IN Cell Analyzer 2500*HS* / 6500*HS*

Customer Instructions

Live Cell Imaging Module (Option)

Contents

IN Cell 2500*HS* / 6500*HS* Live Cell Imaging Module (Option)

- *Introduces the components of the Live Cell Imaging Module*
- *Introduces the Environmental Control Software*
- *Describes and illustrates how to use the Live Cell Imaging Module*
- *Describes how to refill the Live Cell Imaging Module's humidifier bottle*

What is the Live Cell Imaging Module?

Live cell imaging is a critical part of cell biology research. The Live Cell Imaging Module regulates the sample environment by controlling temperature and gas concentration for long- or short-term time-lapse applications.

The system can be set up to control:

- Temperature only
- Temperature, humidity, and CO_2 concentration
- Temperature, humidity, and CO_2/O_2 concentration

The Live Cell Imaging Module is available as an option for the IN Cell Analyzer 2500*HS* and 6500*HS* systems.

Live Cell Imaging Module Components

This section describes the components included with the Live Cell Imaging Module.

Environmental Control Unit

The Environmental Control Unit (ECU) is installed inside the IN Cell Analyzer system and is not accessible to the user. The ECU is controlled by software installed on the IN Cell acquisition workstation and regulates sample temperature, gas mixing, gas flow rate, and humidifier temperature.

The ECU is available in two configurations:

- **CO_2 only.** CO_2 can be controlled from 0-20%. User must provide 100% CO_2 and Clean Dry Air (CDA) as inputs.
- **CO_2 and O_2.** CO_2 can be controlled from 0-20%. O_2 can be controlled from 0-20% but is limited by the O_2 concentration in the CDA (e.g., if the CDA only contains 10% O_2, the system can reach a maximum of 10% O_2 overall.) N_2 is used to regulate O_2 levels. User must provide 100% CO_2, 100% N_2, and Clean Dry Air (CDA) as inputs.

Environmental Control Software

The Live Cell Imaging Module control software is accessed via the IN Cell Acquisition software GUI. The software allows the user to:

- Enable and disable temperature and gas controls
- Define temperature and gas concentration set points
- View real-time feedback on temperature and gas concentration

Humidifier

The Humidifier warms and humidifies the gas flowing to the sample to reduce media evaporation and help maintain sample temperature during live cell experiments. The humidifier lid is equipped with a black heating element to warm the water in the bottle as well as a white stone diffuser which distributes gas into the water. There is also an insulation sleeve included to retain heat and prevent condensation on the bottle. The Humidifier is installed in the storage area on the right side of the IN Cell Analyzer (near the power button, opposite the sample area).

Figure 1. Humidifier and Humidifier Lid with Heating Element and Diffuser

Humidifier

Lid with Heater and Diffuser

Live Cell Sample Lid and Heated Stage

The Live Cell Sample Lid and Heated Stage are heated components that work together to control temperature at the sample.

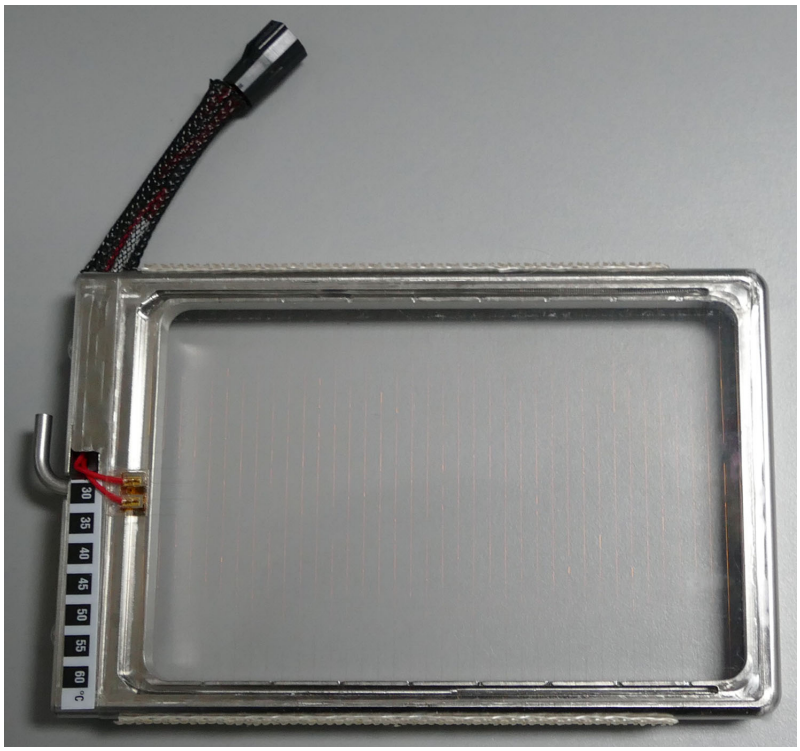
- **Live Cell Sample Lid.** The Live Cell Sample Lid fits tightly on top of the sample plate or slide adapter (if imaging a slide, 35 mm dish, or chambered slide). The lid is heated to control sample temperature from the top and to prevent condensation on the glass window. The lid can reach temperatures close to

50°C during normal operation so it is equipped with a temperature indicator to show when it is safe to touch as well as silica fabric on two sides to provide safe handling. Finally, the Live Cell Sample Lid has a gas input for distribution of humidified gas to the sample.

- **Heated Stage.** On systems equipped with the Live Cell Imaging Module, the stage base plate can be heated to warm the sample from the bottom.

To achieve and maintain the desired temperature at the sample, the sample lid and the stage base plate are heated simultaneously. When the user enters a temperature set point for the sample, a proprietary algorithm determines the appropriate temperatures for the stage base plate and the sample lid, to achieve the desired temperature at the sample. For instance, a temperature set point of 37°C will set the stage plate to 34.3°C and the sample lid to 36.3°C.

Figure 2. Live Cell Sample Lid



Facilities Requirements

In addition to the facilities requirements described in the Site Preparation Guide for the IN Cell 2500*HS* and 6500*HS* (GE Document Number 29254181), operation of the Live Cell Imaging Module requires the following.

Table 1. Facilities Requirements

Parameter	Requirement
CO_2 input*	100%
N_2 input (only required for CO_2/O_2 option)*	100%
CDA (Clean Dry Air) Source*	100% dry - house air or air tank
Pressure Regulators for CDA, CO_2, and N_2 supplies	Gas flow regulated to 25 psi, +/- 1 psi
Regulator Fittings	1/4 in. (6mm) push fittings
Tubing Diameter	1/4 in. outer diameter, 1/8 in. inner diameter (6mm OD 4mm ID)

* To prevent particle buildup on sensitive Live Cell Imaging Module components, we require the following:

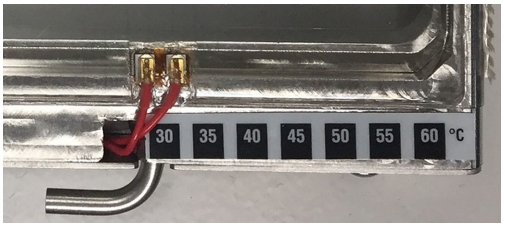
- For the CDA input, we require the use of a water trap/desiccator **and** a 0.2 µm filter between the CDA source and the Live Cell Imaging Module.
- For the N_2 and CO_2 inputs, we require the use of medical grade gas **or** a 0.2 µm filter between the gas sources and the Live Cell Imaging Module.

Non-compliance may result in failure of the Live Cell Imaging Module.

Labels on the Live Cell Sample Lid

The Live Cell Sample Lid shown in Figure 2 includes the safety and operational labels described in the following table.

Table 2. Labels on Live Cell Sample Lid

Label	Description
	Temperature indicator label (from 30°C to 60°C). Temperature values printed on the label change color to indicate temperature of the lid surface. As an example, below, a similar Fahrenheit label indicates a current temperature of about 113°F. 
	CAUTION! Hot Surface. Do not touch the lid surface until the temperature label described in the previous row indicates a safe-to-touch surface temperature.

Using the Environmental Control Software

The control software for the Live Cell Imaging Module is accessed using the Environmental Control icon available within the IN Cell acquisition software. The Environmental Control software is separate from the IN Cell Acquisition software and, therefore, environmental control parameters are not saved in an experiment protocol.

To start the Live Cell Imaging Module's control software and open the Main Status Window:

➢ From the IN Cell acquisition software's main Setup toolbar, click **Environmental Control**  . The Environmental Control software's Main Status Window will display.

The Environmental Control software consists of two primary windows:

- **Main Status Window.** First window displayed when you start the control software. May also be minimized to a status bar.
- **Settings Window.** Allows you to define set points for the temperature, gas, and flow rate.

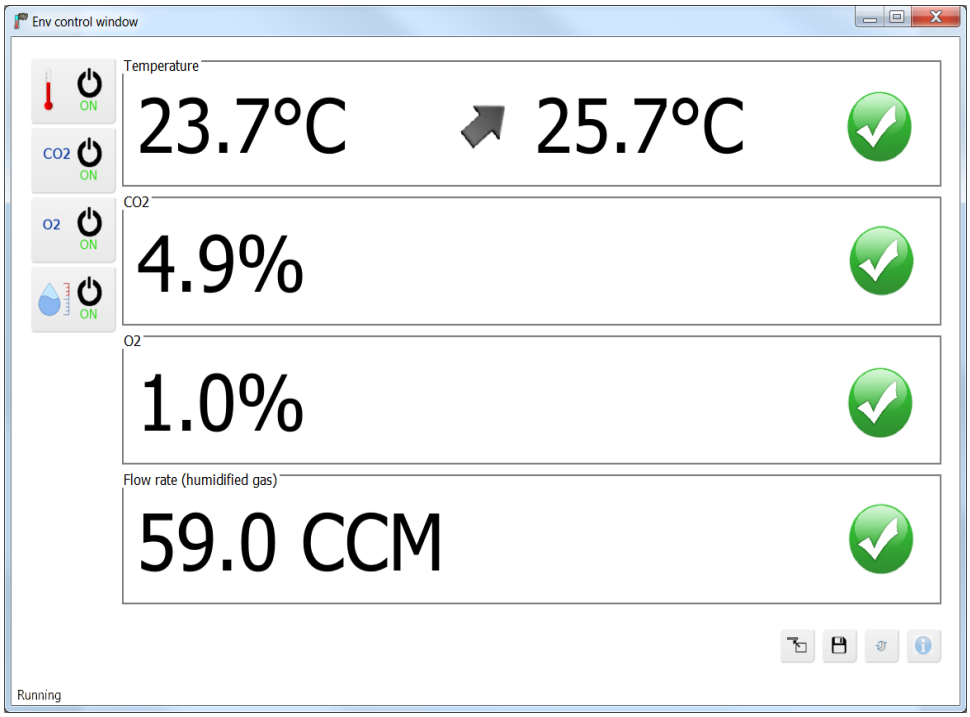
These windows are described in further detail in the following sections.

Main Status Window

The Main Status Window provides a view of the temperature, gas concentration, and flow rate.

Note: The **O2** button will only be displayed if your system is configured with the CO_2/O_2 version of the Live Cell Imaging Module.

Figure 3. Main Status Window



The Component Toggle Bar on the left side of the window allows you to turn the temperature control, CO_2 flow, O_2 flow, and humidifier heater on and off independently.

The Humidifier toggle 

controls the humidifier heater and can be turned on and off only. The humidifier temperature set point is fixed at a temperature that has been shown to provide optimal humidification to the sample.

When live cell components are turned on, current values are displayed to provide real-time feedback. Controls that are turned off display dashes only. The temperature row displays two values. The value on the left displays the heated stage temperature and the value on the right displays the live cell sample lid temperature. These values represent the actual temperatures measured at sensors positioned on the stage and lid. A thermodynamic model is used to determine the target temperature for these sensors so that the desired set point temperature is achieved at the sample. It is normal for neither of these temperature values to exactly match the desired sample temperature value defined in the Settings Window. See previous figure.

Settings Window

The Settings Window allows you to define set points for temperature, gas concentration, and flow rate. Additionally, warning levels can be defined for gas concentration and temperature. When the temperature or gas concentration values move out of the range, a warning will be displayed in the Main Status Window.

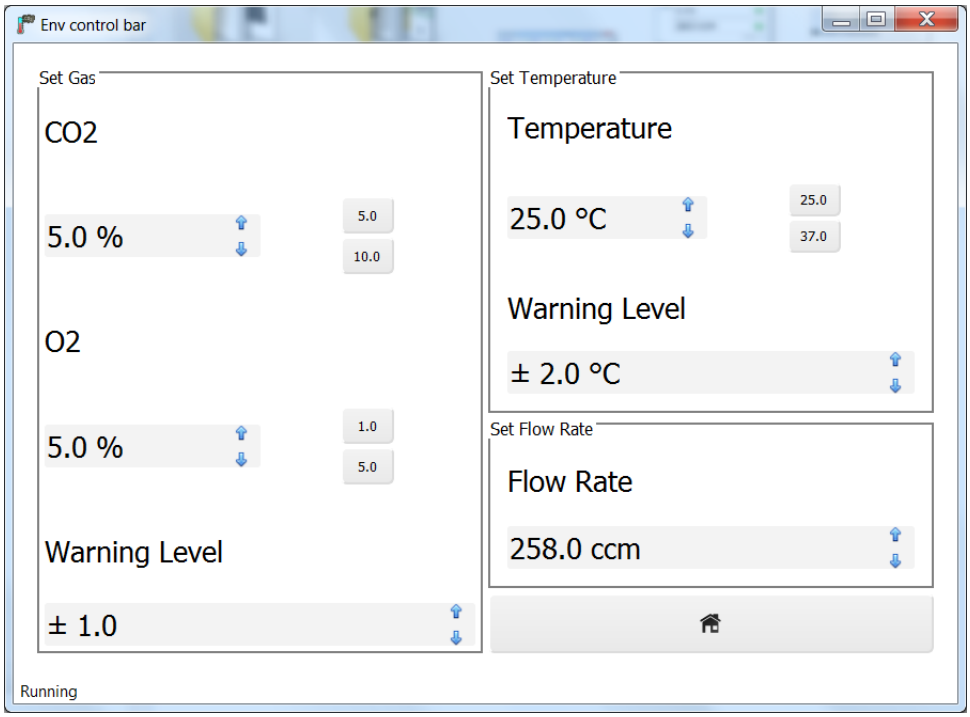
To display the Settings Window:

➢ Click any of the values in the Main Status Window or Status Bar.

To return to the Main Status Window or Status Bar:

➢ Click **Home**  in the Settings Window. The software will return to the Main Status Window or the Status Bar, whichever was originally displayed.

Figure 4. Settings Window



For additional information regarding the Environmental Control software settings, buttons, and features, refer to "Appendix C: The IN Cell Environmental Control Software - Additional Details" on page 18.

Upgrading the Environmental Control Software

The IN Cell Environmental Control Software is periodically updated. To upgrade your version of the software, follow these instructions:

1. Verify that the IN Cell Analyzer system is on.
2. Go to http://incelldownload.gehealthcare.com/download_data/incell/EC/ec_download_page.htm.
3. Click on incellec-setup-XXXX.exe to download the file.
4. Navigate to your download location and double-click on the executable file to open the installer.
5. Follow the on-screen instructions to install the software.

Running a Live Cell Experiment

❖ ***Tips:***

#1 Wait until the environmental control system equilibrates to the defined conditions before placing the sample plate on the stage.

#2 A breathable membrane seal should be used over the sample plate to control evaporation for experiments that last longer than several hours. Breathable membrane

seals are also recommended for shorter experiments to reduce the risk of contamination. An example of such a membrane can be found at https://www.divbio.com/product/bem-1.

To run a live cell experiment:

1. Ensure that the 250 mL humidifier bottle is filled to 50-75% of its max capacity. This ensures that the diffuser is submerged at all times, without allowing water to splash into the gas output tubing. To check the water level, open the access door on the right side of the IN Cell Analyzer system and slide the insulation sleeve down just far enough to have a clear view of the humidifier bottle. See "Refilling the Humidifier" on page 13 for additional information about how to fill the humidifier.

   CAUTION
   Use caution when using any liquids near or on the system.

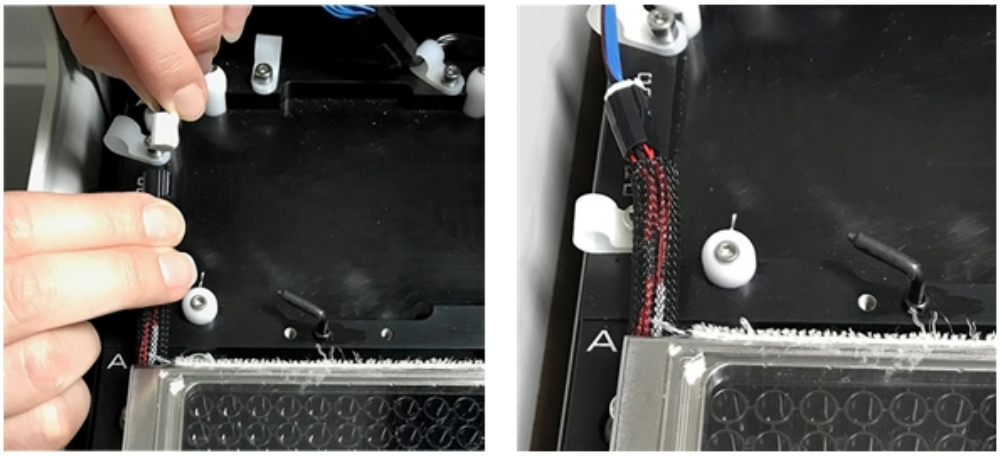
   

   WARNING
   If any liquid is spilled on or around the instrument electronics, unplug the instrument immediately and wipe up the spill. DO NOT PLUG THE SYSTEM INTO ANY POWER MAINS UNTIL THE PROBLEM IS RESOLVED.

2. If your experiment requires gas control, turn on the gas supply.
   - For CO_2 regulation, turn on the 100% CO_2 and CDA, and set pressure at each regulator to 25 psi.

     IMPORTANT: If you have a CO_2/O_2 mixer but are only regulating CO_2 for your experiment, ensure that tubing is connected to the N_2 port on the side of the IN Cell Analyzer system. If not, you must block the port with the provided stainless steel stopper or connect tubing to the port and clamp the tubing. This will prevent gas from leaking from the N_2 port, which could cause inconsistent performance of the CO_2/O_2 mixer.
   - For CO_2 and O_2 regulation, turn on 100% CO_2, 100% N_2, and CDA, and set pressure at each regulator to 25 psi.
3. If your experiment requires temperature control, complete the following steps.
   a. Click **Eject**  on the main toolbar of the IN Cell acquisition software to open the plate access door.
   b. Place a dummy plate onto the stage. Plug the Live Cell Sample Lid's electrical pigtail connector to its outlet.

Figure 5. Connect Electrical Pigtail to Lid



c. Place the Live Cell Sample Lid on top of the dummy plate.

d. Click **Load** on the main toolbar of the IN Cell acquisition software to close the plate access door.

4. In the IN Cell acquisition software, click **Environmental Control** to open the Environmental Control Software.
5. In the Main Status Window, use the Component Toggle bar on the left side of the window to turn the appropriate controls on.
6. Click any of the values in the Main Status Window to open the Settings Window.
7. Ensure that the CO_2, O_2, Temperature, and Flow Rate set points are defined appropriately for your experiment. If required, use the following information to change the set point values.
   - **CO_2.** Type a value into the set point field. Use the up/down arrows or the 5.0/10.0% quick set buttons to change CO_2 concentration. CO_2 concentration can be controlled from 0% to 20%.

     Note: CO_2 is used to keep the culture media at a stable physiological pH (typically 7.2-7.4). 5% CO_2 is commonly used by default; however, media composition and the optimal pH range for a given cell line should be considered if there are indications of suboptimal cell health. Refer to the section "Appendix E: Optimizing CO2 Set Point For Specific Cell Lines" on page 22.
   - **O_2.** Type a value into the set point field. Use the up/down arrows or the 1.0/5.0% quick set buttons to change O_2 concentration. O_2 concentration can be controlled from 0% to 20%.

     Note: For additional information regarding gas mixing for hypoxia applications, refer to the section "Appendix A: Oxygen Concentration Considerations" on page 15.
   - **Temperature.** Type a value into the set point field. Use the up/down arrows or the 25.0/37.0°C quick set buttons to change the temperature. Temperature can be controlled from room temperature to 42°C.

- **Flow Rate.** Type a value into the set point field or use the up/down arrows to set the flow rate in cubic centimeters per minute (ccm). The recommended flow rate for all applications is 25 ccm.

8. Click **Home**  to return to the Main Status Window. If any set point values were changed, it may take a few minutes for the readings in the Main Status Window to match the new set points. An ascending or descending arrow indicates that the value is rising or falling respectively. A green check mark indicates that the measured value matches the defined set point.

   Note: If you are using gas control, the green check mark indicates that the gas concentration coming out of the ECU matches the set point. It is recommended to wait an additional thirty minutes for the rest of the gas delivery system (tubing, humidifier) to equilibrate before loading your sample.

9. Click **Eject**  on the main toolbar of the IN Cell acquisition software to open the plate access door.
10. Remove the dummy plate and load your sample by choosing one of the following

    If using a 96-well plate:

    ➢ Place the plate in the stage opening. If desired, seal the plate with a breathable membrane.

    OR

    If using a 35mm dish, chambered coverglass, or µ-slide adapter:

    a. Load the sample into the appropriate sample holder. If using a 35mm dish or chambered coverglass, secure with the Universal Lid.

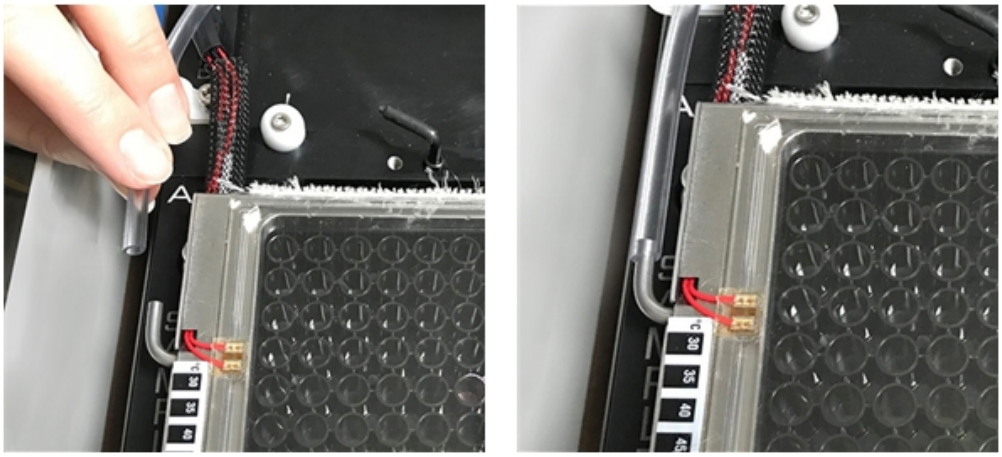
    b. Place the loaded sample holder into the slide adapter.

    c. Place the slide adapter into the stage opening.

    Note: For additional information regarding the specific components of the Sample Holder Kit, refer to the section "Appendix B: IN Cell Sample Holder Kit" on page 16.

11. Install the Live Cell Sample Lid on top of the plate or slide adapter. Ensure that the gasket on the lid is positioned squarely over the rim of the plate or adapter so that the live cell chamber is securely sealed.
12. Disconnect the gas input tubing from its docking location and connect it to the gas input on the Live Cell Sample Lid.

Figure 6. Connect Gas Input Tubing



13. Click **Load** on the main toolbar of the IN Cell acquisition software to close the plate access door.
14. Set up and run your experiment as you normally would.
15. If temperature, CO_2, O_2, or flow rate logs are required, use the **Save** button in the Main Status Window to save this data in a comma-separated-value (CSV) file.
16. When the experiment is complete, turn off the toggle buttons in the EC software and turn off the gases at the source.

Refilling the Humidifier

CAUTION
Use caution when using any liquids near or on the system.

WARNING
If any liquid is spilled on or around the instrument electronics, unplug the instrument immediately and wipe up the spill. DO NOT PLUG THE SYSTEM INTO ANY POWER MAINS UNTIL THE PROBLEM IS RESOLVED.

❖ ***Tip:***

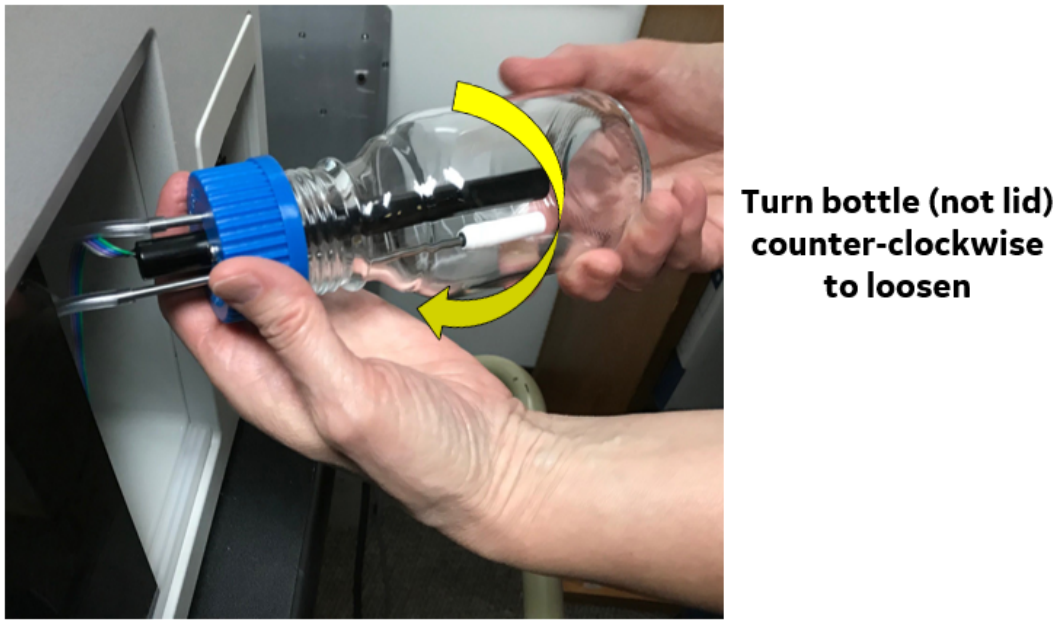
Use sterile, deionized water in the humidifier to prevent accumulation of impurities on the diffuser (white component attached to the bottom of the humidifier lid). Discoloration on the diffuser indicates contamination which may impede gas flow. Contact your GE representative to order a new diffuser.

1. In the Main Status Window of the Environmental Control software, turn off the CO_2, O_2 (if applicable), and Humidifier heater controls.
2. Slide open the access door on the right side of the IN Cell system to access the humidifier.

3. Remove the insulation sleeve from the humidifier by sliding it down the bottle.
4. Open the bottle by turning the glass bottle instead of turning the lid. This helps ensure that the cables do not become tangled and/or break.

   Note: Do not touch the diffuser. Oil and particulates from your hands can damage the diffuser.

Figure 7. Remove Lid (heater and bubbler attached)



5. Fill the bottle about 50% full with sterile, deionized water.
6. Replace the lid by turning the glass bottle instead of turning the lid. Once again, this helps ensure the cables do not become tangled.
7. Replace the insulation sleeve and place the humidifier back into the compartment on the right side of the IN Cell Analyzer system.

Figure 8. Replace Insulation Sleeve and Lid

After refilling the humidifier, it takes about five minutes for the water in the humidifier to return to temperature. Once the water is at temperature, the system is ready to scan a sample.

Appendix A: Oxygen Concentration Considerations

This appendix contains additional information related to regulating O_2 concentration on IN Cell Analyzer 2500HS and 6500HS systems. This section is only relevant for systems with CO_2/O_2 mixers.

Maximum Achievable O_2 Concentration

Maximum achievable O_2 concentration is governed by the O_2 concentration in the CDA and the CO_2 concentration required at the sample.

Of the three inputs on the CO_2/O_2 mixer, only one, CDA, contains O_2. This means that the maximum O_2concentration that can be achieved at the sample will be the same or slightly lower than the O_2 concentration in the CDA.

Maximum O_2 concentration is achieved when the O_2 setpoint is set to 20% and the CO_2 setpoint is set to 0%. In this situation, the ECU would draw in 100% CDA and O_2 concentration at the sample would be the same or slightly lower than the O_2 concentration in the CDA. As the CO_2 setpoint increases, the ECU must draw in a mix of CO_2 and CDA, thus lowering the maximum achievable O_2 concentration at the sample.

See the following table for a few examples of how the CO_2 setpoint affects maximum O_2 concentration at the sample.

Table 3. CO_2/O_2 Setpoint Examples (Assumes O_2 Concentration in CDA is 20%)

O_2 Setpoint	CO_2 Setpoint	Gas Drawn Into ECU		O_2 Concentration at Sample
		CDA	CO_2	
20%	0%	100%	0%	~20%
20%	5%	95%	5%	~19%
20%	10%	90%	10%	~18%

Regulating O_2 Concentration

When attempting to achieve O_2 concentrations lower than what the CDA contains, N_2 is used to displace the air and consequently the O_2.

Appendix B: IN Cell Sample Holder Kit

The IN Cell Sample Holder Kit (GE Part No. 29254209) may be purchased for use with the Live Cell Imaging Module. Though the components of the kit are not required to use the Live Cell Imaging Module, they are designed to minimize gas leakage when imaging 35mm dishes, multi-well chambered slides, and µ-slides.

The kit components are described in the rest of this appendix.

Slide Adapter

The Slide Adapter files directly into the IN Cell stage to allow for slide, dish, or chambered slide imaging. The slide adapter is designed for use with any of the sample holders.

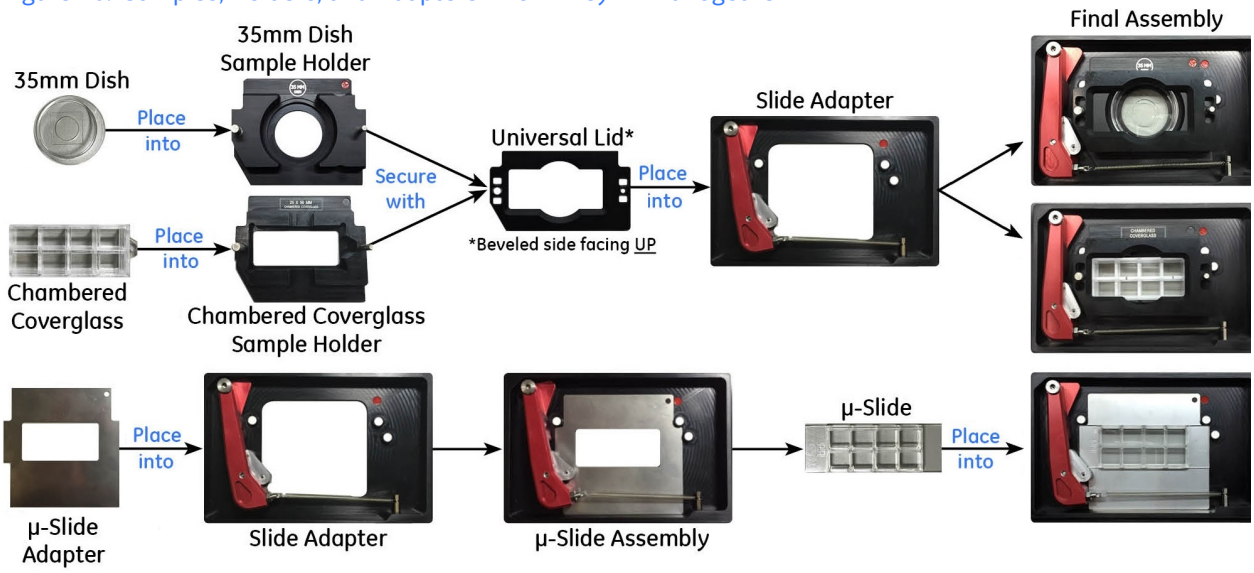
Figure 9. Slide Adapter

Table 4. Sample Holders in the IN Cell Sample Holder Kit

Component	Name	Function
	35mm Dish Sample Holder	Holds 35mm sample dishes in place throughout an experiment.
	Chambered Coverglass Sample Holder	Holds chambered coverglass samples in place throughout an experiment.
	Universal Lid	Used with the 35mm Dish and Chambered Coverglass Sample Holders to secure the sample in the holder throughout an experiment.
	µ-Slide Adapter	Holds µ-slides in place within the Slide Adapter throughout an experiment.

The following figure shows how your samples and the components of the IN Cell Sample Holder Kit all fit together.

Figure 10. Samples, Holders, and Adapters - How They All Fit Together



Appendix C: The IN Cell Environmental Control Software - Additional Details

Figure 11. Main Status Window

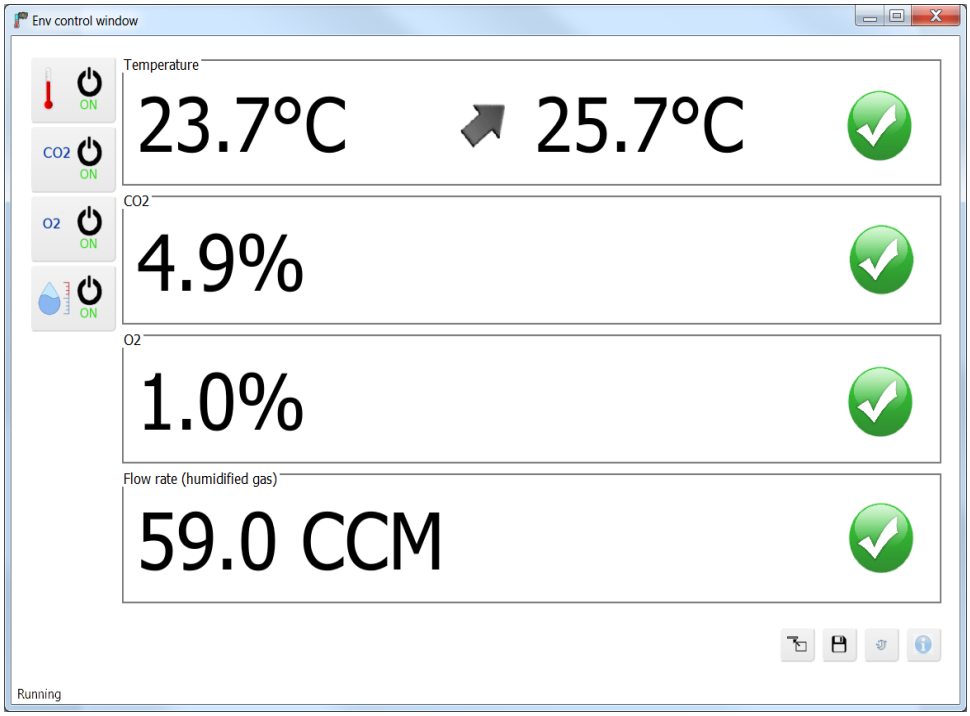
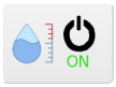


Table 5. Components of the Main Status Window

Icon/Button	Name	Function
	Temperature Toggle Button	Turns on/off the heating elements in the stage base plate and the live cell sample lid.
	Carbon Dioxide Toggle Button	Turns on/off flow of CO_2 and CDA to regulate CO_2 concentration at the sample.
	Oxygen Toggle Button	Turns on/off flow of N_2 and CDA to regulate O_2 concentration at the sample. (CO_2/O_2 configuration only)
	Humidifier Toggle Button	Turns on/off the humidifier heater to warm the water in the humidifier.
	Stable Value	Signifies the value equals the defined set point value and is stable.
	Measurement Rising	Measurement is currently rising to set point value.
	Measurement Lowering	Measurement is currently lowering to set point value.
	Caution and Error(s)	Yellow symbol denotes errors. Red box defines error count (usually 01-09). +10 displayed in the red box means that more than 10 errors have occurred. Errors occur when temperature or gas is out of range and when any other errors occur during operation. ➢ Click the red box to display a dialog box showing the time(s) and value(s) when the error(s) occurred. The error dialog box will remain open on the screen until you click **Reset**.

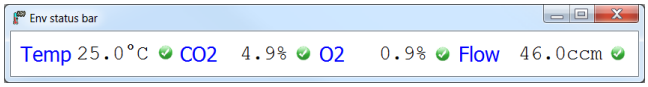
Icon/Button	Name	Function
	Status Bar	Minimizes the Main Status Window to a single-row Status Bar.  ➢ Click the **Status Bar** button to switch the Main Status Window to a single-row status bar. (The Status Bar will always be displayed on top of all other applications open on the screen. This is not an option with the Main Status Window.) Click anywhere in the Status Bar to return to the full size window. If a red error box is displayed on the Status Bar, click it to display the time(s) and value(s) when the error(s) occurred.
	Save	Opens a Save dialog box that saves log files containing temperature, CO_2, O_2, and flow rate data in a comma separated value (CSV) file.
	Day/Night Mode Toggle	Switches the Live Cell Imaging Module software's background screen color from white to black (or black to white) for viewing in varied light situations.
	Info	Displays a dialog box with the software's current version and copyright information.

Figure 12. Settings Window

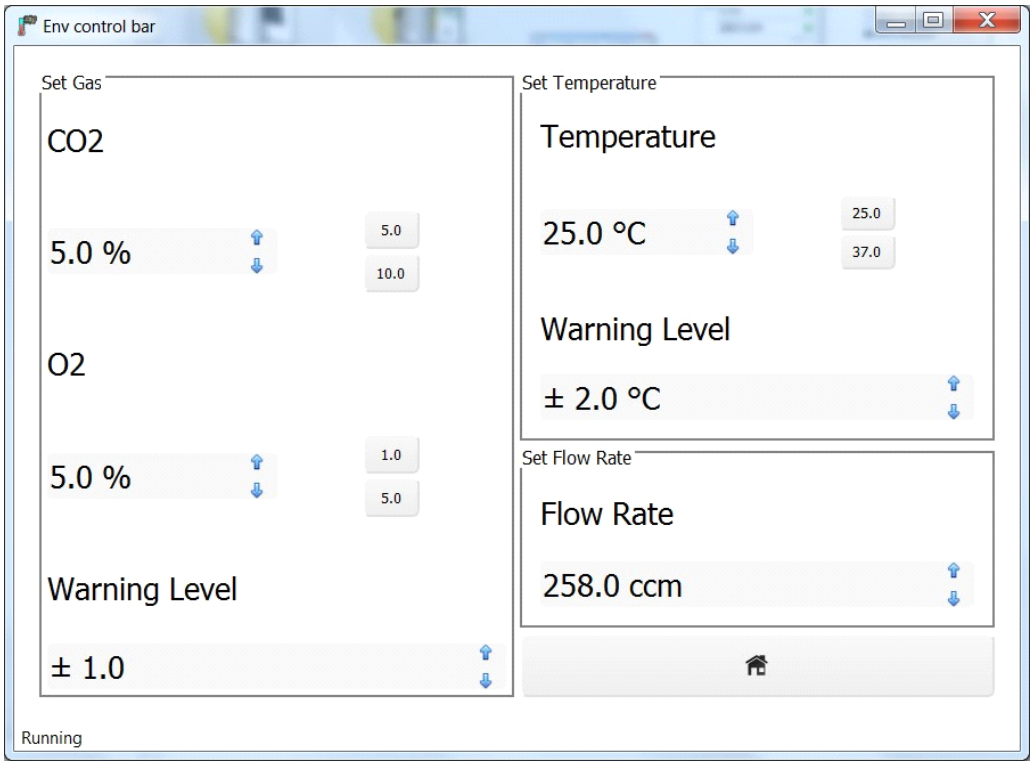
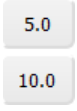


Table 6. Components of the Settings Window

Control	Range	Function
Set Gas - CO_2	0 - 20%	Specifies the percentage of CO_2 for the gas mixture in the sample area.
Set Gas - O_2	0-20%	Specifies the percentage of O_2 for the gas mixture in the sample area.
Set Gas - Warning Level	±0.1 to ±1.0	Specifies the gas accuracy range. If a gas value moves out of this range, an error will display.
	n/a	**Increase/Decrease Set Point Value.** In addition to typing a value directly into a data-entry field, you can click these up and down arrows to increase/decrease the selected value.
	Preset Values	**Quick Set Buttons.** Save time by clicking these buttons to quickly enter the value displayed on the button into the data-entry field.
Set Temperature	Ambient to 42°C	Specifies the desired temperature for the sample area.

Control	Range	Function
Set Temperature - Warning Level	±0.3 to ±3.0°C	Specifies the temperature accuracy range. If the temperature value moves out of this range, an error will display.
Set Flow Rate	0.0 - 800.0 ccm	Specifies the flow rate of the mixed gas into the sample area. The default flow rate is 25 CCM.
Home	n/a	Returns to the Main Status Window.

Appendix D: Cleaning

Glass

To clean the glass bottle on the humidifier, remove the lid as stated in "Refilling the Humidifier" on page 13, by twisting the bottle and not the lid. Wash the bottle using hot water and laboratory-glass-washing detergent, followed by three rinses in sterile, deionized water. Avoid touching the diffuser since debris can cause it to deteriorate. Clean the glass of the Live Cell Sample Lid (inside and outside) gently with 80% ethanol. Do not put too much pressure on the glass surface during cleaning.

Gas Tubing

To ensure gas flows freely during the use of the CO_2/O_2 components, water must not be allowed to pool inside the tubing. During use and cleaning, ensure that water does not enter the tubing.

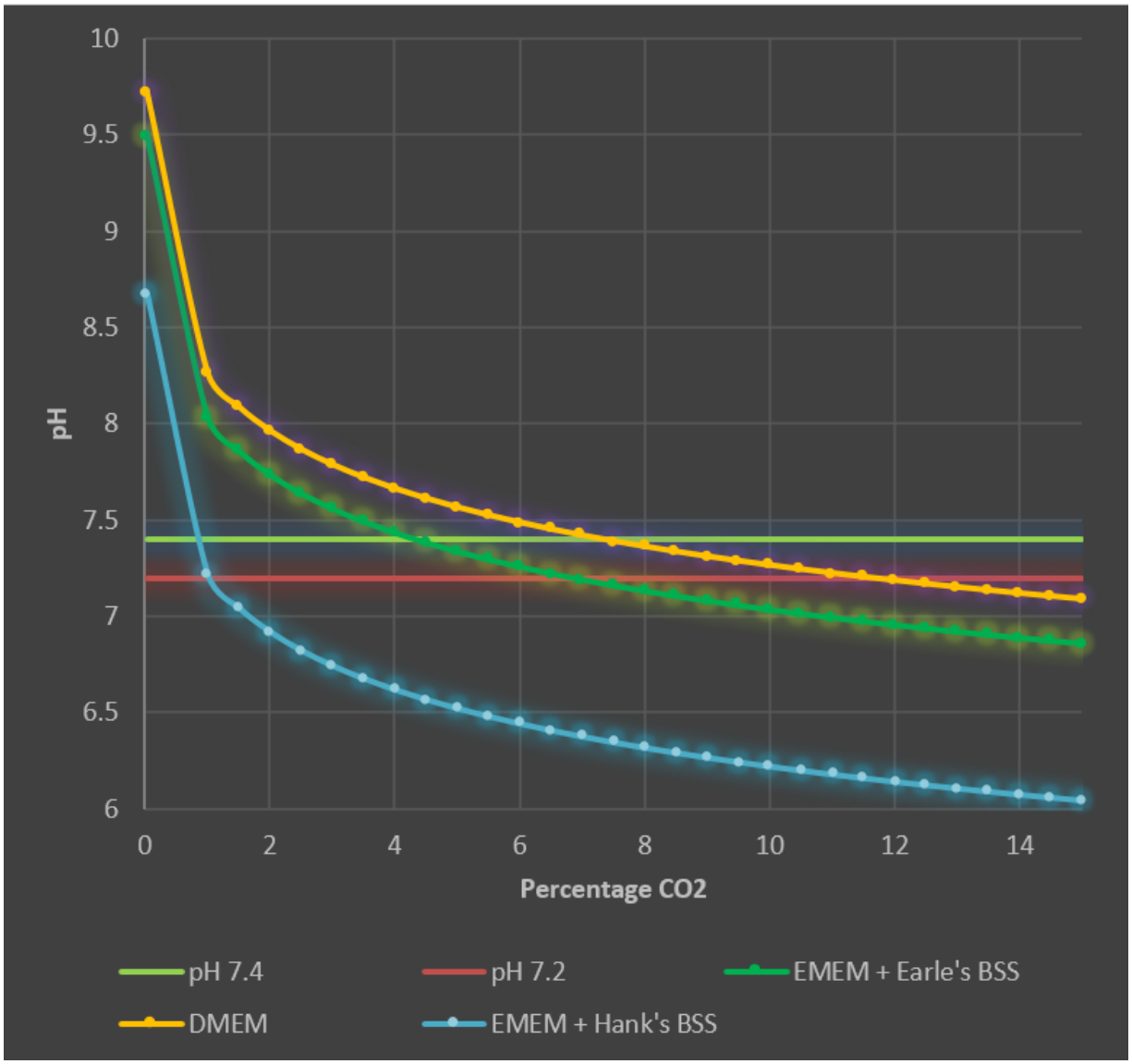
Appendix E: Optimizing CO_2 Set Point For Specific Cell Lines

CO_2 is not a metabolic requirement for cell cultures. The purpose of CO_2 is to dissolve into the cell culture medium where a small proportion of it reacts with water to form carbonic acid. The carbonic acid then interacts with its conjugate base (the dissolved bicarbonate ions in the medium) to control a stable physiological pH through the bicarbonate buffering system.

The amount of sodium bicarbonate (NaHCO3) in the medium dictates the amount of CO_2 that should be used to maintain the pH. Use the following figure as a guide for determining the appropriate CO_2 set point based on the amount of sodium bicarbonate for a given media.

Source: https://www.phe-culturecollections.org.uk/news/ecacc-news/co2-concentration-and-ph-control-in-the-cell-culture-laboratory.aspx

Figure 13. Theoretical pH Ranges for Different Culture Media



Theoretical pH ranges for different culture media in increasing CO_2 levels in a 37°C humidified incubator. The lower and upper physiological range of pH is denoted by the red and green horizontal lines (pH 7.2-7.4). DMEM (44 mM $NaHCO_3$, orange line) is held at a physiological pH at CO_2 concentrations between 7.5% and 11%. Using DMEM in a 5% CO_2 environment will result in a pH of 7.5 which, although acceptable for most cell cultures, is slightly outside the desired range of physiological pH. EMEM + Earle's Balanced Salt Solution (BSS) (25 mM $NaHCO_3$, dark green line) is held at physiological pH with CO_2 levels between 4.5% and 6.5%, whereas a much lower percentage of CO_2 (near atmospheric levels) is required for EMEM + Hank's BSS (4 mM NaCHO3, blue line).

The appropriate physiological pH is typically in the range of 7.2-7.4. Some cell lines, however, require a pH outside this range. Refer to the cell lines datasheet or other associated documentation to determine the optimal pH range and CO_2 concentration recommended for culture media.

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