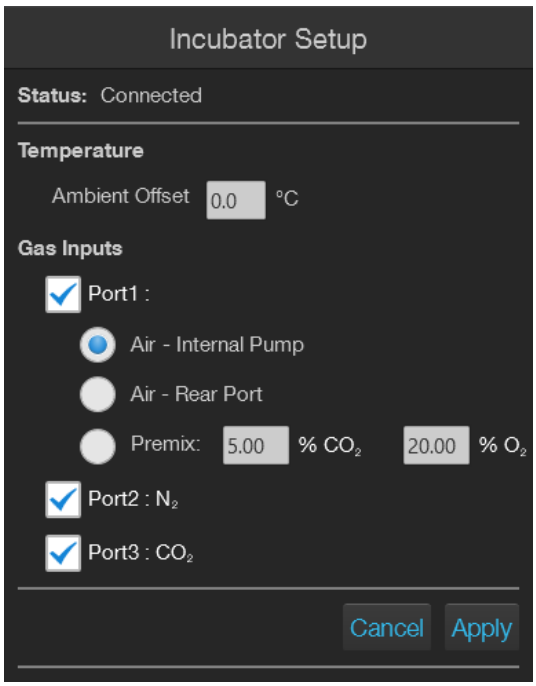
3. Enter the desired temperature for the **Ambient Offset**, then click **Apply**. By default, **Ambient Offset** is set at 0.

Configure gas inputs

1. Go to the **Settings tab**, then click **Incubator** to expand the **Incubator panel**.



2. Click **Incubator Setup** to open the **Incubator Setup window**.



3. Select the appropriate options for the **Gas Inputs** that reflect the set-up for the EVOS™ Onstage Incubator (OSI-2):
   - **Port 1**: Select **Air** or **Premix**, if not using the internal air compressor.
     If you select **Premix**, manually enter the percentage of the **CO_2** and **O_2** to reflect the specifics of the set-up.
   - **Port 2**: Reserved for **N_2** only.
   - **Port 3**: Reserved for **CO_2** only.
4. Click **Apply** after you have configured the gas connections for each port.
5. Open the gas cylinder valve, taking care to shut off or turn down the output pressure before opening.

   Note: The right gauge on the regulator shows the input pressure (tank pressure) and the left gauge shows the output pressure. After the cylinder valve is opened, the output pressure can be adjusted up to 40–50 psi and, if applicable, the output shut-off valve can be opened.
6. Set the **gas flow** on the regulators:
   - **Air**: 40–50 psi
   - **CO_2**: 40–50 psi
   - **N_2**: 40–50 psi

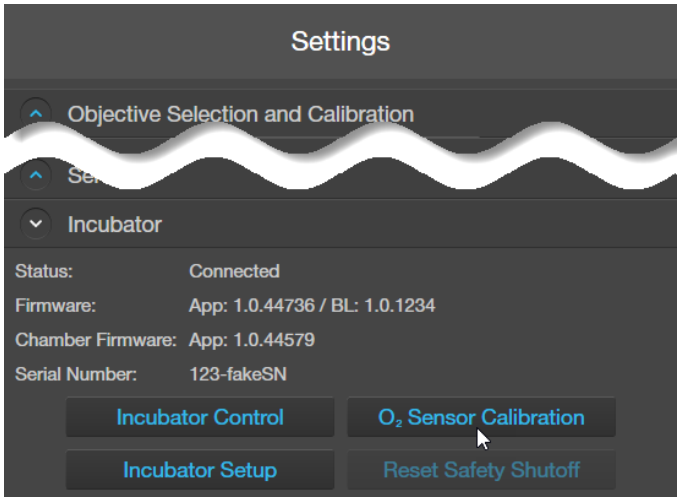
   IMPORTANT! Do **not** exceed 50 psi of pressure. Exceeding 50 psi will damage the incubator. Going below 40 psi will result in suboptimal performance.

Calibrate incubator oxygen sensor

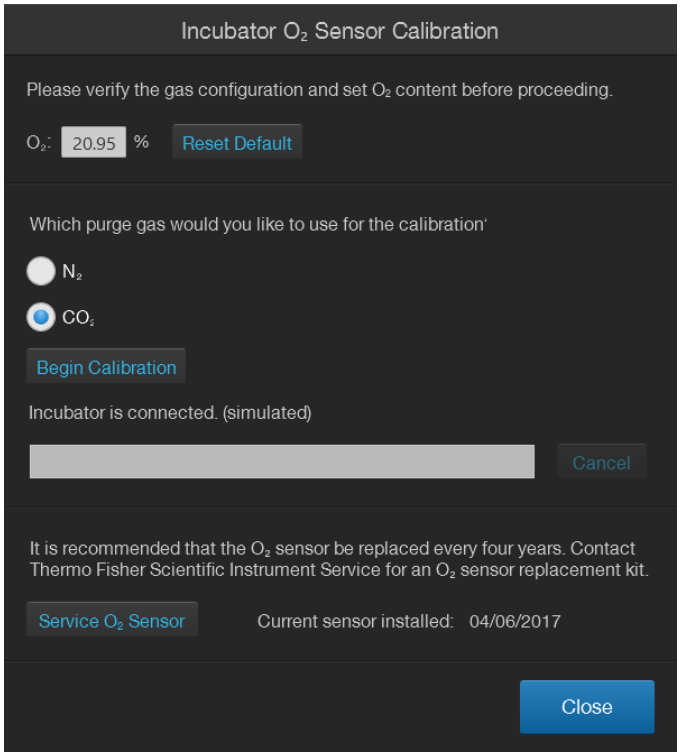
Calibrating the oxygen sensor ensures that the atmosphere in the environmental chamber is replenished with the appropriate gasses in the correct proportion.

Note: We recommend calibrating the oxygen sensor every 6 months or whenever precise control of oxygen is needed (e.g., for hypoxia experiments). The OSI-2 provides a notification if the O_2 sensor has not been calibrated in more than 6 months.

1. Go to the **Settings tab**, then click **Incubator** to expand the **Incubator panel**.



2. Click **Oxygen Sensor Calibration** to open the **Incubator Oxygen Sensor Calibration window**.



3. Verify that the gas connections have been correctly configured.

4. Verify that the **O_2%** is set to a value similar to the types of conditions used, for example hypoxic or normoxic. For normoxic conditions, the laboratory incubator oxygen % value can be used for guidance to set the **O_2%** value of the OSI-2.

5. Select the **purge gas source** for the calibration: **N_2** or **CO_2**.

   Note: A pure CO_2 or N_2 gas tank is required.

6. Click **Begin Calibration**.

   The EVOS™ M5000 Imaging System calibrates the oxygen sensor for the proper functioning of the EVOS™ Onstage Incubator (OSI-2). The entire calibration process takes approximately three minutes.

IMPORTANT! See the *EVOS™ M5000 Imaging System User Guide* (Pub. No. MAN0017563; available for download at **thermofisher.com**) for details on how to set up a time-lapse experiment with the OSI-2.

Use the Onstage Incubator with the EVOS™ M7000 Imaging System

Note: For EVOS™ M5000 Imaging System instructions, see "Use the Onstage Incubator with the EVOS™ M5000 Imaging System" on page 19.

IMPORTANT! Before using the EVOS™ Onstage Incubator (OSI-2) in experiments, ensure that:

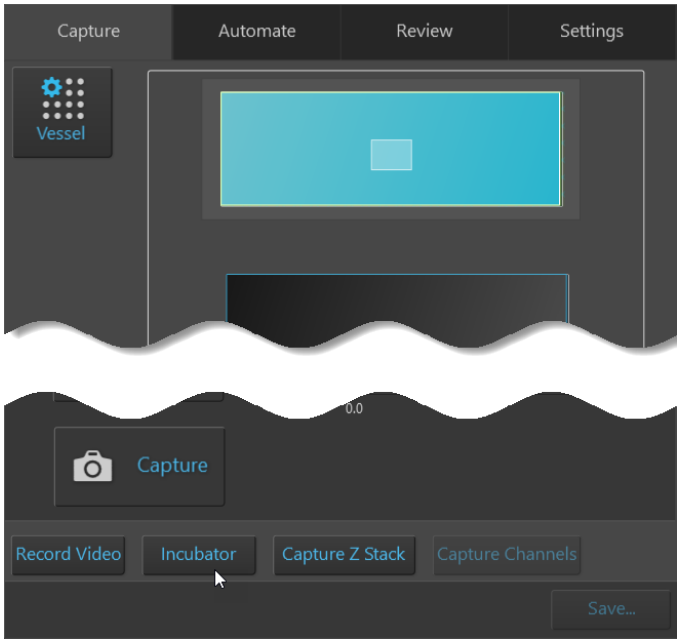
- The gas inputs have been configured (see "Configure gas inputs" on page 29).
- The oxygen sensor has been calibrated (see "Calibrate oxygen sensor" on page 31).

Power on the EVOS™ Onstage Incubator (OSI-2)

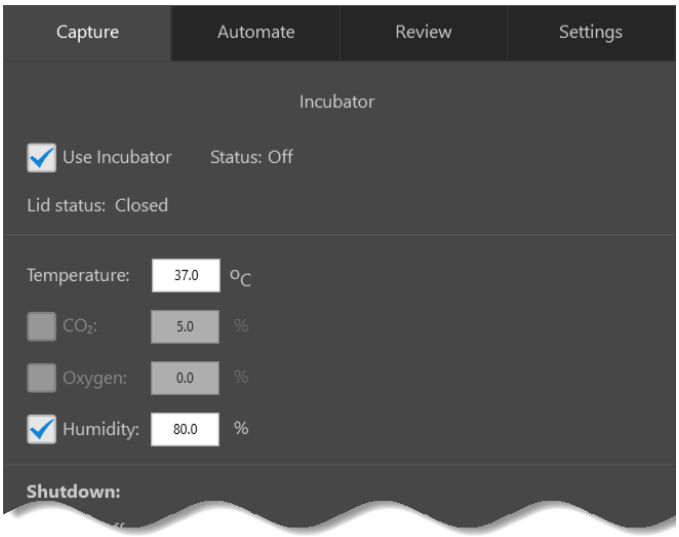
1. Power on the EVOS™ M7000 Imaging System as described in the *EVOS™ M7000 Imaging System User Guide* (Pub. No. MAN0018326).
2. Power on the power switch to the EVOS™ Onstage Incubator (OSI-2) control unit.
3. Start the EVOS™ M7000 Software. The software automatically recognizes the Onstage Incubator on connection.

   Note: If you are using the incubator for the first time or if the configuration of the gas inputs has changed since the last use, configure the gas inputs before using the incubator (see "Configure gas inputs" on page 29).

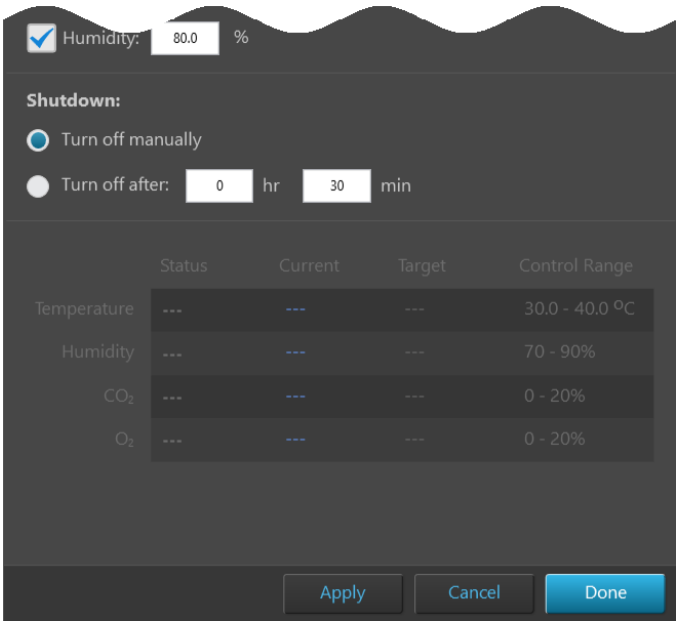
4. On the **Capture** tab, click the **Incubator** button to open the **Incubator Control** panel.



5. Select **Use Incubator** to initiate the incubator, then enter the target values for **Temperature**, **CO_2**, and **Oxygen**. Select **Use humidity** to use a humidified atmosphere in the incubator chamber.



6. Select the desired **Shutdown** option:
   - **Turn off manually**: The incubator remains on until you manually de-select the **Use Incubator** option, then click **Apply**.
   - **Turn off after**: Select **Turn off after**, then enter the time in **Hours** and **Minutes** that must elapse before the incubator is shut down automatically.



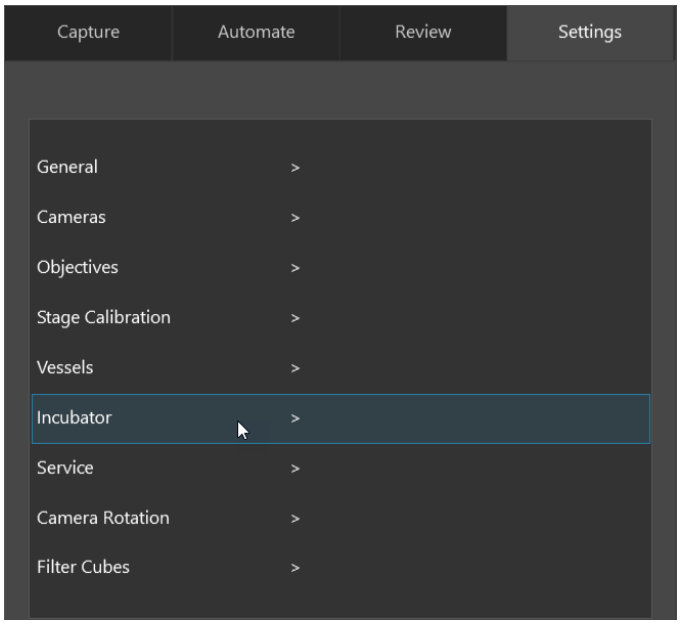
7. Click **Apply**.
   Incubator status changes to **Running** and the control panel displays the **Current**, **Target**, and **Actual** values for **Temperature**, **Humidity**, **CO_2**, and **Oxygen**.

8. Click **Done** to return to the **Capture tab**.

Note: Place an empty "dummy" culture plate into the vessel holder/adaptor for the initial warm up and equilibration to prevent build-up of condensation on the optical components and the inside of the imaging system.

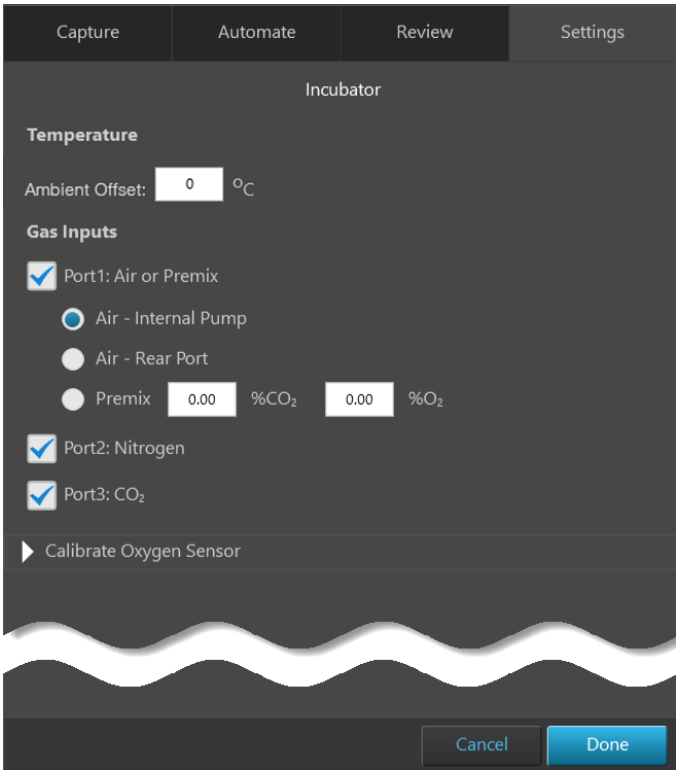
Configure gas inputs

1. Go to the **Settings tab**, then click **Incubator** to open the incubator configuration panel.



2. Select the appropriate options for the **Gas Inputs** that reflect the set-up for the EVOS™ Onstage Incubator (OSI-2):

   - **Port 1**: Select **Air** or **Premix**, if not using the internal air compressor.
     If you select **Premix**, manually enter the percentage of the **CO_2** and **O_2** to reflect the specifics of the set-up.
   - **Port 2**: Reserved for **N_2** only.
   - **Port 3**: Reserved for **CO_2** only.



3. Click **Done** after you have configured the gas connections for each port.

4. Open the gas cylinder valve, taking care to shut off or turn down the output pressure before opening.

Note: The right gauge on the regulator shows the input pressure (tank pressure) and the left gauge shows the output pressure. After the cylinder valve is opened, the output pressure can be adjusted up to 40–50 psi and, if applicable, the output shut-off valve can be opened.

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5. Set the **gas flow** on the regulators:

   - **Air**: 40–50 psi
   - **CO_2**: 40–50 psi
   - **N_2**: 40–50 psi

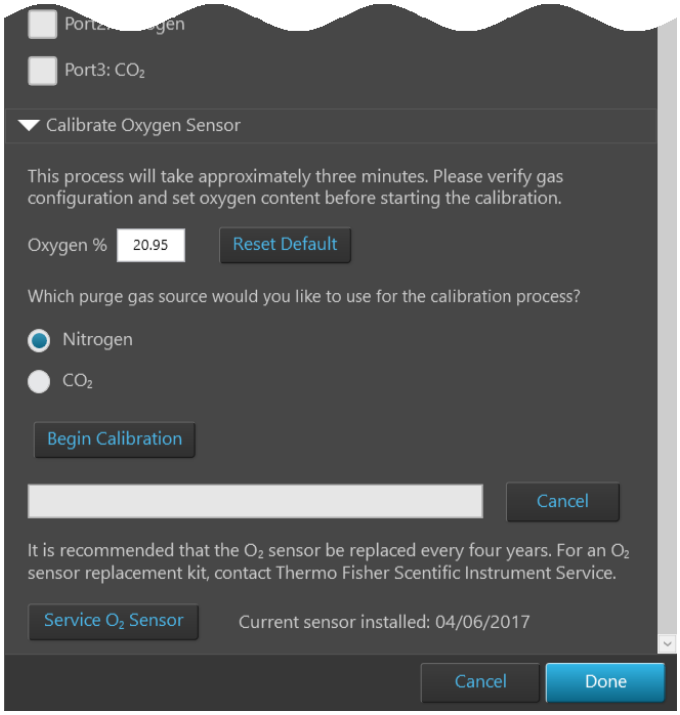
IMPORTANT! Do **not** exceed 50 psi of pressure. Exceeding 50 psi will damage the incubator. Going below 40 psi will result in suboptimal performance.

Calibrate oxygen sensor

Calibrating the oxygen sensor ensures that the atmosphere in the environmental chamber is replenished with the appropriate gasses in the correct proportion.

Note: We recommend calibrating the oxygen sensor every 6 months or whenever precise control of oxygen is needed (e.g., for hypoxia experiments). The OSI-2 provides a notification if the O_2 sensor has not been calibrated in more than 6 months.

1. Go to the **Settings tab**, click **Incubator** to show the incubator configuration panel, then expand the **Calibrate Oxygen Sensor** panel under **Oxygen Sensor**.



2. Verify that the gas connections have been correctly configured.

3. Verify that the **O_2%** is set to a value similar to the types of conditions used, for example hypoxic or normoxic. For normoxic conditions, the laboratory incubator oxygen % value can be used for guidance to set the **O_2%** value of the OSI-2.

4. Select the **purge gas source** for the calibtation: **Nitrogen** or **CO_2**.

   Note: A pure CO_2 or N_2 gas tank is required.

5. Click **Begin Calibration**.
   The EVOS™ M7000 Imaging System automatically calibrates the oxygen sensor for the proper functioning of the EVOS™ Onstage Incubator (OSI-2). The entire calibration process takes approximately three minutes.

IMPORTANT! See the *EVOS™ M7000 Imaging System User Guide* (Pub. No. MAN0018326; available for download at **thermofisher.com**) for details on how to set up a time-lapse experiment with the OSI-2.

3 Troubleshooting

Troubleshooting

Observation	Resolution/Suggestion
Cells appear unhealthy or are dying, or media turning the wrong color.	Check that the media and supplements are appropriate for the cell type.
	Were any dyes, drugs, or other compounds added? If so, test a sample without them to see of they are the cause.
	Check the chamber temperature and the OSI-2 settings. Is the incubator warming correctly? Use your hand to feel the temperature of the glass lid and the warming plate under the water chamber in the control unit to check that they are warming properly.
	Verify gas flow: Check that CO_2 and compressed air gases (or premix gas) are flowing to the control unit, all with 40–50 psi on the regulator.
	Is there water in the water chamber? If not, loss of humidity can be the cause.
Communication error between the OSI-2 and the EVOS™ software.	Check the data cable between the OSI-2 and the computer. Unplug, then replug them. Ensure that both ends of the cable are properly connected to the correct ports.
	Check the data cable between the environmental chamber and the OSI-2 control unit. Unplug, then replug them. Ensure that both ends of the cable are properly connected to the correct ports.
Heavy condensation or opaque mist on the glass lid of the environmental chamber. **Note:** A few droplets inside the environmental chamber or along the edges of the lid are normal.	Verify gas flow: Check that CO_2 and compressed air gases (or premix gas) are flowing to the control unit, all with 40–50 psi on the regulator.
	Is the room cold, or is an air conditioner vent blowing down on the microscope? If so, warm the room or redirect the air, or move the system to a different area.
	If you are using the EVOS™ FL Auto 2 or the EVOS™ M7000 Imaging System, increase the **Ambient Offset** option upward by 1°C in the settings to increase glass lid temperature, then try the system again. Continue increasing the **Ambient Offset** to a maximum of 5°C, until condensation stops.

(continued)

Observation	Resolution/Suggestion
Condensation under the stage, such as on the objectives or on other metal components.	Leakage of humid air from the environmental chamber due to a gap near the sample. Check for gaps, such as from cut off corners of plates, tiny "feet" along the bottom of plate or other vessel, debris along the bottom edge of the vessel, uneven placement of the vessel in the vessel holder, or whether you are using the wrong type of vessel holder for the OSI-2 (see Appendix B, “Compatible vessel holders and stage plates”).
	Is the room cold, or is an air conditioner vent blowing down on the microscope? If so, warm the room or redirect the air, or move the system to a different area. Cold metal under a warm stage will lead to condensation.
"Open lid" error, or status shows as "lid open" when it is not.	Take off the lid and clean the lid contacts with alcohol wipe, then try again.
	Rotate the lid 180-degrees, then try again, to test if the lid contact at one end has failed.
	Have you made any IT changes to the system, such as added software, drivers, or single-user login? If so, reverse these changes, then try again.
EVOS™ Software does not recognize that the OSI-2 is connected or powered on.	Is the power light on the front of the OSI-2 control unit on? If not, check that the power cord and the adapter cords are firmly in their ports and the wall outlet is working properly. Otherwise, the adapter could be faulty.
	Check the data cable between the OSI-2 and the computer. Unplug, then replug them. Ensure that both ends of the cable are properly connected to the correct ports.
	In the **Incubator Control** panel, ensure that the **Use Incubator** option is selected, along with gas options and humidity, then click **Apply**.
EVOS™ system seems to take a long time to adjust to proper humidity or temperature.	It can take as long as 30 minutes for the system to properly adjust. Allow sufficient time after activating the system.
	Use room temperature water in the water chamber. Cold water takes longer to heat to proper temperature, which in turn causes humidity to take longer to reach the proper setting. Do not use hot water, because hot water can overwhelm the sensors in the system.
	Is the room cold, or is an air conditioner vent blowing down on the microscope? If so, warm the room or redirect the air, or move the system to a different area.

Specifications

Physical characteristics

Note: Specifications of the EVOS™ Onstage Incubator (OSI-2) are subject to change without notice. See the EVOS™ product page at **www.thermofisher.com/evos** for the latest product information.

	Stagetop environmental chamber	Control unit
Length:	27 cm (11 in)	45 cm (18 in)
Width:	17 cm (6.8 in)	21 cm (8.3 in)
Height:	4.1 cm (1.6 in)	23 cm (9 in)
Weight:	0.73 kg (1.6 lb)	6.7 kg (15 lb)

Temperature range: Ambient (~20°C) to 40°C (± 1°C)

Humidity: 70–90% relative humidity (RH) at 37–40°C

CO_2 range: 0% to 20%

O_2 range: 0% to ambient (~21%)

Operating power: 100–240 VAC, 1.8 A

Frequency: 50–60 Hz

Electrical input: 24 VDC, 7.5 A

Hardware

Compatible vessels: Multiwell plates, 35-mm Petri dishes, T-25 flasks (See Appendix B, “Compatible vessel holders and stage plates” for a complete list of recommended vessels and vessel holders.)

Gas input ports: Air or air-CO_2 premix, CO_2-only, and N_2-only (range 40–50 psi)

Stagetop environmental chamber accessories: Master stage plate, vessel holder for multiwell plates, light shield

B Compatible vessel holders and stage plates

EVOS™ Onstage Incubator (OSI-2)-compatible vessel holders and stage plates

	Capacity	Catalog No.	Adapter option
Slides[1]			
	One Nunc™ Lab-Tek™ Chambered Coverglass	AMEPVH055	EVOS™ Vessel Holder for Automated Stage (Cat. No. AMEPVH054) required for EVOS™ M5000 Imaging System
	One Nunc™ Lab-Tek™ II Chambered Coverglass	AMEPVH039	
	One 75 mm × 25 mm slide	AMEPVH058	
	One 75 mm × 50 mm slide	AMEPVH056	
	Two 75 mm × 25 mm slides	AMEPVH057	
	Two Nunc™ Lab-Tek™/Lab-Tek™ II chambered coverglasses	AMEPVH069	
Well plates/well dishes[1]			
	One 128.2 mm × 86.2 mm multiwell plate	AMEPVH028	AMEPVH054 required for EVOS™ M5000 Imaging System

(continued)

	Capacity	Catalog No.	Adapter option
	One Nunc™ multiwell dish	AMEPVH040	AMEPVH054 required for EVOS™ M5000 Imaging System
Dishes[1]			
	One BD Falcon™/Corning™/Nunc™ 35-mm Petri dish	AMEPVH029	AMEPVH054 required for EVOS™ M5000 Imaging System
	One BD Falcon™/ibidi/MatTek™/Nunc™/WillCo Wells™ 35-mm dish (gasket sealed)	AMEPVH070	
	One Nunc™ 60-mm Petri dish	AMEPVH031	
	One Nunc™ 100-mm Petri dish	AMEPVH037	
	Two BD Falcon™/Nunc™ 35-mm Petri dishes	AMEPVH030	
	Two Nunc™ 60-mm Petri dishes	AMEPVH032	
Flasks			
	One BD Falcon™/Corning™ T-25 flask	AMEPVH033	AMEPVH054 required for EVOS™ M5000 Imaging System
	Two BD Falcon™/Corning™ T-25 flasks	AMEPVH034	

(continued)

	Capacity	Catalog No.	Adapter option
Master/stage plates			
	One BD Falcon™ T-75 flask or one 128.2 mm × 86.2 mm multiwell plate or AMEPVH001–AMEPVH020	AMEPVH054	For automated stages and EVOS™ M5000 Imaging System only
	One EVOS™ Onstage Incubator (OSI)	AMEPVH035	
	One EVOS™ Onstage Incubator (OSI-2)	AMEPVH106	

[1] Secured with retention clips.

C

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words.

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Standard safety symbols

Symbol and description
CAUTION! Risk of danger. Consult the manual for further safety information.
CAUTION! Risk of electrical shock.
CAUTION! Hot surface.
CAUTION! Pinch point.
CAUTION! Potential biohazard.

Location of safety labels

Label and location


Figure 6 Rear panel of EVOS™ Onstage Incubator (OSI-2)

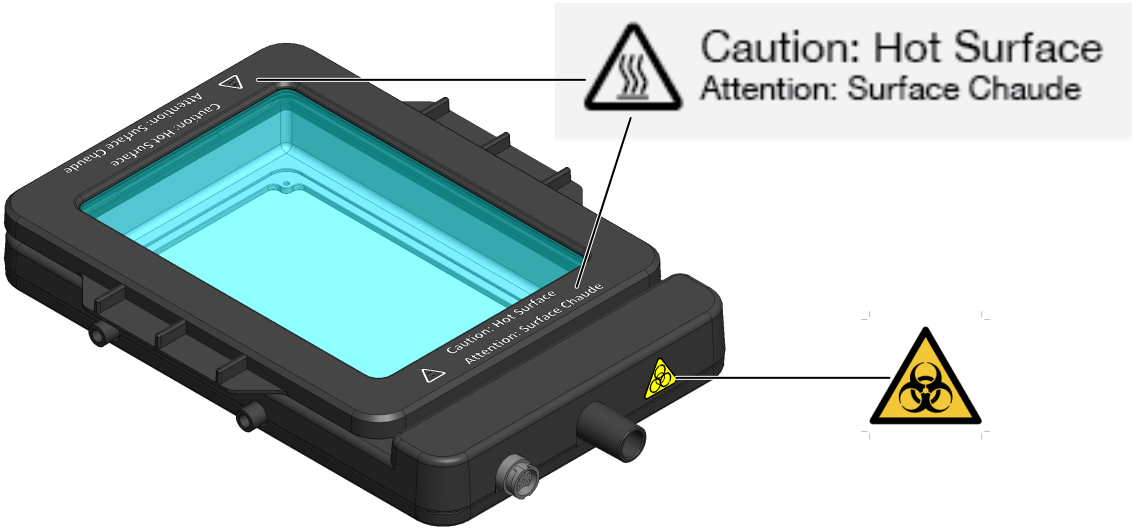


Figure 7 EVOS™ Onstage Incubator (OSI-2) stagetop environmental chamber

Control and connection symbols

Symbols and descriptions	
	On (Power)
	Off (Power)
	Earth (ground) terminal
	Protective conductor terminal (main ground)
	Direct current
	Alternating current
	Both direct and alternating current

Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with China RoHS requirements.
	Indicates conformity with European Union requirements.
	INDICATES CONFORMITY WITH UNITED KINGDOM REQUIREMENTS
	Indicates conformity with Australian standards for electromagnetic compatibility.
	Indicates conformity with the WEEE Directive 2012/19/EU. **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

If covers are removed, do not use the instrument. Contact Technical Support.

Electrical safety

WARNING! Fuse Installation. Before installing the instrument, verify that the fuses are properly installed and the fuse voltage matches the supply voltage. Replace fuses only with the type and rating specified for the unit. Improper fuses can damage the instrument wiring system and cause a fire.

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility. If the line cord is damaged, contact Technical Support.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

Overvoltage rating

The EVOS™ M5000 Imaging System, the EVOS™ M7000 Imaging System, and the EVOS™ Onstage Incubator (OSI-2) have an installation (overvoltage) category of II, and are classified as portable equipments.

Safety requirements for EVOS™ Onstage Incubator (OSI-2)

WARNING! Thermo Fisher Scientific recommends the use of nitrogen, oxygen, and carbon dioxide gas with the Onstage Incubator. The use of alternative gasses is currently not supported and can adversely affect system performance.

Gas cylinders

You must supply the required nitrogen, oxygen, and carbon dioxide gas cylinders and accessories for the installation. This instrument requires pressurized house lines, or one size 1-A gas cylinder that holds approximately 7.2 m^3 (257 ft^3) of gas when full for each gas. Use only pre-purified gasses of 99.9% or greater purity.

CAUTION! Damage to the instrument and its products can result from using impure gas, gases other than specified, or an inadequate amount of gas.

WARNING! EXPLOSION HAZARD. Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use, and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

WARNING! Gas cylinders are heavy and CAN topple over, potentially causing personal injury and tank damage. Firmly secure cylinders to a wall or work surface. Contact your environmental health and safety coordinator for guidance on the proper installation of a gas cylinder.

Pressure regulator

A multistage regulator must be supplied for each cylinder to be used with the OSI-2. The regulator must be able to regulate the output pressure to between 40–50 psi. An appropriate fitting must be connected to the regulator output to connect to the ⅛ in. (3.175 mm) ID, ¼ in. (6.35 mm) OD gas line.

Attaching the cylinder

Attach the pressurized gas cylinder firmly to a wall or gas cylinder cart by means of approved straps or chains.

Ventilation requirements

WARNING! The Onstage Incubator should be installed and operated in a well-ventilated environment as defined as having a minimum airflow of 6–10 air changes per hour. Contact your environmental health and safety coordinator to confirm that the instruments will be installed and operated in an environment with sufficient ventilation.

Allow at least 50 cm (20 in.) of clearance around the instrument for ventilation.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods that are specified in the manufacturer user documentation. It is the responsibility of the operator (or other responsible person) to ensure that the following requirements are met:

- No decontamination or cleaning agents are used that can react with parts of the equipment or with material that is contained in the equipment. Use of such agents could cause a HAZARD condition.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) before the instrument is serviced at your facility or is sent for repair, maintenance, trade-in, disposal, or termination of a loan. Request decontamination forms from customer service.
- Before using any cleaning or decontamination methods (except methods that are recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.

Instrument component and accessory disposal

To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety standards

Reference	Description
EU Directive 2014/35/EU	European Union “Low Voltage Directive”
IEC 61010-1 EN 61010-1 UL 61010-1 CAN/CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010 EN 61010-2-010	*Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use Part 2-010: Particular Requirements for Laboratory Equipment for the Heating of Materials*
IEC 61010-2-081 EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*

EMC standards

Reference	Description
EU Directive 2014/30/EU	European Union “EMC Directive”
EN 61326-1 IEC 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
AS/NZS CISPR 11	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*
ICES-001, Issue 4	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*
FCC Part 15 Subpart B (47 CFR)	*U.S. Standard Radio Frequency Devices* This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Environmental design standards

Reference	Description
Directive 2012/19/EU	European Union “WEEE Directive”—Waste electrical and electronic equipment
Directive 2011/65/EU & Commission Delegated Directive 2015/863	European Union “RoHS Directive”—Restriction of hazardous substances in electrical and electronic equipment
SJ/T 11364-2014	“China RoHS” Standard—Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical Products For instrument specific certificates, visit our customer resource page at **www.thermofisher.com/us/en/home/technical-resources/rohs-certificates.html**.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Chemical waste safety

Chemical waste hazard

CAUTION! HAZARDOUS WASTE!

Refer to Safety Data Sheets (SDSs) and local regulations for handling and disposal.

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis, if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  www.who.int/publications/i/item/9789240011311

D Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.