



ECU

Environmental Control unit



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INSTRUCTION MANUAL

Serial No. _____

050214

World Precision Instruments

FluoroDish™

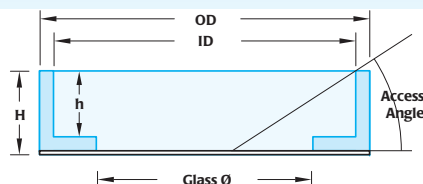
Cover-glass bottom for observing and growing cells in imaging related research

- **Optical quality glass bottom for better imaging quality (RI=1.525)**
- **Low sample volume for expensive chemicals**
- **Lowest access angle for micropipette**
- **Low toxicity adhesive for embryo research**



Part Number	ID (mm)	OD (mm)	Glass Ø (mm)	height (inside)	Height (outside)	Access Angle
FD35	33	35.5	23.5	7.8	9	29°
FD3510	10	35.5	10	1.5	4.65	17°
FD5040	47.5	49.82	35	7.25	7.4	17°

- **Choose from poly-D-lysine or collagen coated, or uncoated**



FD35-100	FluoroDish Sterile Culture Dish, clear wall, 35 mm, 23 mm well, box of 100	\$159
FD35PDL-100	FluoroDish Sterile Culture Dish, Poly-D-Lysine Coated, 35 mm, 23 mm well, box of 100	\$229
FD3510-100	FluoroDish Sterile Culture Dish, clear wall, 35 mm, 10 mm well, low sidewall, box of 100	\$195
FD5040-100	FluoroDish Sterile Culture Dish, clear wall, 50 mm, 35 mm well, box of 100	\$299

These polycarbonate dishes provide exceptional imaging quality for many applications requiring the use of inverted microscopes. FluoroDish uses a specially formulated adhesive that is optically clear, durable and with extremely low toxicity. Tests by an independent laboratory have shown that the 96-hour surviving rate of embryos is 100% when kept in FluoroDish. The bottom glass has superior UV transmission (30% transmission at 300 nm, compared to less than 7% for the most popular German glass).

The 35 mm dish has outside dimensions similar to that of a Corning 35 mm dish and has ø23.5 mm glass window (**FD35**) or ø10 mm glass window (**FD3510**). Most heaters and perfusion adapters designed for the Corning 35 mm dish will also fit this dish. The 23.5 mm glass window dish is available uncoated or poly-D-lysine-coated. Certain types of cell lines (e.g., PC3 and HEK) adhere well to the uncoated glass bottom dish. The poly-D-lysine coating has been reported to improve the adhesion of neuron cells.

The 10 mm glass window dish (**FD3510**) has low sidewall for easy microelectrode access and low solution volume. The low microelectrode access angle is the lowest among all of 35 mm dishes on the market (very close that of a 50 mm dish). The dish needs only 100 ~ 200 µL to cover the bottom well.

The 50 mm dish (**FD50**) has a large growth area (35 mm well diameter), a low access angle for microelectrodes, and grips for easy handling.

WARRANTY

WPI (World Precision Instruments, Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of 30 days* from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within ten (10) days after receipt of shipment. Claims for lost shipments must be made within thirty (30) days of receipt of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim is settled. In some instances, photographic documentation may be required. Some items are time-sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container.

Do not return any goods to us without obtaining prior approval and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. Goods accepted for restocking will be exchanged or credited to your WPI account. Goods returned which were ordered by customers in error are subject to a 25% restocking charge. Equipment which was built as a special order cannot be returned.

Repairs

Contact our Customer Service Department for assistance in the repair of apparatus. Do not return goods until instructions have been received. Returned items must be securely packed to prevent further damage in transit. The Customer is responsible for paying shipping expenses, including adequate insurance on all items returned for repairs. Identification of the item(s) by model number, name, as well as complete description of the difficulties experienced should be written on the repair purchase order and on a tag attached to the item.

* Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.

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ABOUT THIS MANUAL

The following symbols are used in this guide:



This symbol indicates a CAUTION. Cautions warn against actions that can cause damage to equipment. Please read these carefully.



This symbol indicates a WARNING. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and TIPS contain helpful information.



Fig. 1—The Stagetop Environment (STEV) and ECU (Environmental Control Unit) comprise the basic incubator system for growing cells under controlled conditions.

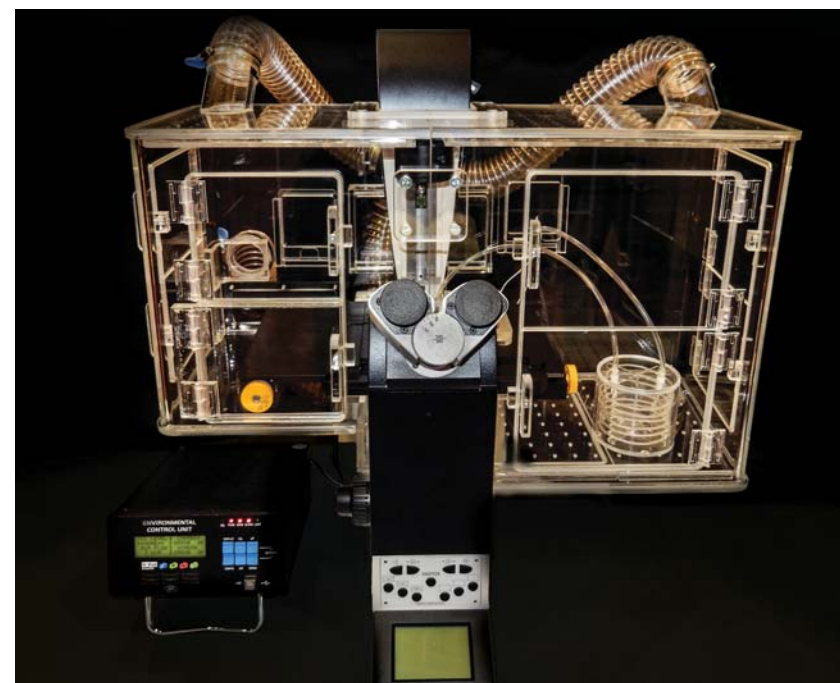


Fig. 2—A complete environmental control system includes the ECU (Environmental Control Unit), a microscope with chamber and a STEV stagetop environment.

INTRODUCTION

The **Environmental Control Unit (ECU)** can be used with either the **Stagetop Environment** incubator (**STEV**) or with a full microscope enclosure system.

- For short term or long term studies of living cell cultures under a microscope or for time lapse video research, a microscope **Stagetop Environment** incubator (**STEV**) is essential.
- Perfect for Live Cell Imaging, **STEV** is a compact environmental incubation chamber that houses your culture wells and fits on a microscope stage inside the live cell microscope incubator.

This system offers precision control of both temperature and carbon dioxide, as well as remote control and data logging via a USB connection. The system is flexible and easy to configure for a variety of experimental conditions.

The carbon dioxide sensor is located in the incubator chamber (**STEV**) wall. Feedback from this sensor allows the **ECU** to control the concentration of CO₂ from 0–20%. This controller offers a background flow rate of 0–950 SCCM.

NOTE: THE **ECU-HCP** has an external CO₂ sensor.

The system includes the **Environmental Control Unit (ECU)** electronics which use four programmable loops to control the temperatures of the incubation chamber (base) and the lid, CO₂ within the environmental incubation chamber and airflow within the incubator.

The system comes in four configurations, and all four are discussed in this manual.

- **ECU-H5**—Pre-mixed CO₂ gas of the desired concentration is pumped into the system, and this **ECU** regulates the airflow at the desired level. It also controls the heating and monitors the stagetop environment chamber.
- **ECU-HC**—This control unit mixes the air and CO₂ gas to the desired concentration. An internal CO₂ sensor (inside the **ECU** controller) monitors the concentration of the gas. The **ECU-HC** regulates the airflow at the desired level, controls the heating of the system and monitors the stagetop environment chamber.
- **ECU-HCP**—This unit performs like the **ECU-HC**, except this unit comes with a remote CO₂ sensor that is positioned inside the stagetop environment chamber.
- **ECU-HOC**—This unit performs like the **ECU-HC**. In addition to controlling the temperature and CO₂, this unit also controls the O₂ level. Nitrogen is used to displace oxygen from the background air, which generally has about 20.7% oxygen. The O₂ level of the background gas can be regulated down to as low as 1%.

Features

- Up to five programmable digital control loops
 - Independent incubator base temperature PID control with $\pm 0.1^\circ\text{C}$ precision
 - Independent incubator lid temperature PID control with $\pm 0.1^\circ\text{C}$ precision
 - CO₂ digital PID control with $\pm 0.1\%$ precision
 - O₂ digital PID control with $\pm 0.1\%$ precision
 - Airflow digital PID control from 0–900 SCCM

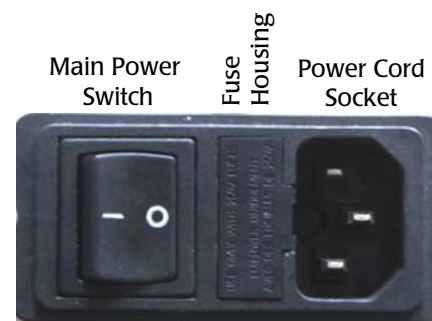


Fig. 60—Unplug the power cord to access the fuse housing release.

3. Insert a small flat blade screwdriver under the lip on the right side of the fuse housing cover (Fig. 61).



Fig. 61—Insert the screw driver under the fuse housing lip and pry the housing open.

4. Pull the fuse housing out as far as it will go and rotate it to the right. There is a catch to keep the housing from coming completely out (Fig. 62).



Fig. 62—Open the fuse housing and rotate it right to remove the fuse.

5. Remove the bad fuse. It is the one on the top. A spare fuse is stored in the bottom slot of the fuse housing.
6. Use the spare fuse provided to replace the bad fuse. Slide it into the top slot of the fuse housing.
7. Rotate the fuse housing and slide it back into position.
8. Reinstall the power cord.
9. Turn the power switch on to verify that the **Air-Therm** has power again.



Fig. 57—(Left) Insert the screw driver under the fuse housing lip and pry the housing open.

Fig. 58—(Right) The fuse housing slides out.

4. Pull the fuse housing out of its socket (Fig. 59).



Fig. 59—Remove the fuse housing to access the fuses.

5. Remove the bad fuse.
6. Replace the bad fuse with another 5x20mm metric fuse, 4A, 250V.
7. Position the power switch/fuse housing and slide it back into its socket until it snaps into place.
8. Reinstall the power cord.
9. Turn the power switch on to verify that the **Air-Therm** has power again.

Changing a Fuse on the AirTherm Satellite 110V

A spare fuse is provided in the fuse housing (Fig. 60).

1. Turn the main power switch off (I).
2. Unplug the power cord from the power cord socket on the back of the **Air-Therm** (Fig. 60).

- Probe tracking adaptive mode automatically adjusts the setpoints for both the chamber and the window to maintain the desired well temperature
- USB-based remote control and data logging
- Electronic flow meter
- Programmable alarm for out of tolerance condition on all four channels
- Compact and lightweight

Options

The standard environmental control unit has a heated incubator (with a heated glass lid) and CO₂ control. In the event that your application uses a heated microscope chamber, this system is also available without the heating option.

Notes and Warnings



CAUTION: Always remove the lid on the Stagetop Environment (**STEV**) by using the thumb hold indentation in the bottom left corner of the incubation chamber.

Parts List

After unpacking, verify that there is no visible damage to the unit. Verify that all items are included:

- (1) **STEV** – Stagetop environmental control unit with lid
- (1) **ECU** – Environmental Control Unit electronics control
- (1) Temperature sensor
- (1) CO₂ Sensor Cable (orange) – This is **ONLY** provided with the **ECU-HCP** systems.
- (1) Accessory Kit that includes a tubing kit with connectors, and a syringe kit
- (1) Instruction Manual

Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled “Claims and Returns” on page 35 of this manual. Please contact WPI Customer Service if any parts are missing at 941.371.1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100mm (four inches) of shock absorbing material. For further details, please read the section entitled “Claims and Returns” on page 35 of this manual.

INSTRUMENT DESCRIPTION

This system is comprised of the Stagetop Environment (STEV) and the Environmental Control Unit (ECU) electronics.

STEV

The Stagetop Environment Incubator has an airtight lid that snaps into place.

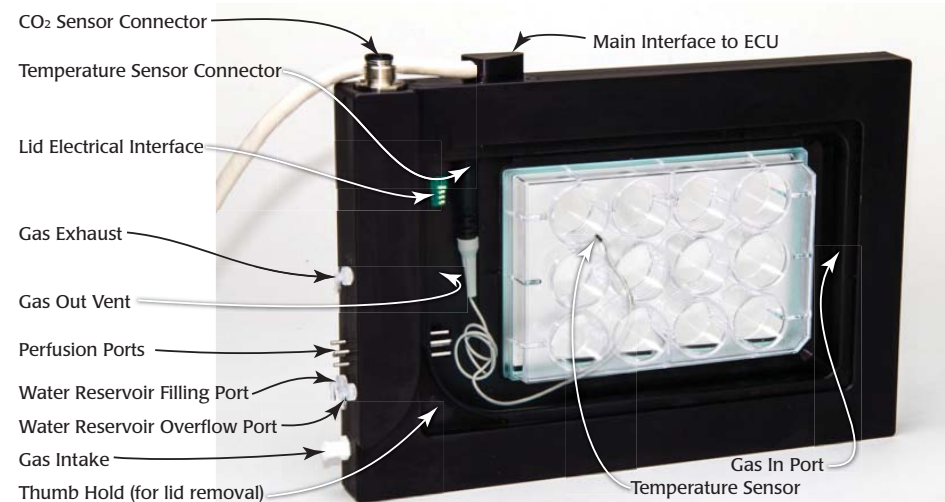


Fig. 3—The Stagetop Environment incubator for the ECU-HCP is shown with the cover removed.

CO₂ Sensor Connector—The CO₂ sensor is housed inside the incubator wall. The CO₂ gas passes from the **Gas Intake** to the **Gas In Port** over the culture wells to the **Gas Out Vent**. Then, it passes over the CO₂ sensor as it exits through the **Gas Exhaust** tube. Attach the orange CO₂ sensor cable from the ECU to this port.

Temperature Sensor Connector—Two ports are located on the inside of the chamber wall. The first (left) is for future use. Plug the temperature sensor into the second one (right). The tip of the sensor can be positioned inside the chamber, as desired.

Lid Electrical Interface—When the lid is properly installed, this interface allows the ECU to control the lid temperature.

Gas Exhaust—Mixed gas travels through the system and exits through this exhaust port. It can be vented to the air or connected to an exhaust system, if desired.

Gas Out Vent—Mixed gas inside the incubation chamber exits the chamber through this vent hole.

Perfusion Ports—These ports can be used for microfluidic experiments and gas transfer.

AirTherm Satellite.

- Place the sensors inside the microscope chamber.
- Connect the power cord to the **AirTherm Satellite**, and plug it into an AC wall outlet. Turn the power switch on. When the power is on, the yellow Power LED Indicator is illuminated.
- Adjust the temperature setpoint to the desired target value. See “Auxiliary Setpoint” on page 20. By default the temperature setpoint is set at 37°C. If the temperature reading is below the setpoint, the heater turns on and the green Heater LED indicator begins to flash.
- Adjust the fan speed to control the air flow rate. See “Fan Air Flow Setpoint” on page 20. The airflow rate can be adjusted from 20–50 ft³/minute (CFM). For safety reasons, the fan flow rate cannot be set at less than 10%.

TIP: A lower flow rate offers a quiet operation and is suitable for most applications. However, the higher flow rate can generate more turbulence, so that the temperature in the chamber is more uniform. The higher air flow speeds up the heating process and makes it possible to heat larger chambers.

CAUTION: Avoid blocking the airflow. Although a built-in thermal cutoff switch is designed to reduce the hazard, a fan failure or obstruction in the airflow has the potential for overheating the system and damaging the instrument or even causing a fire.

- Set the display to show the **AirTherm Satellite** as the auxiliary heater when you toggle through the display menu. See “Auxiliary Source” on page 25.
- Allow the temperature to reach the setpoint and wait one hour.

Changing a Fuse on the AirTherm Satellite 220V

- Turn the main power switch off (I).
- Unplug the power cord from the power cord socket on the back of the **AirTherm** (Fig. 56).



Fig. 56—Unplug the power cord to access the fuse housing release.

- Insert a small flat blade screwdriver under the orange lip on the left side of the main power switch/fuse housing (Fig. 57). Gently pry upwards until the power switch slides out.

APPENDIX A: AIRTHERM SATELLITE HEATER

If desired, an **AirTherm Satellite** heater may be plugged into the Auxiliary Heater port on the ECU, and the ECU can then be used to regulate the temperature of a microscope enclosure chamber used in live cell imaging.

Setting Up an AirTherm Satellite Heater

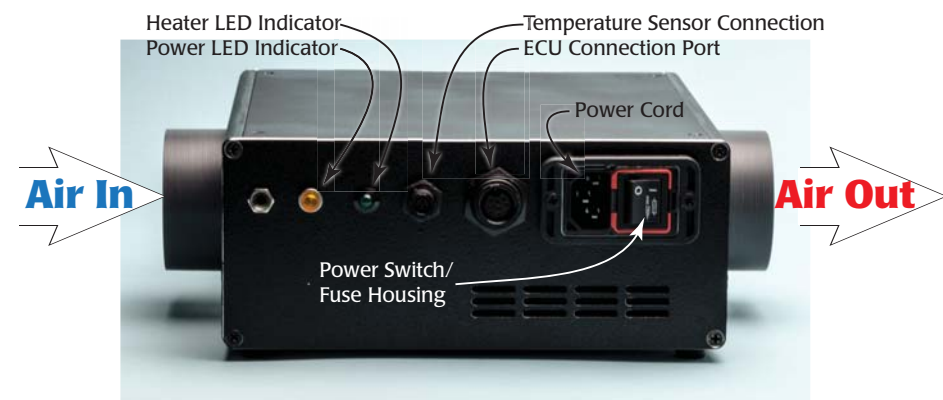


Fig. 54—All the AirTherm Satellite connections are on the same face of the unit.

1. Connect tubing with the included clamps as shown in Fig. 55.

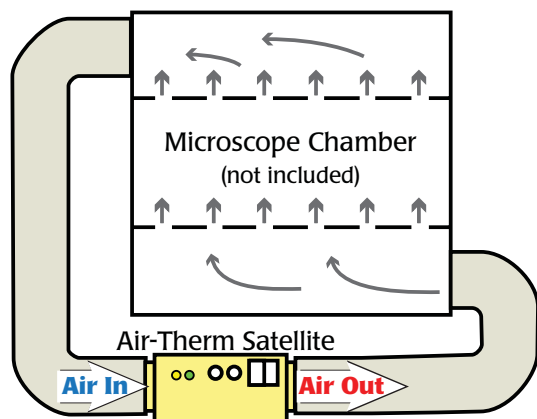


Fig. 55—Connect the tubing to the air inlet and outlet ports on the AirTherm Satellite to the air outlet and inlet ports on the microscope chamber.

2. To connect the **AirTherm Satellite** heater to the ECU, connect one end of the ECU cable supplied with the **AirTherm Satellite** to the ECU Connection Port on the **AirTherm Satellite**. Connect the other end to the Auxiliary Heater Connection on the ECU.
3. Connect the temperature sensor to the Temperature Sensor Connection on the

Water Reservoir Ports—The water reservoir inside the incubator wall serves two purposes. When it is heated, it helps to maintain the temperature of the chamber. Second, it humidifies the mixed gas as the gas travels through water permeable tubing inside the water reservoir. Connect a syringe filled with distilled water to the luer fitting on the **Water Reservoir Filling** port. Fill the chamber until the water comes out of the Water Reservoir Overflow Port.



Fig. 4—Use a large syringe filled with distilled water to fill the water reservoir. When it is full, water will come out the overflow port.

Gas Intake—The mixed gas enters the incubation chamber through this port. Use 1/4" OD tubing to connect this port to the **Mixture Out** port on the back of the ECU.

Thumb Hold—Insert your thumb or finger and gently lift the chamber lid when you need to remove the lid.

Main Interface to ECU—The cable allows the STEV to communicate with the ECU electronics.

Gas In Port—Mixed gas enters the incubator chamber through this hole.



Fig. 5—The STEV is shown with the chamber cover in position.

ECU

The **ECU** houses all the electronics for:

- Regulating the CO₂ flow and temperature of the incubation chamber
- Controlling the temperature of the chamber lid and an auxiliary heater
- Monitoring the air flow, CO₂ level, and four temperatures.
- **ECU-HOC** also regulates and monitors the O₂ level.

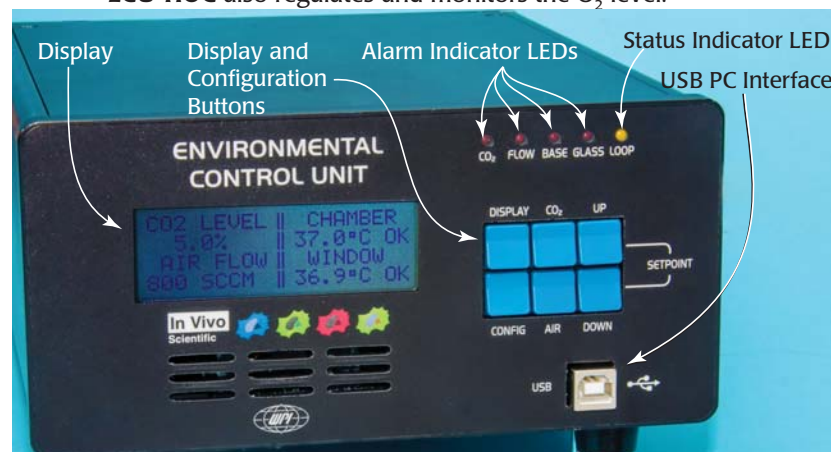


Fig. 6—The front panel of most ECUs displays four monitored parameter: CO₂ level, air flow, chamber temperature and temperature of the chamber's heated glass lid (window). The ECU-H5 does not display CO₂ because a pre-mixed gas of known concentration is used, and CO₂ is not monitored. The ECU-HOC also displays the oxygen level.

Display—The main display shows the CO₂ level in the incubator chamber, the O₂ level in the incubator chamber (**ECU-HOC** version only), the air flow (in standard cm³), the temperature inside the chamber and the temperature of the heated glass lid (window).

Display and Configuration Buttons—

Display: Press this button to toggle between the main display and a set of individual temperatures, including the probe temperature (positioned inside the chamber), the chamber temperature (measured by a fixed RTD in the chamber wall), the window temperature (lid), the adaptive mode display, and an optional auxiliary temperature which can be connected to the system using the **Auxiliary Heater** port on the back of the **ECU**.

Config: This button toggles through a list of configuration parameters like setpoints and alarms.

CO₂: To quickly see the CO₂ level, press this button. On the **ECU-HOC**, this button displays both the CO₂ level and the O₂ level.

Air: To quickly see the air flow, press this button.

Up/Down: These two buttons are used to adjust configuration parameters.

Chamber never reaches setpoint	The factory default parameters may be corrupt	Reset the parameters to the factory settings. See "Resetting Factory Defaults" on page 26.
System does not stably regulate CO ₂	Parameters have changed or the setup has changed.	Reset the parameters to the factory settings. See "Resetting Factory Defaults" on page 26.
	Incorrect pressure set for CO ₂ , background air or any other gas	Check the pressure. Then, check the connections on all the gas ports for leaks.

NOTE: If you have a problem/issue with that falls outside the definitions of this troubleshooting section, contact the WPI Technical Support team at 941.371.1003 or technicalsupport@wpiinc.com.

SPECIFICATIONS

This unit conforms to the following specifications:

Power	110/240V, 50/60Hz
Operating Temperature (ambient)	10 – 50°C (50 – 122°F)
Operating Humidity (ambient)	15 – 70% RH, non-condensing
Warm up Time	20 minutes
Computer Interface	USB via external USB/RS232 converter
Sensor	Non-dispersive infrared (NDIR), dual beam, 20s response time

CO₂ Sensor (ECU-HCP and ECU-HC only)

Sensor Range	0 – 20% CO ₂
Control Range	0 – 20%
Control Precision	0.1% CO ₂
Control Accuracy	0.1 – 3% of reading
Drift	<2.5% reading/year

O₂ Sensor (ECU-HOC only)

Sensor Type	Zirconium Dioxide, diffusion, 4s response time
Sensor Range	0–25%
Control Range	0–25%*
Control Precision	0.1% O ₂
Control Accuracy	±0.5% (2% of the full scale)

*The upper limit of the ECU-HOC oxygen control range is constrained by the oxygen content in the background gas. For example, if the background air has 20.7% oxygen, the ECU-HOC can only control up to 20.7% oxygen.

Resetting Factory Defaults

To restore the original factory settings on the **ECU**:

1. Power down the **ECU**.
2. Restart the system while depressing both the **Up** and **Down** buttons. The display indicates that the default settings have been restored.

ACCESSORIES

Part Number	Description
3245	Quick fit reducers 1/4" OD, 5/32" ID
400195	0.156" OD Tubing
AIRTHERM-SAT-1W	AirTherm Satellite Heater (110V)
AIRTHERM-SAT-2W	AirTherm Satellite Heater (230V)
97777	AirTherm Satellite ECU Interface cable
XXXXXX	Microscope objective (lens) warmer
97799	Lens warmer dongle
3301	European power cord
3302	UK power cord
3006	US power cord
13208	Hose clamps
14088	Australian power cord
15590	Clear hose, 2.5" diameter, 4.5'
98727	Temperature probe for the AirTherm Satellite
802269	AirTherm Satellite 220V Fuse, slow blow, 250V, 4A
802270	AirTherm Satellite 110V Fuse, slow blow, 250V, 1.2A

TROUBLESHOOTING

Issue	Possible Cause	Solution
No Power	Power cord is improperly connected	Verify that the power cord is securely connected and plugged into a live wall socket.
Error on the display	The CO ₂ Level display shows "ERROR" because the CO ₂ sensor is not properly plugged in.	Check the CO ₂ sensor connection on the STEV . This applies to the ECU-HCP only.
	The Probe Temperature display shows "ERROR" because the temperature sensor is improperly installed.	Check the temperature probe connection inside the STEV .

Alarm Indicator LEDs—The red LEDs illuminate to indicate an alarm state.

CO₂: This red LED illuminates when the CO₂ level falls more than $\pm 0.5\%$ outside the high and low alarm limits set using the configuration menu.

Flow: This red LED illuminates when the measured air flow is more than 100 SCCM (Standard Cubic Centimeters/Minute) greater or less than the setpoint.

Base: This red LED illuminates when the incubation chamber (base) temperature falls outside the high and low alarm limits set using the configuration menu.

Glass: This red LED illuminates when the chamber lid (glass) temperature falls outside the high and low alarm limits set using the configuration menu.

Status Indicator LED—This yellow heartbeat LED blinks continuously to indicate that the unit is operational and the control loops are running.

USB PC Interface—Connect the **ECU** to a computer using a standard USB cable. This interface can be used to monitor and control the ECU. It is intended for integration with software packages like a microscope imaging software.



Fig. 7—The ECU-HCP back panel has the ports and connectors to connect the STEV chamber with the electronic controls. The ECU-H5 has no Gas Inlet port or CO₂ Sensor Cable. Because the ECU-HC has an internal sensor, it will not have a CO₂ Sensor Cable. The ECU-HOC has an additional port for nitrogen inlet.

Power Switch—This toggle button powers on the electronic controls.

Auxiliary Heater Connection—An auxiliary heater (like the AirTherm or a lens warmer) can be plugged into this port to allow the **ECU** to control it. For example, if used with a satellite heater, this could be used to regulate the temperature of a microscope enclosure chamber used in live cell imaging. Alternatively, it could be used to regulate a lens warmer.

Background Gas Inlet pressure fit connector—Connect the pressurized background gas to this port using 1/4" OD tubing. If you are using the **ECU-H5**, connect the pre-

mixed gas to this port. With the other ECUs, connect the background air tank to this port.

CO₂ Inlet pressure fit connector—Connect the CO₂ tank to this port using 1/4" OD tubing. The ECU-H5 does not have this port.

N₂ Inlet pressure fit connector—Connect the N₂ tank to this port using 1/4" OD tubing. The ECU-HOC is the only unit with this port. The nitrogen is used to displace oxygen.

Mixed Gas Outlet—Connect this port to the **Gas Intake** on the STEV chamber using 1/4" OD tubing.

CO₂ Sensor Cable (ECU-HCP only)— Connect this orange cable to the **CO₂ Sensor Connector** on the STEV chamber.

Tubing Kit

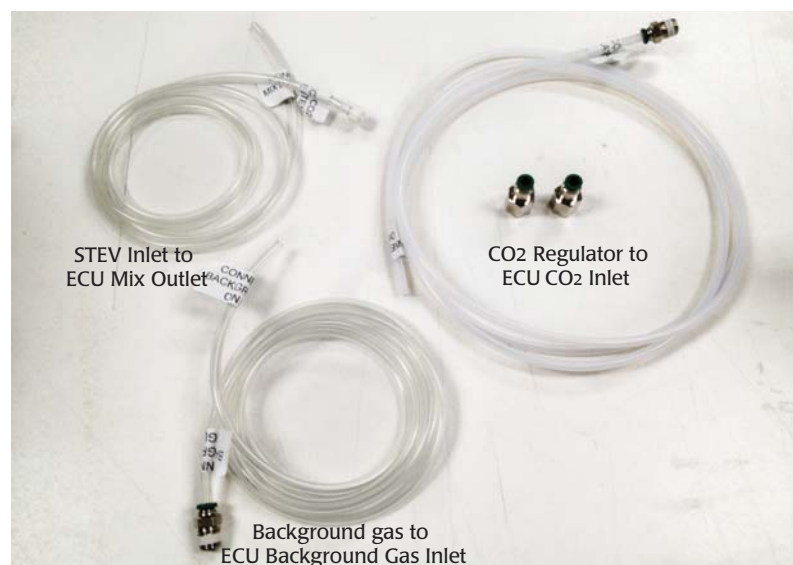


Fig. 8—The tubing kit contains all the tubing to connect the gas tanks, the ECU and the STEV. This kit will vary somewhat depending on which ECU you ordered.

Auxiliary Source

Set the display for the auxiliary source so that “AirTherm” or “Lens Heater” appears when you toggle through the display options. By default the ECU is configured for use with an AirTherm. If you want to control a lens heater instead, you must also configure the controller. See “Configuring the ECU for a Lens Warmer” on page 25.

1. Simultaneously press the **Up** and **Down** buttons to access the Hardware Setpoint menu.



Fig. 51—Press the Up and Down buttons together to enter the Hardware Setpoint menu.

2. Press the **Config** button until Auxiliary Source appears.



Fig. 52—Choose AirTherm or Lens Heater by pressing the Up or Down button.

3. Press the **Up** or **Down** button to select AirTherm or Lens Heater.
4. Press the **Display** button to save the new parameters or press the Config button to toggle through the rest of the parameters.

Configuring the ECU for a Lens Warmer

1. Power down the ECU and unplug it from the AC power source.
2. Remove the four screws on the top of the ECU control box to remove the cover.
3. Locate and remove the J19 and J20 jumpers.

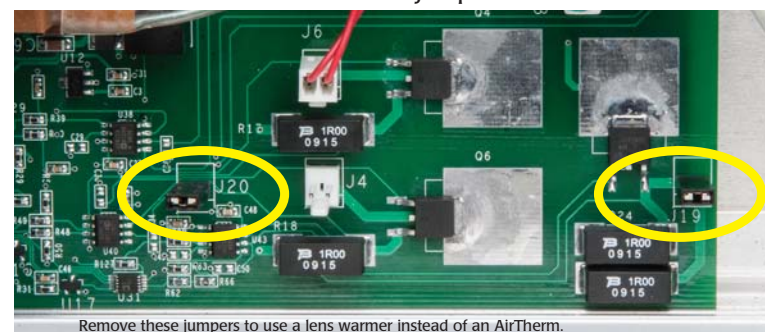


Fig. 53—Remove the J19 and J20 jumpers to use a lens warmer.

4. Reinstall the cover and secure it with the four screws.
5. Power up the controller and set the auxiliary source. See “Auxiliary Source” on page 25.

required gas volumes in an acceptable range. Fig. 48 shows the suggested air flow setting to achieve the oxygen concentrations shown for CO₂ levels of 5%, 10% and 15%. Slight deviation from these values is acceptable.

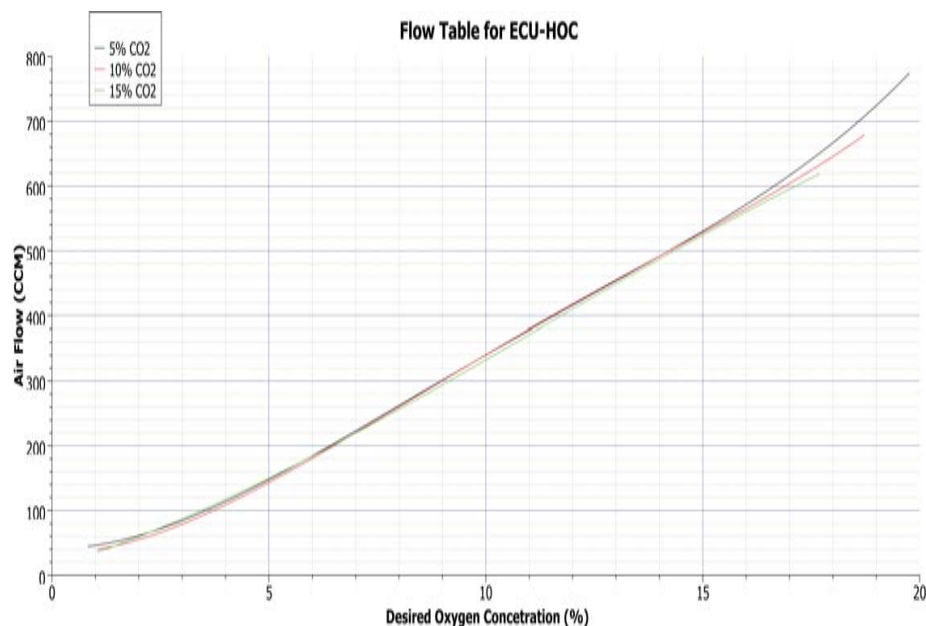


Fig. 48—This graph shows how to choose a Flow Setpoint based on the level of CO₂ and the desired concentration of O₂ in the air mix.

1. Simultaneously press the **Up** and **Down** buttons to access the Hardware Setpoint menu.



Fig. 49—Press the Up and Down buttons together to enter the Hardware Setpoint menu.

2. Press the Config button to display the O₂ Level Setpoint.



Fig. 50—The setpoint can be adjusted with the Up/Down buttons.

3. Press the **Up** or **Down** button to change the setpoint.
4. Press the **Display** button to save the new parameters or press the **Config** button to toggle through the rest of the parameters.

Syringe Kit

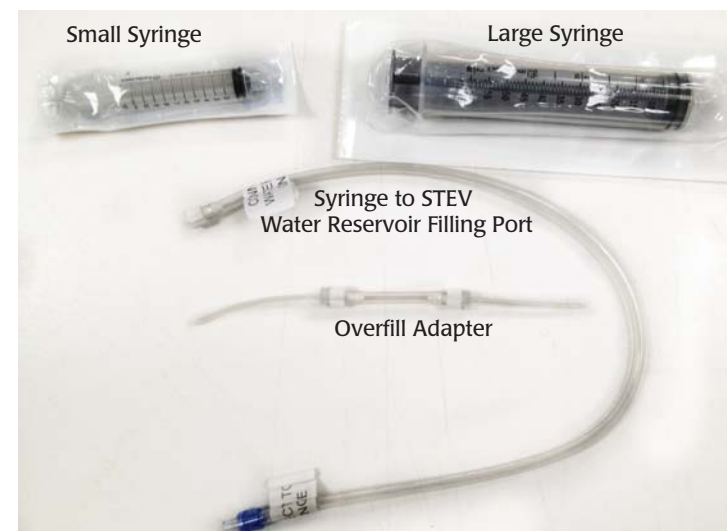


Fig. 9—The syringe kit is used for filling the STEV water reservoir.

OPERATING INSTRUCTIONS

System Setup

The basic setup of the environment control system is shown below. Gas connections can be made using 1/4" OD tubing. The **ECU** is optimized at the factory for 5% CO₂, however, a range of other control percentages may be used. During the initial setup, the setpoint should be set to 0% CO₂ concentration and allowed to settle for 15 minutes. For the **ECU-HCP**, the **CO₂ flow control should be fully open**,* and the background air should be running at 800CCM. The background gas for all the other configurations should be set at 400CCM. Once the system settles, adjust the setpoint to the desired concentration.

***NOTE:** For the system to operate correctly, it is important that the pressure for the CO₂ flow control and the back-ground air be set at 25 PSI. For the **ECU-HOC**, the pressure for the nitrogen flow must also be set to 25 PSI.

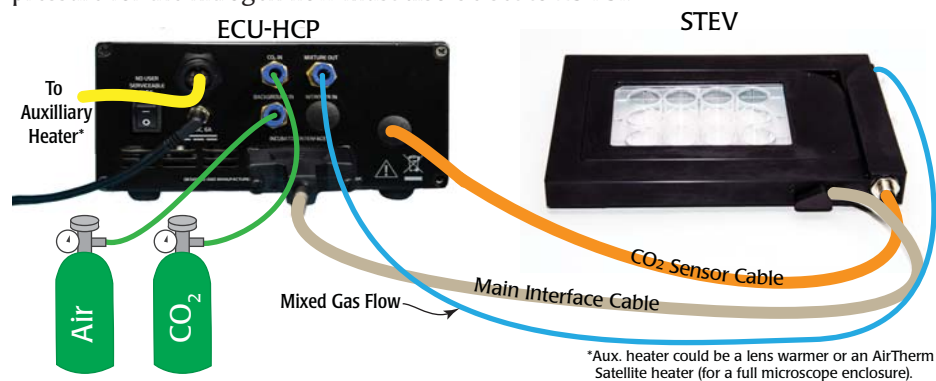


Fig. 10—The ECU-HCP connections

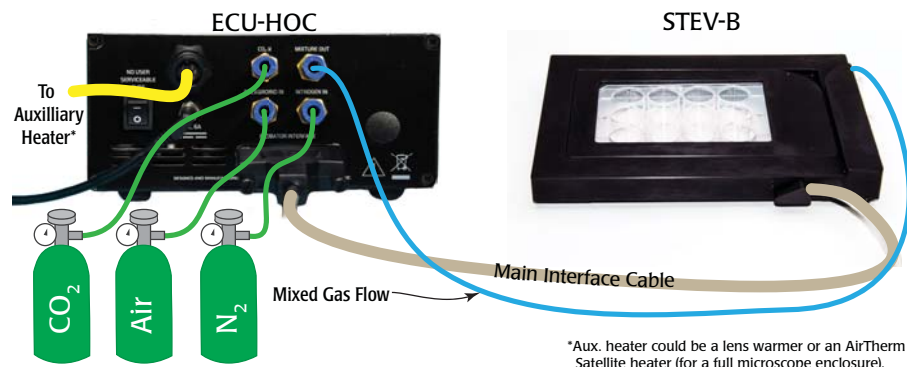


Fig. 11—The ECU-HOC connections

3. Press the **Up** or **Down** button to change the setpoint.
4. Press the **Display** button to save the new parameters or press the **Config** button to toggle through the rest of the parameters.

O₂ Level Setpoint (ECU-HOC only)

The **ECU-HOC** controls the oxygen level down to 1% oxygen. This is accomplished by displacing the oxygen in the background gas with nitrogen. It can be set in increments of 0.1%. The default is 15%.

NOTE: For setpoint levels of 5% or less, reduce the background gas air flow to 100 CCM to improve system stability. See "Flow Level Setpoint" on page 22.

Oxygen Concentration versus Flow Considerations

The gas mixture out of the **ECU-HOC** consists of a mixture of air, nitrogen and carbon dioxide. The concentration of CO₂ is determined directly by adding CO₂ to the air. Therefore, a mixture of 5% CO₂ (with no N₂ added) is 95% air and 5% CO₂. The concentration of O₂ is determined by the amount of O₂ present in the air. The standard concentration of O₂ in air is 20.8%. Because of this, the maximum concentration of O₂ in a 5% CO₂ mixture is 19.76% ($0.95 \times 20.8 = 19.76$). As the CO₂ increases, the maximum obtainable O₂ decreases.

To control the O₂ concentration in the gas mixture, we pump N₂ into the gas mixture to displace the air and consequently the O₂.

NOTE: During this process, the N₂ displaces CO₂, but the CO₂ control loop restores the balance by adding CO₂ until the desired concentration is achieved.

The **ECU's** flow meter measures the input flow of air into the unit. The output flow rate on the **ECU** is the sum of the incoming air plus the required CO₂ to achieve the desired concentration of CO₂ plus the necessary N₂ to displace the required amount of air in order to regulate the oxygen content. The lower the required O₂ concentration, the greater the amount of N₂ required to displace the O₂. This increases the volumetric flow into the incubation chamber. Since the incubation chamber opposes the air flow, pressure increases.

For example, let's say that our setup is as follows:

- Air flow is 400 CCM
- CO₂ concentration is 5%
- Desired O₂ concentration is 1%

To achieve this, we would need to configure the system as follows:

- 7200 CCM of N₂
- 400 CCM of CO₂

This would result in a total gas mixture flow rate of 8000 CCM (400 CCM air + 7200 CCM N₂ + 400 CCM CO₂). This volumetric flow significantly increases the pressure at the **STEVE**. This volume is not achievable with the specified input gas pressures. In order to mitigate this problem, the input air flow must be reduced to keep the

CO₂ Level Setpoint (Not Available on the ECU-H5)

For the **ECU-HCP**, the carbon dioxide sensor is located in the incubator chamber (**STEV**) wall. For the **ECU-HC**, the sensor is located inside the **ECU** controller. Feedback from this sensor allows the **ECU** to control the concentration of CO₂ from 0–20%. It can be set in increments of 0.1%. The default is 5%.

1. Simultaneously press the **Up** and **Down** buttons to access the Hardware Setpoint menu.



Fig. 44—Press the Up and Down buttons together to enter the Hardware Setpoint menu.

2. The CO₂ Level Setpoint displays after a couple seconds.

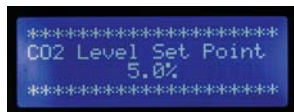


Fig. 45—The setpoint can be adjusted with the Up/Down buttons.

3. Press the **Up** or **Down** button to change the setpoint.
4. Press the **Display** button to save the new parameters or press the **Config** button to toggle through the rest of the parameters.

Flow Level Setpoint

This controller offers a background flow rate of 0–950 SCCM. It can be set in increments of 10 CCM. The default is 800 SCCM for the **ECU-HCP**, and 400 SCCM for the **ECU-HOC**, the **ECU-HC** and the **ECU-H5**.

This controller offers a background flow rate of 0–950 CCM. It can be set in increments of 10 SCCM. The default is 800 SCCM for the, 400 for the **ECU-HC**, and 400 for the **ECU-H5**.

1. Simultaneously press the **Up** and **Down** buttons to access the Hardware Setpoint menu.



Fig. 46—Press the Up and Down buttons together to enter the Hardware Setpoint menu.

2. Press the **Config** button to display the Flow Level Setpoint.



Fig. 47—The setpoint can be adjusted with the Up/Down buttons.

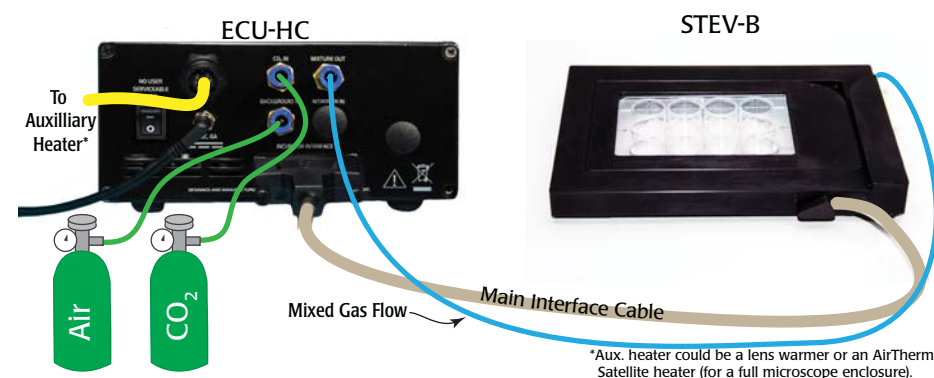


Fig. 12—The ECU-HC connections.

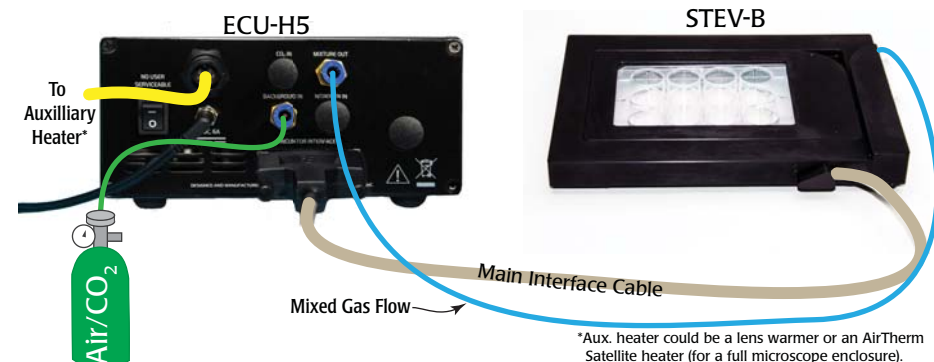


Fig. 13—The ECU-H5 connections

1. Connect one end of the overflow adapter to water reservoir filling port and the other end to the overflow port. The overflow adapter allows the water in the reservoir to expand as it heats without spilling onto the microscope stage.



Fig. 14— The overflow adapter has two luer fittings used to connect to a syringe.



Fig. 15—The overfill adapter is connected to the STEV filling and overflow ports.

2. To fill the water reservoir:
 - Disconnect the luer fitting on the overfill adapter in order to fill the reservoir.
 - Attach the white fitting on the reservoir filling tubing to the water intake tubing.



Fig. 16—The white end of the reservoir filling tube attaches to the STEV water intake tubing, and the blue end attaches to a syringe.

- Fill the 100CC syringe filled with warm, distilled water. Attach the blue luer lock on the filling tube to the syringe. Inject water into the reservoir filling port until water without bubbles comes out of the reservoir overflow port on the top of the stagetop environment.

Window Alarm

By default alarms are disabled on startup, because they would beep continuously until the control loops stabilize the conditions in the stagetop environment system. When the window alarm is enabled, the controller will beep if the temperature of the heated glass lid is outside the range set by the high and low alarms.

1. Press the **Config** button on the **ECU** to access the Configuration menu. Press the **Config** button again until the Window Alarm parameter displays.



Fig. 41—Enable or disable the alarm using the Up/Down buttons.

2. Press the **Up** or **Down** button to change the setpoint.
3. Press the **Display** button to save the new parameters or press the **Config** button to toggle through the rest of the parameters.

Adaptive Mode

When enabled, the probe tracking adaptive mode automatically adjusts the setpoints for both the chamber and the window to maintain the desired well temperature. By default the adaptive mode is disabled. Once the system stabilizes, the adaptive mode may be enabled. When it is enabled, the system samples the probe temperature periodically based on the configured adaptive interval. Each time it samples the probe temperature, the ECU automatically adjusts the window and lid setpoints to achieve (and then maintain) the desired well temperature.

1. ALLOW THE SYSTEM TO STABILIZE BEFORE YOU ENABLE ADAPTIVE MODE.
2. Press the **Config** button on the **ECU** to access the Configuration menu. Press the **Config** button again until the Adaptive Mode Enable parameter displays.

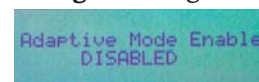


Fig. 42—Enable or disable the Adaptive Mode by pressing either the Up or Down button.

3. Press the **Up** or **Down** button to enable (or disable) the adaptive mode.
4. If necessary, modify the Adaptive Interval. By default it is set to 10 minutes, but the range is from 1–20 minutes. Press the **Config** button again to display the Adaptive Interval parameter.

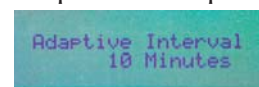


Fig. 43—The adaptive interval can be adjusted with the Up/Down buttons.

5. Press the **Up** or **Down** button to change the adaptive interval in one minute increments.
6. Press the **Display** button to save the new parameters or press the **Config** button to toggle through the rest of the parameters.

Auxiliary Setpoint

The auxiliary setpoint is the target temperature of the auxiliary heater that is connected to the **Auxiliary Heater** port on the back of the **ECU**. If you are using a microscope chamber, you may connect a heater to this port so that the **ECU** can maintain the temperature of the large microscope chamber, too.

1. Press the **Config** button on the **ECU** to access the Configuration menu. Press the **Config** button again until the AUX Setpoint parameter displays.



Fig. 38—The setpoint can be adjusted with the Up/Down buttons.

2. Press the **Up** or **Down** button to change the setpoint.
3. Press the **Display** button to save the new parameters or press the **Config** button to toggle through the rest of the parameters.

Fan Air Flow Setpoint

1. Press the **Config** button on the **ECU** to access the Configuration menu. Press the **Config** button again until the Fan Air Flow Setpoint parameter displays.



Fig. 39—The setpoint can be adjusted with the Up/Down buttons.

2. Press the **Up** or **Down** button to change the setpoint.
3. Press the **Display** button to save the new parameters or press the **Config** button to toggle through the rest of the parameters.

Chamber Alarm

By default alarms are disabled on startup, because they would beep continuously until the control loops stabilize the conditions in the stagetop environment system. When the chamber alarm is enabled, the controller will beep if the temperature inside the chamber is outside the range set by the high and low alarms.

1. Press the **Config** button on the **ECU** to access the Configuration menu. Press the **Config** button again until the Chamber Alarm parameter displays.



Fig. 40—Enable or disable the alarm using the Up/Down buttons.

2. Press the **Up** or **Down** button to change the setpoint.
3. Press the **Display** button to save the new parameters or press the **Config** button to toggle through the rest of the parameters.



Fig. 17—Connect the syringe to the blue connector on the filling tube.

- Disconnect the filling tube, and reconnect the overfill adapter.

TIP: If you prefer, cap the inlet port, and leave the outlet port open. Be sure to route the water away from the microscope. The water in the chamber may expand on initial heating and overflow the chamber. The moist air in the chamber transmits heat much better than dry air.

3. Using the labeled tubing kit, make the gas connections for the **ECU**:
 - (**ECU-HCP, ECU-HOC** and **ECU-HC** only.) Connect the CO₂ tank to the **CO₂ In** port. Press the opaque Tygon tubing all the way into the port.
 - (**ECU-HOC** only.) Connect the nitrogen gas tank to the **N2 In** port.
 - Connect the background gas tank to the **Background In** port. For the **ECU-H5**, connect the pre-mixed gas to this port.
 - Connect the **Mixture Out** port to the **Gas Input** port on the **STEV** chamber.
4. Connect the **STEV** with the **ECU**:
 - (**ECU-HCP** only.) Line up the pins and notch on the sensor connector (on the **STEV**) with the holes on the sensor cable to connect the sensor.
 - Connect the **Main Interface** cable on the **STEV** chamber to the **Incubator Interface** port on the **ECU**.



Fig. 18—The ECU-HCP (shown) has the connector for the external CO₂ sensor.

5. If desired, connect an auxiliary heater to the Auxiliary Heat Connector on the back of the **ECU**. This could be an **AirTherm Satellite** unit for heating a microscope chamber, or it could be a lens warmer. To setup an **AirTherm Satellite** heater, see "Appendix A: AirTherm Satellite Heater" on page 28. If you are using a lens warmer, a dongle is required.



Fig. 19—The AirTherm Satellite heater can be used as an auxiliary heater which can be controlled by the ECU



Fig. 20—A lens warmer is plugged into the ECU dongle.

6. Open the lid of the chamber by placing your thumb or index finger in the thumb hold and gently lifting the lid.
7. Position the culture well plate and the temperature sensor as desired inside the chamber. Usually the sensor is positioned near the center of the well plate. Fill the temperature sensor well with distilled water.



Fig. 21—(Left) Position the temperature sensor in one of the culture wells.

Fig. 22—(Right) This STEV has a 35mm well adapter plate.

1. Press the **Config** button on the **ECU** to access the Configuration menu. Press the **Config** button again until the Window Setpoint parameter displays.



Fig. 35—The setpoint can be adjusted with the Up/Down buttons.

2. Press the **Up** or **Down** button to change the setpoint.
3. Press the **Display** button to save the new parameters or press the **Config** button to toggle through the rest of the parameters.

Window High Alarm

If the heated glass lid temperature climbs above the high alarm temperature, the **Glass** LED illuminates. If the Window Alarm is enabled, the **ECU** will also beep continuously until the temperature falls back into the acceptable range. See "Window Alarm" on page 21.

1. Press the **Config** button on the **ECU** to access the Configuration menu. Press the **Config** button again until the Window High Alarm parameter displays.



Fig. 36—The alarm parameter can be adjusted with the Up/Down buttons.

2. Press the **Up** or **Down** button to change the setpoint.
3. Press the **Display** button to save the new parameters or press the **Config** button to toggle through the rest of the parameters.

Window Low Alarm

If the heated glass lid temperature falls below the low alarm temperature, the **Glass** LED illuminates. If the Window Alarm is enabled, the **ECU** will also beep continuously until the temperature returns to the acceptable range. See "Window Alarm" on page 21.

1. Press the **Config** button on the **ECU** to access the Configuration menu. Press the **Config** button again until the Window Low Alarm parameter displays.



Fig. 37—The alarm parameter can be adjusted with the Up/Down buttons.

2. Press the **Up** or **Down** button to change the setpoint.
3. Press the **Display** button to save the new parameters or press the **Config** button to toggle through the rest of the parameters.

2. Press the **Up** or **Down** button to change the setpoint.
3. Press the **Display** button to save the new parameters or press the **Config** button to toggle through the rest of the parameters.

Chamber High Alarm

If the chamber temperature climbs above the high alarm temperature, the **Base** LED illuminates. If the Chamber Alarm is enabled, the **ECU** will also beep continuously until the temperature falls back into the acceptable range. See "Chamber Alarm" on page 20.

1. Press the **Config** button on the **ECU** to access the Configuration menu. Press the **Config** button again until the Chamber High Alarm parameter displays.



Fig. 33—The chamber alarm parameter can be adjusted with the Up/Down buttons.

2. Press the **Up** or **Down** button to change the setpoint.
3. Press the **Display** button to save the new parameters or press the **Config** button to toggle through the rest of the parameters.

Chamber Low Alarm

If the chamber temperature falls below the low alarm temperature, the **Base** LED illuminates. If the Chamber Alarm is enabled, the **ECU** will also beep continuously until the temperature returns to the acceptable range. See "Chamber Alarm" on page 20.

1. Press the **Config** button on the **ECU** to access the Configuration menu. Press the **Config** button again until the Chamber Low Alarm parameter displays.



Fig. 34—The chamber alarm parameter can be adjusted with the Up/Down buttons.

2. Press the **Up** or **Down** button to change the setpoint.
3. Press the **Display** button to save the new parameters or press the **Config** button to toggle through the rest of the parameters.

Window Setpoint

The window setpoint is the target temperature of the glass lid of the **STEV** chamber.

NOTE: The setpoint for the window regulates the temperature of the **ECU** lid. The temperature at the well plate is lower because of a temperature gradient between the plate and the body of the chamber. Setting the lid and chamber setpoints to 38.5°C usually allows the **ECU** to maintain the well plate temperature around 37°C.

NOTE: Adapters are available that allow you to use 35mm culture dishes inside a **STEV**. If you use an adapter, verify that the dish is placed inside the adapter so that it sits flat. Push it in place with enough force so that the gasket seals around it. Because it is a snug fit, press it straight down. If it is tilted, it is harder to install. It must sit flat on the bottom of the **STEV**.

8. Reposition the chamber lid and press it securely into place. The rubber gasket on the lid does not conduct heat. Most of the heat is transmitted through the moist air and the glass radiating onto the well plate.

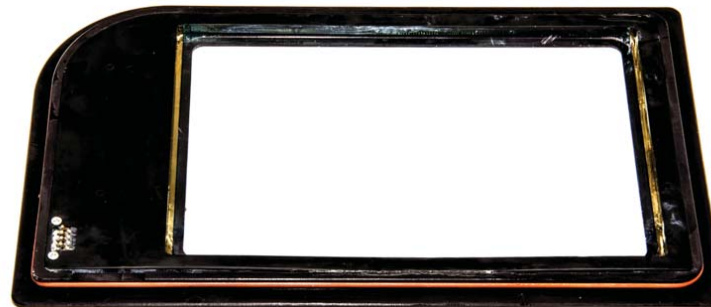


Fig. 23—The rounded corner of the lid fits into the thumb hold on the base of the stagetop environment. Notice the electronic interface connection in the bottom left corner of the image. This interface allows the controller to communicate with the lid and control the lid temperature.

CAUTION: Avoid touching the electronic interface connector on the underside of the lid and on the base. Keep moisture out of this interface, especially when you are cleaning the lid and base unit.

9. Plug the power cable from the **ECU** into an AC power outlet.
10. Turn the power switch on the back of the **ECU** unit on.
11. Set the configuration parameters, as needed. See "Setting Configuration Parameters" on page 17.

NOTE: The setpoints for the chamber and the lid regulate the temperature at those structures. The temperature at the well plate is lower because of a temperature gradient between the plate and the body of the chamber. Setting the lid and chamber setpoints to 38.5°C usually allows the **ECU** to maintain the well plate temperature around 37°C. If you prefer, you can set the Probe tracking adaptive mode to automatically adjust the setpoints for both the chamber and the window to maintain the desired well temperature.

12. Allow the system to run for 20 minutes so that the CO₂ level stabilizes.

Display Options

The main controller display of the ECU shows the current measured CO₂ level, air flow level, incubation chamber temperature (chamber) and temperature of the heated lid (window). The **ECU-HOC** also displays the O₂ level.



Fig. 24—(Left) The default display of the ECU controller continuously updates to show four measured parameters. The ECU-H5 displays “None” for the CO₂ value, because a pre-mixed gas of known concentration is used, and CO₂ is not monitored.

Fig. 25—(Right) The ECU-HOC also displays the oxygen level.

Press the **Display** button to toggle through the display options and monitor the current values of the system temperatures:

- Probe Temperature is measured by the sensor positioned inside the chamber. The free probe temperature is not used as feedback for the temperature control circuit.
- Chamber Temperature is monitored by a sensor mounted inside the chamber wall.
- Window Temperature registers the temperature of the heated glass lid of the chamber.
- Probe Tracking display shows if the adaptive control mode is enabled and the chamber and window setpoint temperatures.
- Auxiliary Temperature shows the reading from an optional auxiliary heater connected to the back of the **ECU**. This could be an AirTherm satellite heater or a lens warmer.



Fig. 26—Press the **Display** button to toggle from the default display to the Probe Temperature, Chamber Temperature, Window Temperature, Probe Tracking and Auxiliary Temperature back to the default display.

Viewing the CO₂ Measurement

The CO₂ level measured inside the incubation chamber (**STEV**) is always available in the upper left corner of the main display. To quickly view the CO₂ level alone, press the **CO₂** button on the **ECU**.



Fig. 27—(Left) Press the CO₂ button to display only the CO₂ level.

Fig. 28—(Center) The CO₂ level is shown in the upper left corner of the main display.

Fig. 29—Fig. 18- (Right) When you press the CO₂ button on the ECU-HOC, you can view the O₂ level also.

Viewing the Air Flow

The air flow level of the mixed air entering the incubation chamber (**STEV**) is always available in the lower left corner of the main display. To quickly view the air flow alone, press the **Air** button on the **ECU**.



Fig. 30—(Left) Press the Air button to display only the current air flow to the chamber.

Fig. 31—(Right) The air flow is shown in the lower left corner of the main display.

Setting Configuration Parameters

Press the **Config** button on the **ECU** to access the Configuration menu. From this menu, you can adjust the setpoints, high alarms and low alarms for the chamber and the glass lid of the chamber (window). The auxiliary temperature setpoint and fan air flow setpoint can be set, and the alarms can be enabled or disabled.

Chamber Setpoint

The chamber setpoint is the temperature to be maintained inside the **STEV** incubation chamber.

NOTE: The setpoint for the chamber regulates the temperature of the **ECU** base. The temperature at the well plate is lower because of a temperature gradient between the plate and the body of the chamber. Setting the lid and chamber setpoints to 38.5°C usually allows the **ECU** to maintain the well plate temperature around 37°C.

- Press the **Config** button on the **ECU** to access the Configuration menu. The chamber setpoint displays.



Fig. 32—The chamber setpoint can be adjusted with the Up/Down buttons.