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H301-T-UNIT-BL-PLUS

Electric Top Stage Incubation System

bold line

Manual

Vers. 02.14

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Index

1 Revision table

Revision Number	Additions or changes	Date	Editor
01	Edited	October 2014	Amelia Liccardo
02	Review	November 2014	Silvia Foppiano Amelia Liccardo

2 Preface

The **H301-T-UNIT-BL-PLUS** is designed to maintain all the required thermal conditions for live cell imaging; hence it is ideal for time-lapse imaging. It controls the temperature in the range of 25-50°C. The system is composed of the main unit, **H301-T-UNIT-BL-PLUS**, a fine gauge thermocouple to monitor the temperature of a blank sample or Reference Well located in close proximity to the experimental one, a thermistor to monitor Room Temperature, a micro environmental chamber 'incubator' and the Touch Screen Operation Board (OKO-TOUCH). An Objective Heater must be included when using oil immersion objectives. The Control unit **H301-T-UNIT-BL-PLUS** is compatible with any Bold Line Gas Controller; Temperature and Gas controllers are stackable and connect through a bus connector (see paragraph 8). The Touch Screen interface (OKO-TOUCH) must be connected via RS232 serial cable to the any one of the Control Units on the 'stack' and it allows controlling the complete system.

3 Symbols descriptions

3.1.1 Symbols in this manual and on the product:

The following symbols identify important information to note:

CAUTION or WARNING: This symbol warns you about the risk of electrical shock.

CAUTION or WARNING or IMPORTANT: This symbol warns you of circumstances or practices that can affect the instrument's functionality and must refer to accompanying documents.

Tip ► Supplies you with helpful suggestions.

Note ► Supplies you with important information to successfully setup and use the instrument.

3.1.2 Symbols on the product label:

CE MARKING: This symbol indicates a product's compliance with EU legislation.

PRODUCT DISPOSAL: This symbol indicates that this product must not be disposed as urban solid waste.

IP 30

This symbol indicates the protection degree against ingress of solids or liquids inside the product.

4 Safety Notes

Before operating the equipment please read carefully the instructions and the safety notes. If you have any questions, please contact Okolab.

The equipment must only be used as intended and as described in this Manual.

Equipment should only be operated by technically qualified personnel.

Do not start up the equipment if some of its parts are damaged.

This instrument is not intended for use in locations where flammable or explosive gases are present.

Handle the equipment with care.

Equipment and its internal parts can be damaged by dropping and by shock.

Do not use a volatile solvent such as paint thinner to clean the instrument. Deformation or discoloration will occur. Use a soft, dry cloth to remove stains from the instrument. Not following these instructions can result in damage or breakdown of the device and its accessories.

The products labels can be found on the bottom panel of the Main Unit.

Do not exceed voltage indicated in this manual and on the product label.

Avoid excessive induction noise, static electricity, magnetic fields.

Do not expose this instrument to rain or moisture.

Prevent throttling and kinking of tubing.

Check the cables are well fixed to their own connectors so they cannot slip off

This device is not designed for use under medical conditions.

Power cord of unit should be unplugged from electrical outlet when left unused for long period of time.

VENTILATION, unit should be situated so that its' location or position does not interfere with proper ventilation.

Unit should be situated away from heat sources such as radiators, heat registers, stoves, or other appliances or processes that produce heat

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Do not start up the equipment if the supply cable is damaged.

Connect the equipment only to grounded mains power socket.

Do not disconnect cables while in operation.

Do not open the unit. Do not remove cover or back.

Prevent metal fragments or lead wire scraps from falling inside instrument case to avoid electric shock, fire or malfunction.

No user serviceable parts inside.

Before starting, assemble the equipment while unplugged from an outlet.

Unit should never be used where it can fall or be pushed into water.

International caution symbol marks this device. IMPORTANT: read the "Safety Notes" before installing, using and commissioning this device, as the notes contain important information relating to safety and EMC. Not following these instructions can result in damage or breakdown of the device and its accessories.

We reserve the right to make technical variations.

IN NO EVENT SHALL OKOLAB S.R.L. BE LIABLE FOR ANY DIRECT, INCIDENTAL OR CONSEQUENTIAL DAMAGES OF ANY NATURE, LOSSES OR EXPENSES RESULTING FROM ANY DEFECTIVE PRODUCT OR THE USE OF ANY PRODUCT.

5 AC section wiring diagram

Figure 1 shows the alternating current (AC) section, designed to connect internal power supplies (see Figure 1 label C) in parallel to low voltage power grid.

A. **Power Connector,** OM-SP8210263
B. **AC-DC Relay**, Crydom DRA1-CX240D5.
C. **Power supplies**, TDK-Lambda-ZWS150-24 and ZWS5-5.

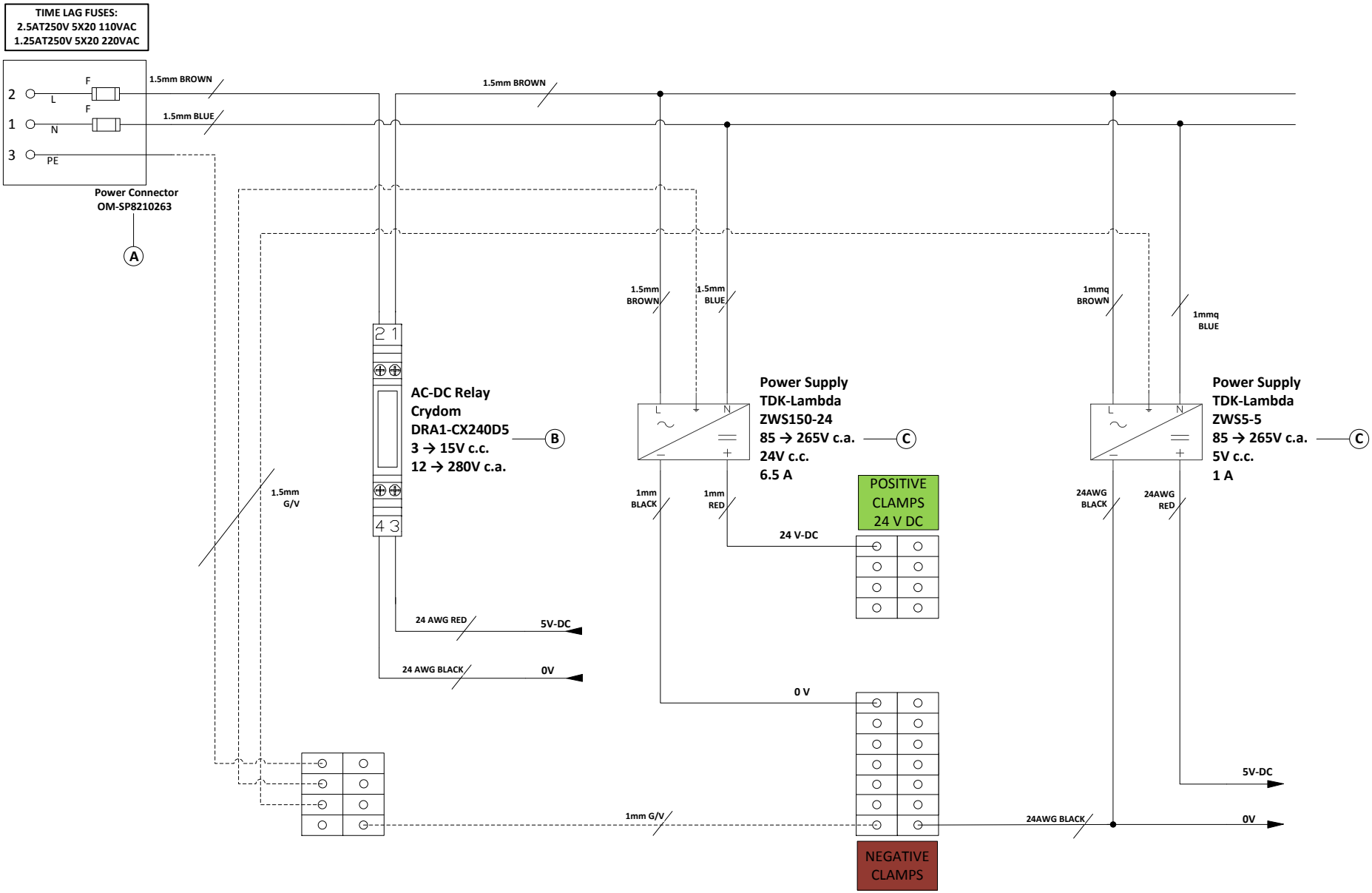


Figure 1 AC section wiring diagram

6 Equipment and connections

6.1 Equipment supplied

H301-T-UNIT-BL-PLUS includes:

1. **T-Unit**
2. **Fine Gauge Thermocouple**. To measure specimen temperature
3. **NTC Thermistor**.To measure ambient temperature
4. **#1 power supply cable**.

It must be equipped with:

A. **OKO-TOUCH**

1. -**Touch Screen Interface**
2. **#1 5V-DC Power adapter**. To connect the Touch Screen.
3. **#1 RS232 Serial cable**. To connect the Touch Screen to the T-Unit

B. **Incubating chamber** with heated lid, chamber riser and one or more plate adapters

Optional accessories:

Objective heater. Required ONLY when using oil immersion objectives.

SmartBox . Data logger, web server, remote assistant

1. SmartBox Unit
2. #1 USB cable. To connect the SmartBox to the T-Unit (optional)

7 ~~Digital~~ Temperature Controller connection ports.

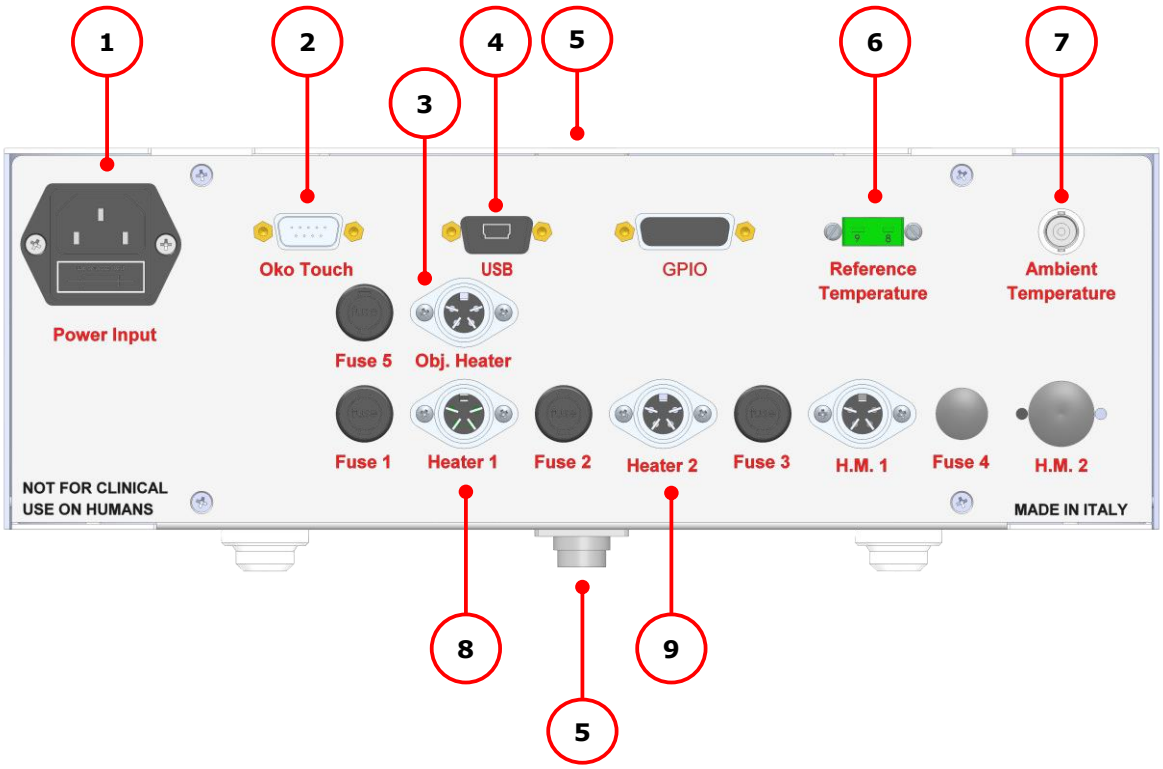
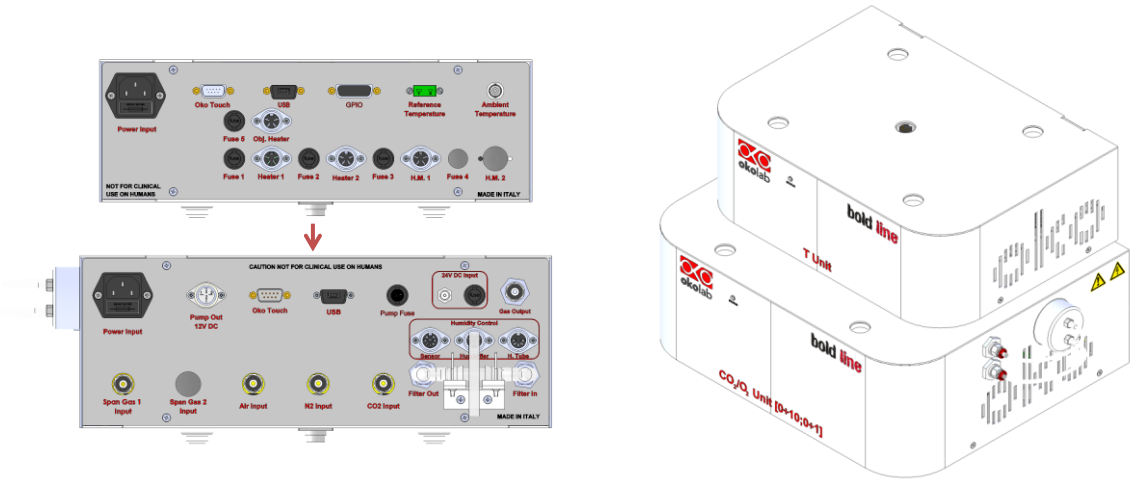


Figure 2: Control Unit Rear Panel Overview.

1. Power Input.
2. OKO-TOUCH RS232 serial port.
3. Objective Heater connector (only if you have ordered also an OBJ-COLLAR-####)
4. USB port.
5. 5-pole bus port used to connect digital controllers to each other by stacking them.
6. Gauge Thermocouple port.
7. NTC Thermistor port.
8. Chamber base connector.
9. Lid chamber connector.

8 How to connect H301-T-UNIT-BL-PLUS with a BOLD LINE Digital Gas Controller

The H301-T-UNIT-BL-PLUS can be used as a stand-alone device or in combination with any one of the Bold Line Gas Controllers. Figure 3~~Figure 3~~ shows how to assemble the H301-T UNIT-BL-PLUS, for example, with a CO2-O2 Unit BL.



a. CO2-O2 UNIT-BL [0-10; 0-1] and H301-T-UNIT-BL. Units rear panels.

b. CO2-O2 UNIT-BL [0-10; 0-1] and H301-T-UNIT-BL. 3D view.

Figure 3. CO2-O2 UNIT-BL [0-10; 0-1] and H301-T-UNIT-BL.

The T-Unit and Gas Controller must stack on top of each other by lining up the bus ports located on the top and bottom surface of each. When the units are properly connected the Temperature, CO2 and O2 parameters will appear on the OKO-TOUCH Touch Screen interface Home page. If not all the parameters appear on OKO-TOUCH Home Page then the Control Units are not properly connected. Please check that the bus ports are properly aligned with each other.

If you have a Gas Controller and a Temperature Control Unit, it is essential to read both this manual and the Gas Controller Manual to familiarize yourself with the functions and the operation of the devices before use.

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9 H301-T-UNIT-BL-PLUS. Installation and user guide

The following paragraphs illustrate how to install and use the Temperature Control Unit.

9.1 H301-T-UNIT-BL-PLUS. Installation guide

The following instructions will guide you through a quick installation. For safe operation of the unit it is absolutely necessary to read carefully all the instructions and safety notes**.**

1. Connect the base of the incubation chamber to the port labeled 'Heater 1' on the rear panel of the Temperature Control Unit (see Figure 4).
2. Connect the lid of the incubation chamber to the port labeled 'Heater 2' on the rear panel of the Temperature Control Unit (see Figure 4).

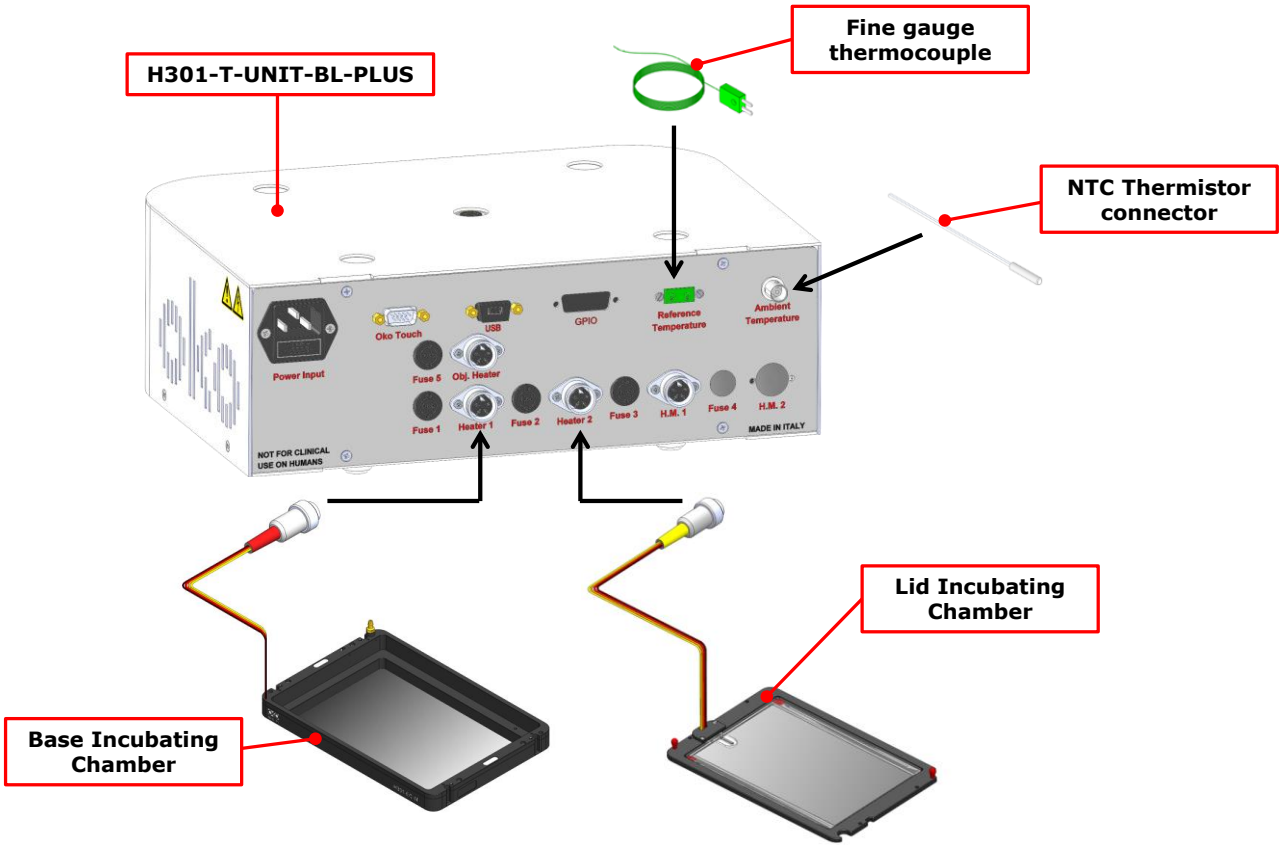

Figure 4. H301-T-BL-PLUS. Standard Connection.

3. Plug the NTC Connector on the port labeled 'Ambient Temperature' located on the rear panel of the Temperature Control Unit (see Figure 4). This temperature sensor must be placed in air close to the illumination pillar in proximity to the microscope stage. This thermistor is monitoring the room temperature.

Note ► Stage Top Chambers for most brands/models are available. See www.oko-lab.com for details.

4. The Fine Gauge Thermocouple (green) plugs into the green port labeled 'Reference Temperature' located on the rear panel of the Temperature Control Unit (see Figure 4). When operating the device in 'Sample Feedback Mode' place this thermocouple in a Reference Well filled with water. Make sure that the end of the Fine Gauge Thermocouple is fully immersed.

Tip ► The Fine Gauge Thermocouple may be placed into a Reference Well if running the "Sample Feedback Mode" (see paragraph 9.2.5.2). The Fine Gauge Thermocouple may also be used to calibrate the Stage Top Incubation Chamber and/or the Objective Heater (OBJ-COLLAR ####), when present. The Fine Gauge Thermocouple may be left in air if the system is operated in 'Chamber feedback mode' (see paragraph 9.2.5.2)

5. When using oil immersion objectives an Objective Heater should also be purchased. If your system is also equipped with the Objective Heater, (OBJ-COLLAR ####, optional) its objective collar should be installed before connecting the heater to rear panel of the Temperature Control Unit. Follow the steps shown in Figure 5. Once you have assembled it, plug it into the port labeled 'Obj. Heater' located on the rear panel of the Temperature Control Unit (see Figure 6).

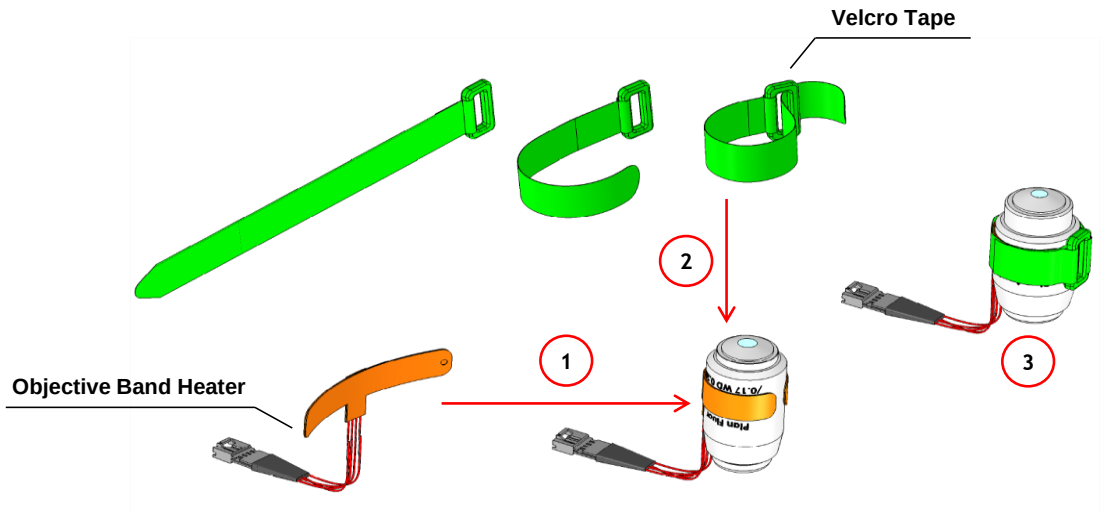

Figure 5.Objective Heater (OBJ-COLLAR ####) Assembly.

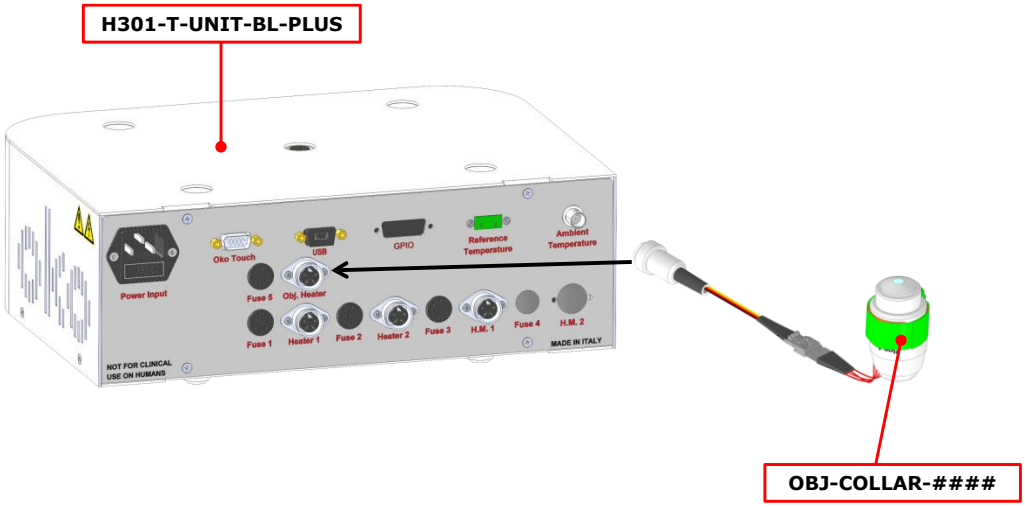

Figure 6. OBJ-COLLAR Connection (Optional).

6. Connect the touch screen interface OKO-TOUCH to the Temperature Control Unit using the serial cable provided (see Figure 7).

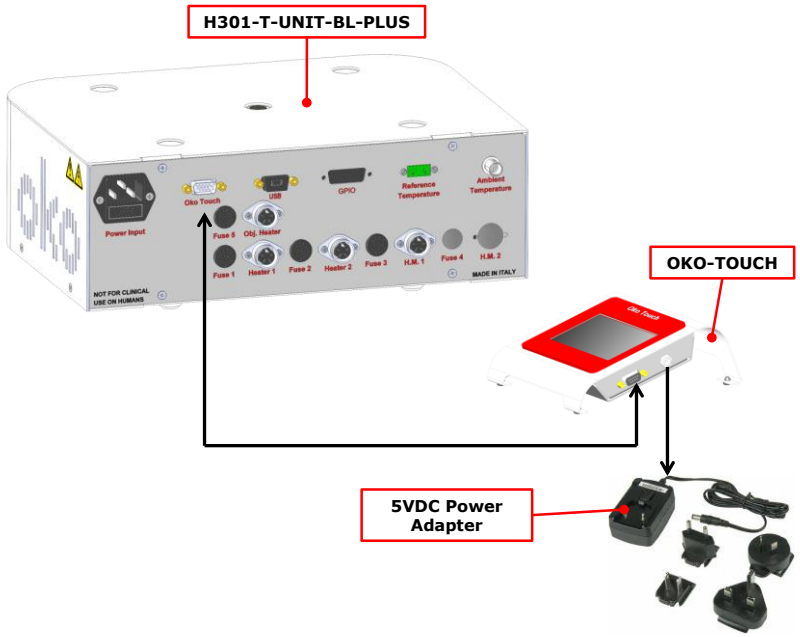

Figure 7. OKO-TOUCH Connection

7. Connect the power adapter to OKO-TOUCH. Connect the additional power cord to the Temperature Control Unit.
8. Turn the OKO-TOUCH on (rocking switch is located on the left side of OKO-TOUCH). A small green LED light will blink on the front of the Temperature Control Unit. This means that the system is initializing and the following screen will appear on the Touch Screen (OKO-TOUCH).

Once the system has been initialized the LED on the Temperature Control Unit will no longer blink and will be steadily on. The OKO-TOUCH monitor will display its homepage and the indicator light on the temperature tab will be initially yellow. When the temperature set point is reached, the indicator light will turn green.

Tip ►Wait until system has reached the steady state before loading your samples.

9.2 Unit operation through Touch Screen Interface OKO-TOUCH.

9.2.1 Home page.

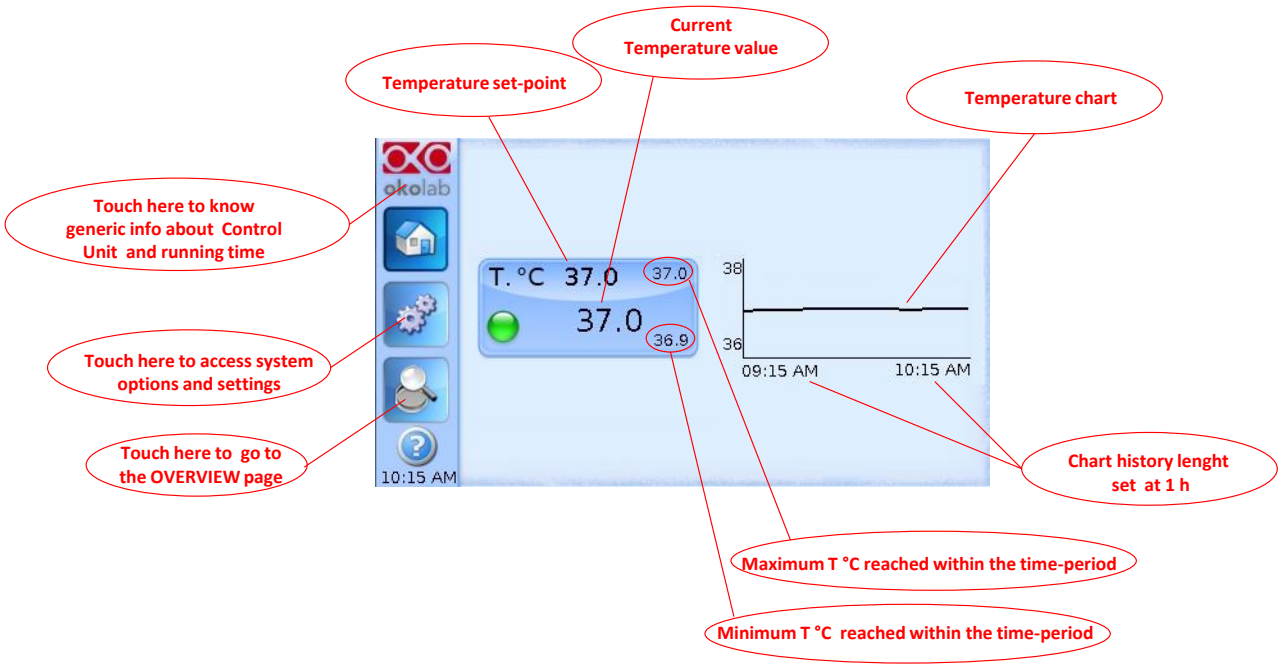

Figure 8. Homepage of the Control Unit Touch Screen Display.

On the right side of the screen you will see the graph of Temperature value over time. The X axis displays the scale of the duration of time you selected (in this example 1 hour). The Y axis, shows the temperature scale.

Tip ►The Touch Screen is pre-set at the following temperature: 37°C. Once you turn it on it will start operating to reach this set-point value.

9.2.2 Colours meaning.

A GREEN indicator means that set-point value has been reached (within the tolerance you've set) and that the system is working properly.

A YELLOW indicator means that the system is working towards reaching set points. NO actions on your part are required. Please note the Yellow light will also appear every time you change the Temperature set point.

An ORANGE indicator means that the current gas concentration is not correct and its value is out of the set tolerance (see section "ALARMS" in paragraph 9.2.4.3). While giving time to the system to recover from this out-of-range value, please double check if there may be reasons not depending on the unit for which it is not properly working. Verify if all cables are correctly connected.

A RED indicator means that something may have been damaged inside the unit. If after few hours the Red light doesn't turn to Green, turn off the system and then turn it on again. If the problem persists, please contact Okolab for assistance http://oko-lab.com/onlineSupport.page

9.2.3 Changing Set Points

To change a set point, simply touch the corresponding tab. A new page will open where you can press + and – to adjust the temperature to the desired value in the range 25-45°C. Press "Set" to save the new set point or "Cancel" to undo.

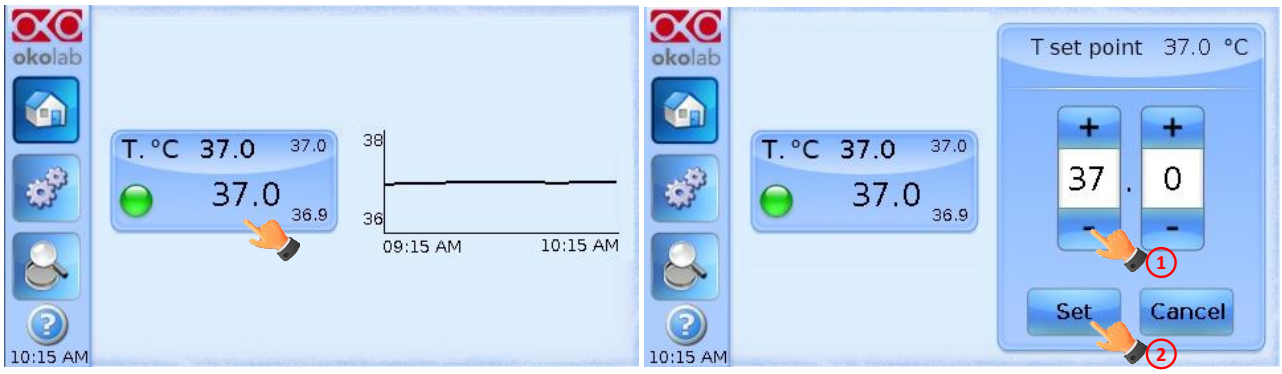

Figure 9. How to change the Temperature set point.

9.2.4 Touch Screen Settings

Touch Screen Settings are adjusted by following the two steps below (look at the touching finger)

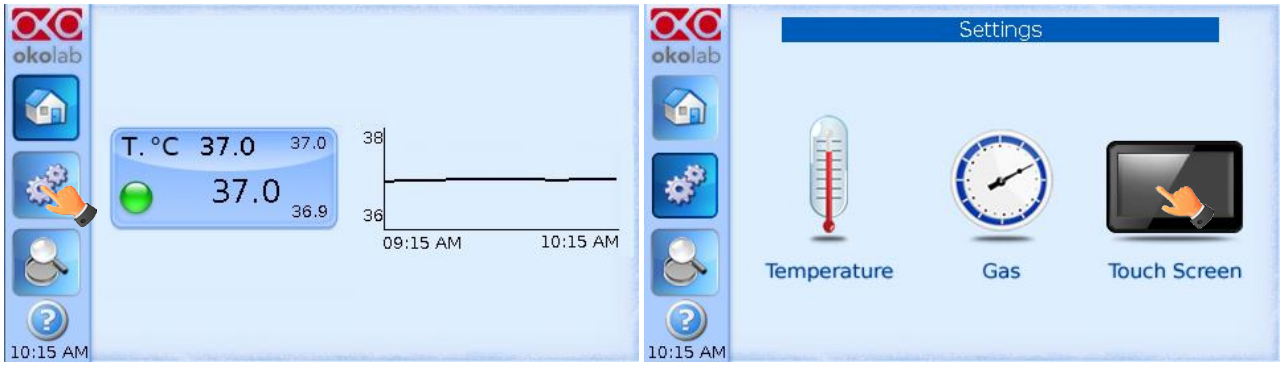

Figure 10.Touch screen settings.

9.2.4.1 Setting Date and Time

The first time you turn the system on, it is important to set date and time, use the + and – to set correct date/time and then press "Save".

Figure 11. Touch screen settings. Date and Time..

Press "Home" (first one on the left menu) to go back to the main page.

9.2.4.2 Options Submenu

In this window you can set the scale of the X axis (time duration) of the graphs displayed in the main page

Figure 12. Chart Options.

Press and slide your finger along the setting bar or simply click +/- to set desired values, then press "Save".

Tip ► Recommended value for the chart history length is 60 or 120 minutes.

9.2.4.3 Alarms settings

This page allows you to set deviation values (from set-point) beyond which you want the system to trigger an Alarm.

Figure 13. How to enter in Alarms page.

Tip ►Flag "Buzzer" if you want the Alarm to be acoustical as well rather than just being displayed.

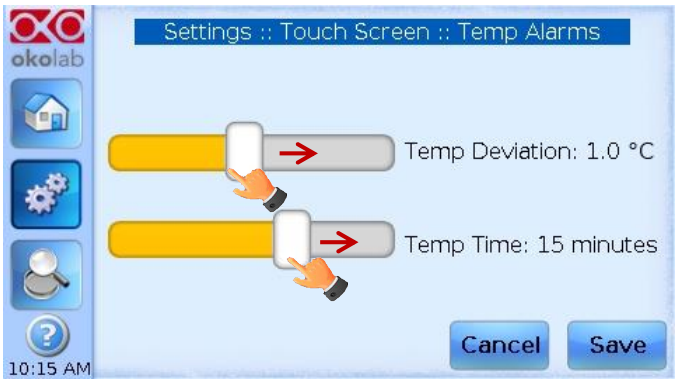

Figure 14. How to set the Deviation and Time Alarm.

Figure 14. In this example the following settings are displayed (valid only after the system has reached its stationary state): if Temperature Deviation from set point is 1°C or greater (i.e. if set point temperature is 37°C and the temperature reaches a value equal or less than 36.5°C or equal or more than 37.5°C) for a period of time equal or longer than 15 minutes ("Temp time" set in this example) then the system triggers an Alarm.

9.2.5 Temperature Settings

Temperature Settings are adjusted by following the two steps below (look at the touching finger)

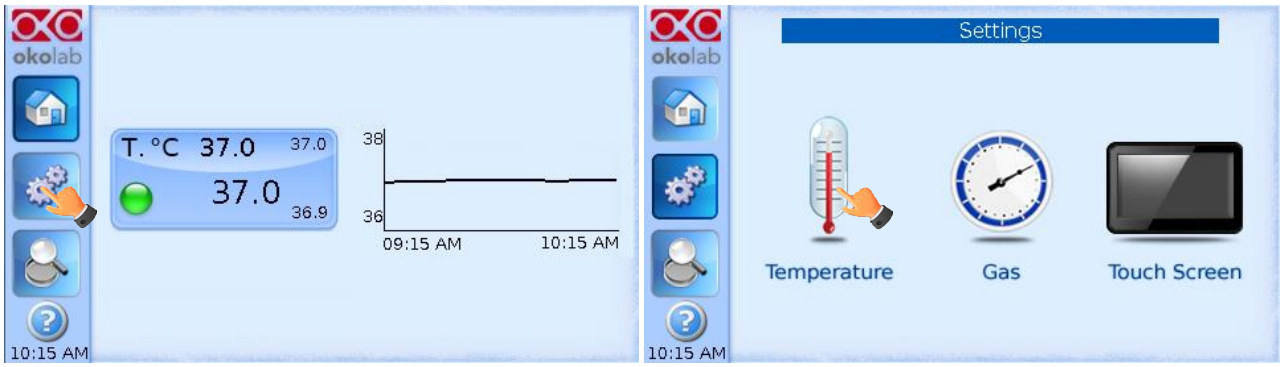

Figure 15. Temperature settings.

9.2.5.1 Chamber And Adapter Settings

Figure 16. Here you have to set the model of incubating chamber (it depends on your XY stage/piezo) that you have purchased and the type of sample adapter being inserted into it. Depending on these selections, the thermal controller will automatically change settings. To switch configuration click "Change" (see Figure 16), then scroll among different chambers using the arrows on the left and right sides of the screen. When the chamber you have purchased appears on the screen press "Next". Repeat this sequence to find and select the sample adapter (see Figure 17) and press "Ok" (see Figure 18). The unit will save these settings.

Figure 16. Chamber & Adapter change.

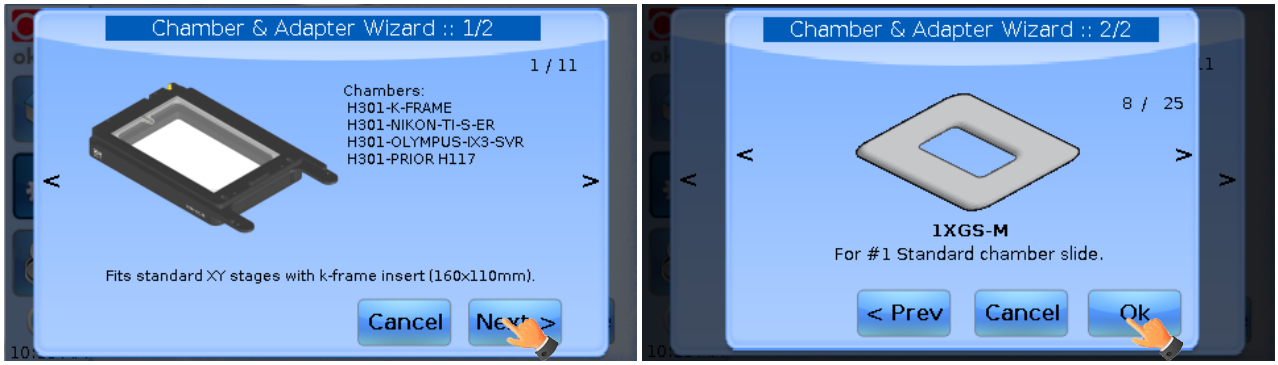

Figure 17 Chamber & Adapter settings.

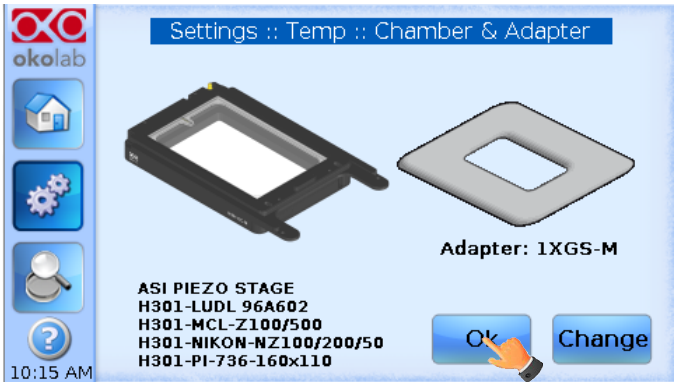

Figure 18. Chamber and adapters saving.

Tip ►The system is shipped from the factory already set according to the incubating chamber model and sample adapter you have ordered. Keep in mind that thermal control settings change when changing the adapter in use. Hence, every time you change adapter you must input the correct one using the procedure above. In case you purchase additional incubating chamber models make sure to input the correct chamber/sample insert in the Setting prior to your experiment.

9.2.5.2 Sample or Chamber Feedback Mode

The Temperature Control can run in two different modalities, please choose which one you want to use, Sample or Chamber Feedback Mode.
Figure 19 shows how to select **Chamber Feedback Mode**.

Figure 19. Chamber Mode Setting.

In this configuration the temperatures of both the lid and the base of the incubating chamber are strictly controlled. A careful calibration performed in our laboratories between these temperatures and that of the sample, guarantees that sample temperature is maintained at the desired set point value. The advantage of this solution is that you don't have to manually place the Fine Gauge Thermocouple into a Reference Well.

Tip ► Advantages of this Control Mode are: fast experiment start up and no specific action required in multi user applications. The disadvantage is that it needs calibration in case of room temperature variations beyond +/- 1 degree Celsius.

When selecting Chamber Feedback Mode the following Screen will appear:

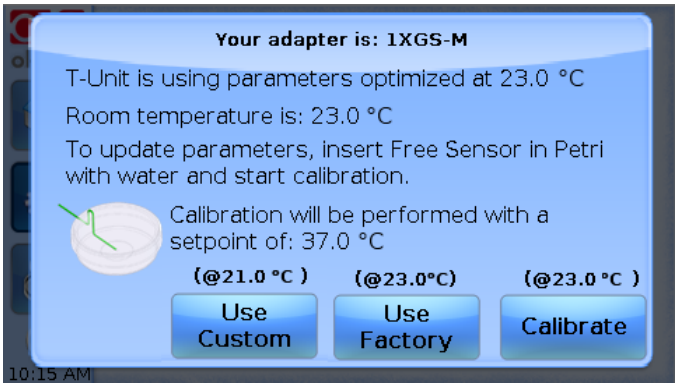

Figure 20. Chamber calibration start.

It states that the system has been calibrated at the factory at a Room Temperature of 23°C. In case your Room Temperature, measured by the included thermistor, is different from 23°C (i.e. 20.3°C) you have 3 options:

1. Use Custom Calibration: this means recalling the last saved calibration settings. Once you calibrate the system (see point 3 below) these settings will be automatically stored. The system saves only the last calibration performed. Please note that calibration depends on i) set-point temperature; ii) room temperature; iii) type of sample adapter in use. Hence using 'Custom Calibration' is NOT recommended in any of the following as it will yield inaccurate results:

A. If the recalled calibration was performed with a different sample adapter than what you intend to use
B. If the room temperature on the Custom Calibration is significantly different than the current one (i.e. more than 1°C)
C. If the Custom Calibration was performed with a different set point temperature than what you plan on using (e.g Custom Calibration had set point of 37C but new experiment requires a set point temperature of 39C).

2. Use Factory settings optimized at 23°C Room Temperature. This is acceptable if your room temperature is within 1°C from 23°C. However, for room temperature more than 1°C from 23°C this may be not acceptable depending on the sensitivity of your sample.
3. For maximum of accuracy when using "Chamber Feedback Mode", you can calibrate the system in your lab at your Room Temperature. To do so follow the instructions on paragraph 10 (Chamber Self-Calibration) and then press the "Calibrate" button; the automatic calibration procedure will start.

Tip ► Note please it may take 1-2 hours to complete the calibration.

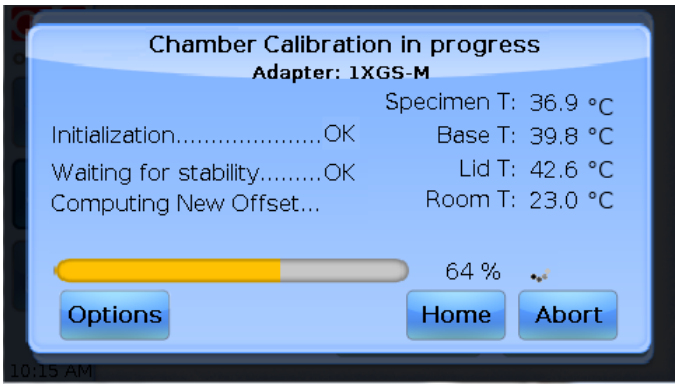

Figure 21. Chamber calibration progress

Just wait until the following dialog box appears:

Figure 22. Chamber Calibration result.

Figure 23~~Figure 23~~ shows how to select operating in **Sample Feedback Mode**.

Figure 23. Sample Mode setting.

In this configuration, every time you run an experiment, you must place the Fine Gauge Thermocouple (thin green wire) into a Petri dish or well adjacent to your sample (from here on this will be referred to as Reference Well). Fill the Reference Well with distilled water to a level allowing full immersion of the fine gauge thermocouple (see Figure 44). When possible close the petri with its own plastic lid (or with SENSOR LID, see **Tip** in paragraph 10) and place it in the appropriate holder. Using a Reference Well in close proximity to your Experimental Well provides active Sample Feedback Temperature Control. This ensures that your sample will be within 0.1°C from the temperature set point regardless from room temperature variations.

Tip ► Advantages of Sample Feedback Control Mode are: 1) direct monitoring and active control of sample temperature and 2) independence from room temperature variations. The disadvantage is that it requires manual operation for correct placement of the fine gauge thermocouple in a Reference Well.

The Control Mode page also includes an **Open Incubator** button (see Figure 24). Press Open Incubator before opening the lid of the Incubation Chamber (e.g. in order to add culture medium). This places the thermal controller in stand-by and avoids overheating the sample. **Note ►**: this feature is available only when operating in **Sample Feedback Mode** Control.

Figure 24.Open Incubator.

After closing the incubation chamber wait a couple of minutes and press "Incubator Closed". Now you can continue your experiment.

Figure 25.Incubator Closed.

9.2.5.3 Objective Heater

Figure 26 shows how to enable/disable the Objective Heater (OBJ COLLAR ####). The Objective Heater is disabled by default. Read this paragraph only if your system is equipped with Objective Heater (OBJ COLLAR ####).

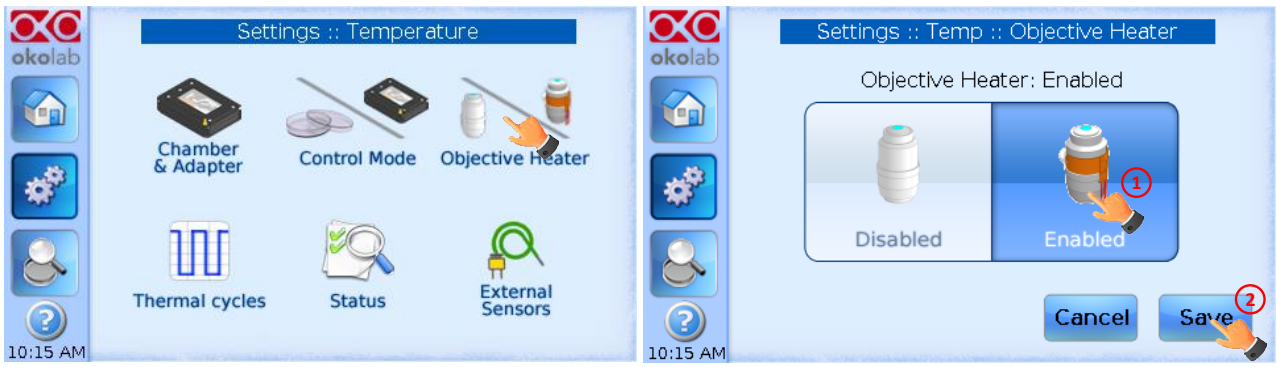

Figure 26. Objective Heater enabling.

Tip ► Before enabling it, make sure it is already installed around your objective, and that it has been correctly connected to the Temperature Control Unit.

When enabling and saving the Objective Heater the following Screenshot will appear:

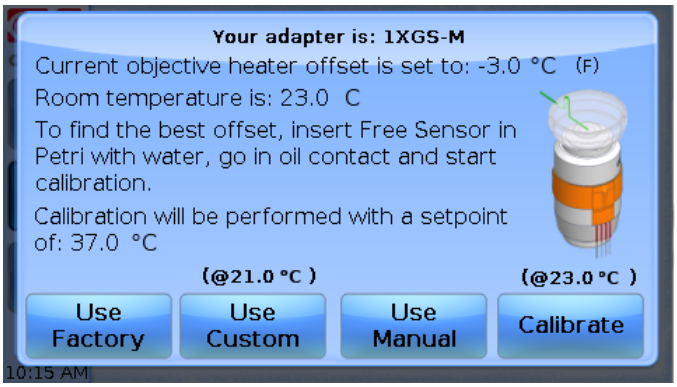

Figure 27. Objective Heater Calibration Page.

The temperature of the Objective Heater is factory calibrated at room temperature of 23°C, with a 3 degrees offset relative to the sample set-point temperature of 37°C. This offset value is appropriate for a set point of 37°C. If your set point is different than 37°C the system will automatically apply the correct offset. See Figure 27 above. This means that if the temperature is set at 37°C the Objective Heater is kept at 40°C in order to maintain the sample at the desired temperature while imaging with an oil immersion objective. To accept this offset you can press "USE FACTORY" and return to the Homepage. Alternatively the following options are available:

1. USE CUSTOM: Selecting "USE CUSTOM" recalls parameters saved with the last calibration. The temperature in parenthesis above the "Use Custom" button shows the room temperature of the last calibration. "Use Custom" button appears only if the temperature set point (in this example 37°C) is the same as in the last calibration.
2. USE MANUAL: Selecting "USE MANUAL" allows searching for the best offset value manually, step by step. See Figure 28 below and paragraph 11 for instructions on how to manually calibrate the Objective Heater (OBJ-COLLAR ####).

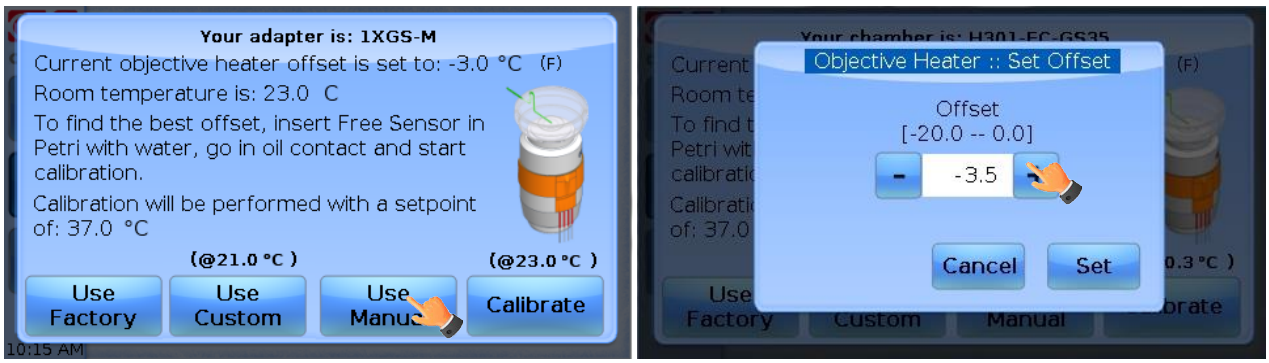

Figure 28. Objective Heater Manual Offset.

3. CALIBRATE: Selecting "CALIBRATE" automatically finds the best offset according to your specific experimental conditions. For details on this calibration procedure please follow the instructions on paragraph 12. If you are working in "Chamber Feedback Mode", the

starting Self-Calibration message will be displayed as in Figure 29. If you are working in "Sample Feedback Mode" it will be displayed as in Figure 30.

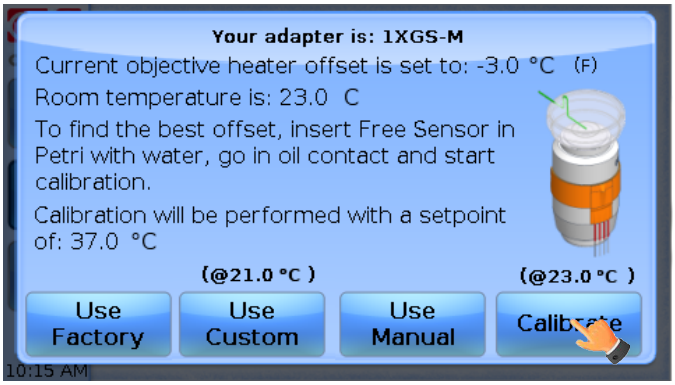

Figure 29.Objective Heater Self-Calibration Starting Message in Chamber Mode.

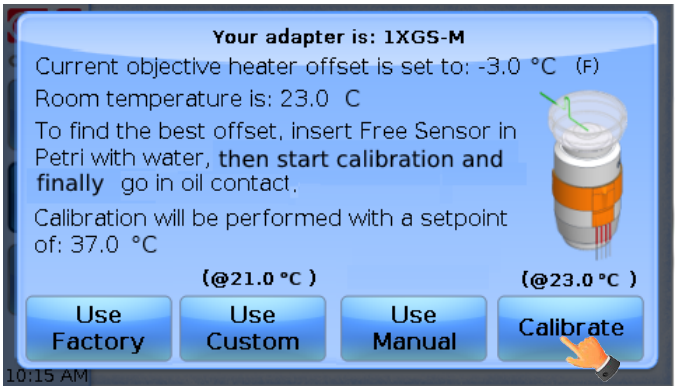

Figure 30.Objective Heater Self-Calibration Starting Message in Sample Mode.

Figure 31 shows the warning message that will appear once you have pressed "Calibrate" if you are working in Chamber Feedback Mode. This message reminds you that you have to ensure that a Chamber Calibration has already been completed. Or alternatively, that the factory calibration is appropriate for your laboratory room temperature (i.e. that, even in Chamber Mode, the sample is close to the Set Point temperature). If you are unsure it is recommended to re-check the room temperature and decide whether to re-calibrate the chamber.

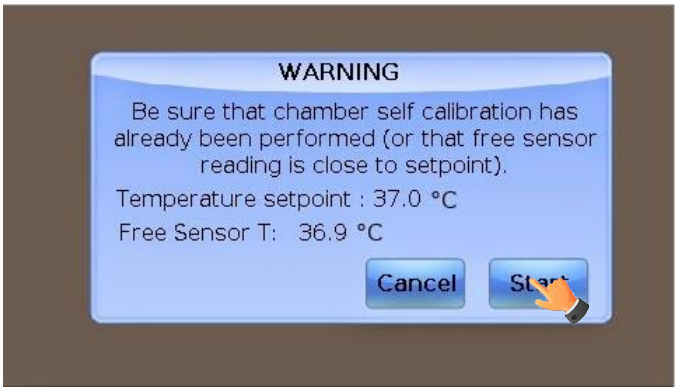

Figure 31. Self-calibration Warning in Chamber Mode.

Figure 32 shows the warning message that will appear once you have pressed "Calibrate" if you are working in Sample Feedback Mode. This message appears only if the Fine Gauge Thermocouple is far from the Set Point value. If the Fine Gauge Thermocouple is close to the set Point value, the Self-Calibration start will automatically.

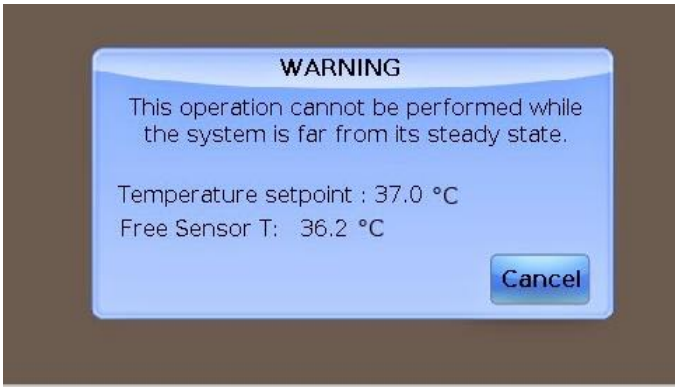

Figure 32. Self-Calibration warning in Sample Mode.

The calibration procedure typically takes more than one hour. When the calibration is in process a reminder icon will appear on the Temperature tab. Once the calibration is completed the reminder icon will disappear (see Figure 33).

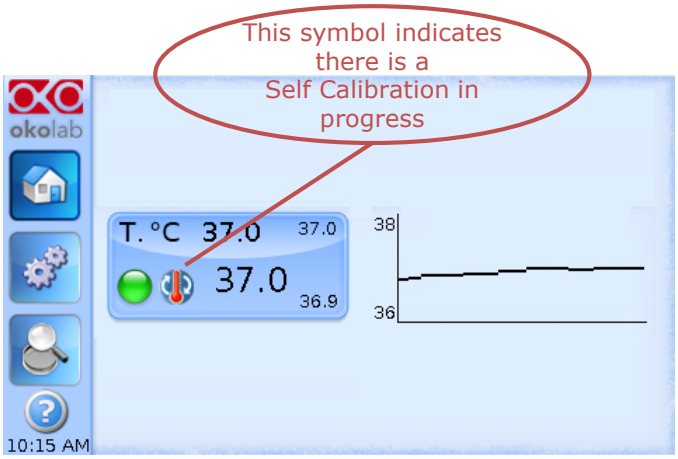

Figure 33. Homepage during Objective Heater Calibration.

In the Objective Heater Calibration progress window press "Options" to access "Advanced Calibration Parameters". In this window you can adjust Accuracy and Standard Deviation, which will be calculated over 10 minutes of readings. This allows setting more stringent parameters to the objective heater calibration.

Figure 34. Objective Heater Self Calibration parameters.

Tip ►The higher these values (therefore the less accurate and precise is the calibration), the less time is taken by the calibration procedure to complete. Use default values to get the most accurate calibration.

Once the calibration procedure is completed, the following message appears.

Figure 35. Objective Heater Self-Calibration end.

9.2.5.4 Thermal Cycles

This function allows the incubator to switch between two different temperatures (both temperatures must be at least 3°C above Room Temperature).

Figure 36.Thermal Cycles Subpage.

You can decide whether setting a new thermal cycle ("New") or recalling a previously saved one ("Load"). To set a new thermal cycle, first choose the two temperatures between which

you want to switch (SP1: Set Point 1 and SP2: Set Point 2). Next, set the duration in hours and minutes you want the system to dwell on each temperature. Lastly set the number of thermal cycles ("Number") you want the system to perform (see Figure 37).

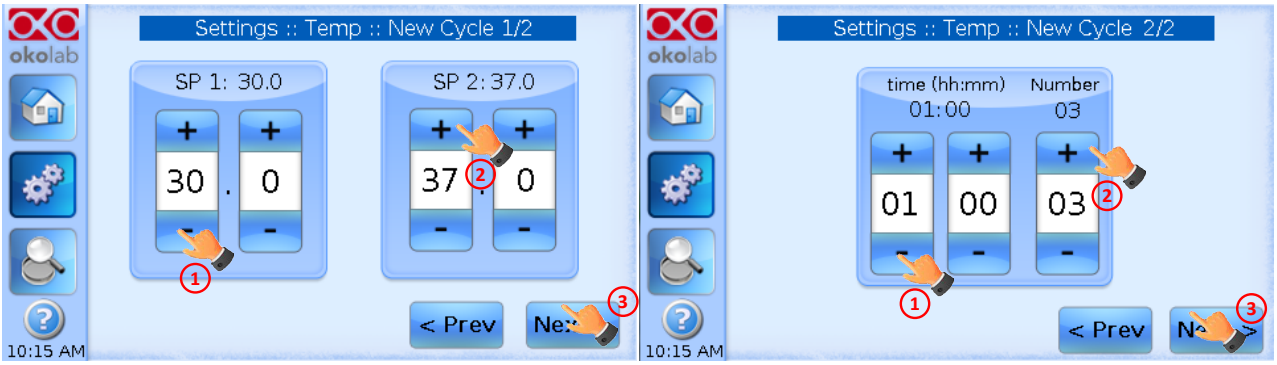

Figure 37.Thermal cycles settings.

Tip ► The system switches from a higher to a lower temperature by dissipation; hence it requires some time (the amount of time required depends on several factors such as control mode, room temperature and set-points). Please keep this in mind when setting the "time" between the two set points.

Once you have adjusted the settings, a "Cycle Summary" page will be displayed. Press "Save As" if you want to save the Thermal Cycle program to be recalled for future experiments. You can save up to 4 different thermal cycle programs. Saving a 5th one will overwrite the first saved one.

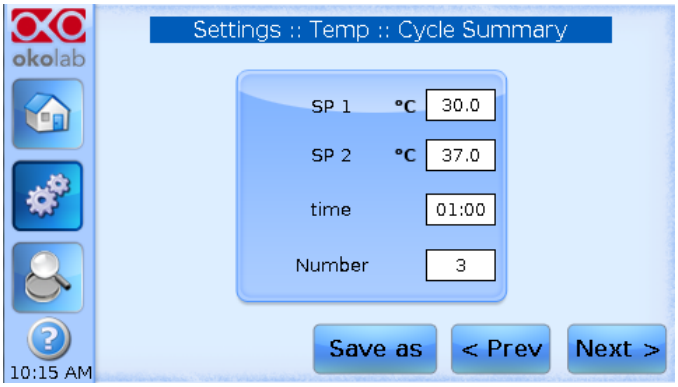

Figure 38.Thermal cycle Summary.

In the last step you can schedule the time you want the thermal cycle to start.

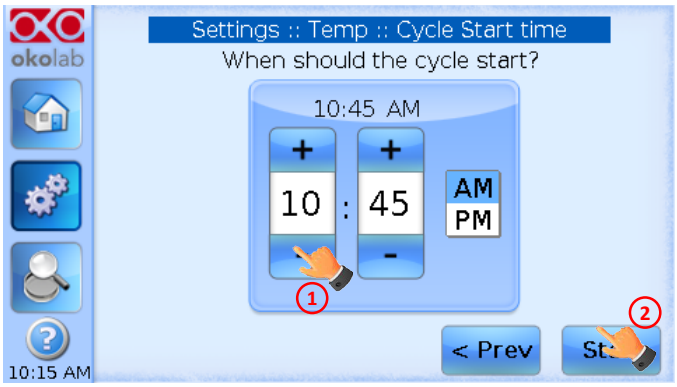

Figure 39. Cycle Start Time.

At set time the cycle will start. A thermal cycling icon will be displayed on the Homepage as a reminder. Trying to change set points or starting a new thermal cycle while a different one is still running will open the window in Figure 40. You can press Abort Cycle to modify it or start a new one. Pressing 'View' will open the current thermal cycle settings.

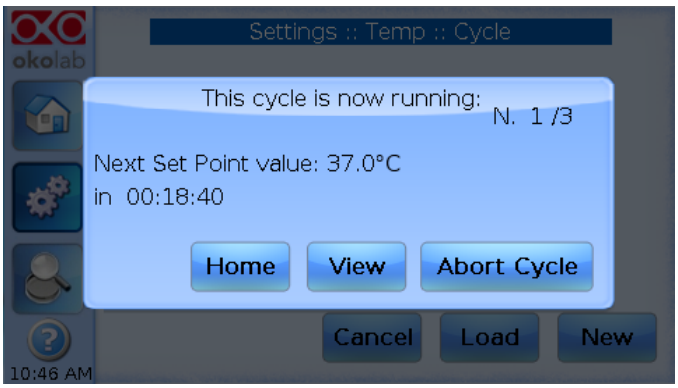

Figure 40. Running Cycle window.

9.2.5.5 Status Page

To open the 'Status' page press the tab with gears on the left side of the screen. The 'Settings: Temperature' page will open, next select 'Status'. On this page you can see the status of the Temperature Control Unit, with a summary of all parameters allowing you to check that all is working properly. You may have to refer to this window when asking for Okolab technical support.

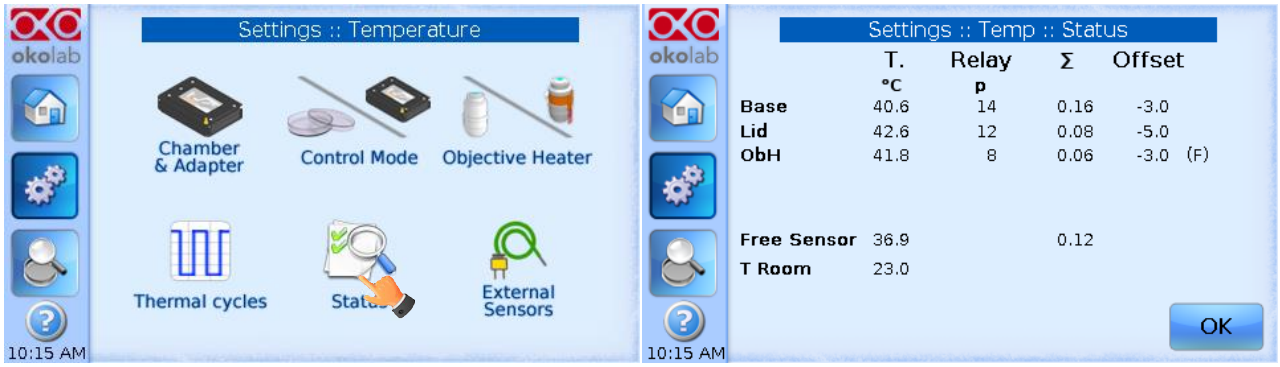

Figure 41. Status page.

9.2.5.6 External Sensors

There are two external temperature sensors connected to the Temperature Control Unit. A white thermistor is to be placed in air to monitor the Room Temperature. A green Fine Gauge Thermocouple, to be placed into a reference well if running the "Sample Control Mode" or to run Chamber or Objective Heater calibrations. **IMPORTANT: when running the Temperature Control Unit in Sample Feedback Mode the green Fine Gauge Thermocouple MUST be immersed in liquid in a Reference Well. Failure to do so will result in overheating of the chamber.** When running in "Chamber Feedback Mode" the green Fine Gauge Thermocouple can be placed in any area of the system to monitor temperature in that area. With this setup you can monitor both temperatures read by the thermistor (T Room) and by the thermocouple (Free Sensor) (see Figure 42).

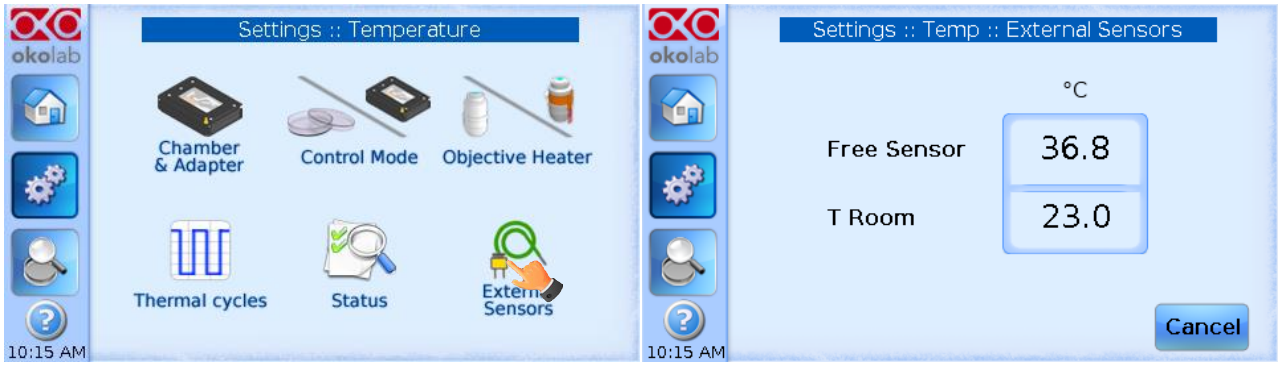

Figure 42. External sensors supage.

9.2.6 Overview Page

Figure 43 shows an overview of the complete system displaying current values of all controlled parameters. Similarly to the Status Page you may have to refer to this window when asking for Okolab technical support.

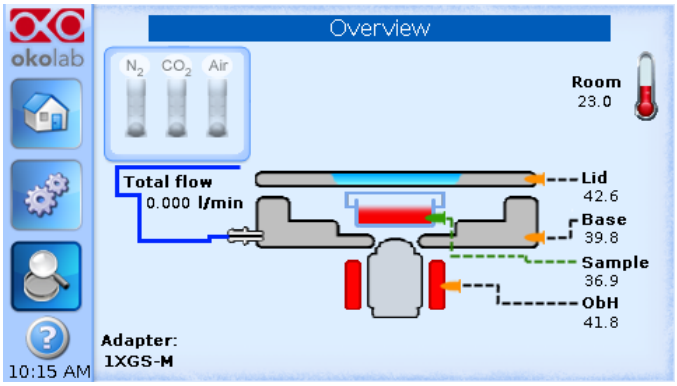

Figure 43.Overview page

Figure 43 shows the Overview page when your system is equipped with the Objective Heater (OBJ COLLAR ####).

10 Chamber Self-Calibration

Carefully read the instructions given in this paragraph and in paragraph 9.2.5.2 before starting the Chamber Calibration.

Note ► It is important to monitor the room temperature during the calibration, as well as reducing air drafts and forced convection. If possible divert any draft coming from the air conditioning system in the room, from the incubation system.

The goal of this procedure is to automatically adjust the temperature offset of the Base and Lid of the incubation chamber hence maintaining the sample at the desired temperature (e.g. 37°C) during imaging. Before starting the Chamber Self-Calibration:

1. Plug the green Fine Gauge Thermocouple in the port labeled "Reference Temperature" located in the rear panel of the Temperature Control Unit, as illustrated in Figure 4.
2. Insert the Fine Gauge Thermocouple into the chamber by threading the wire through one of the screw holes on the chamber riser.
3. Secure the end of the Fine Gauge Thermocouple to the bottom of a 35mm or similar Petri dish using some tape making sure to avoid covering the head of the probe (see Figure 44).

Figure 44.Free Sensor attached on the internal base of the Petri Dish.

4. Fill the Petri dish with distilled water to a level sufficient to fully immerse the Fine Gauge Thermocouple. This is a Reference Well simulating experimental conditions. Place the lid on the Petri dish, and insert the dish in the incubation chamber. You can also use SENSOR LID-# to close the dish (see the "**Tip**" below).

Tip ► It is recommended using an Okolab SENSOR LID (available for purchase) to secure the Fine Gauge Thermocouple in place in the Reference Well during calibration or operation in Sample Feedback Mode (see Figure 45).

Sensor lids are available for the following: 35 mm, 60 mm Petri-dish, chamber slide and chambered cover glass. Sensor Lids are ordered separately using the codes in following table.

SENSOR LID CODES	
SENSOR LID-35	Temperature sensor lid. To be used in specimen feedback in a 35 mm Petri Dish
SENSOR LID-60	Temperature sensor lid. To be used in specimen feedback in a 60 mm Petri Dish
SENSOR LID-GS	Temperature sensor lid. To be used in specimen feedback in chamber slides and chambered cover glass

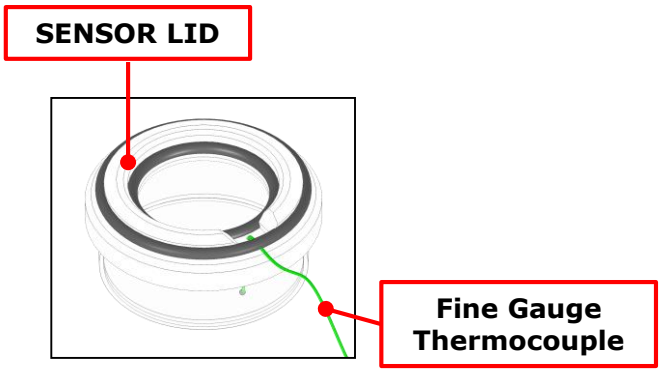

Figure 45. SENSOR LID-35. For 35 mm Petri-dish.

5. Go in Control Mode page as shown in Figure 19 and enable "Chamber Mode", the page shown in Figure 20 will open.
6. Next press "Calibrate" to start the Self-Calibration program.
7. Self-Calibration may take 1-2 hours to complete, during this time the dialog shown in Figure 21 will be displayed. Once the Self-Calibration procedure is complete the window shown in Figure 22 will be displayed.
8. You can remove the Fine Gauge Thermocouple from the Reference Well petri-dish inside the incubating chamber. When running in Chamber Feedback Mode the system will use the parameters from the calibration at your Room Temperature.

11 Objective Heater Manual Calibration

Carefully read the instructions given in this paragraph and in paragraph 9.2.5.3 before starting the Objective Heater Manual Calibration.

The goal of this procedure is to adjust the controlled Objective Heater temperature manually, varying its offset; while maintaining the sample at the desired temperature (e.g. 37.0 °C) once the oil immersion objective is in contact with the bottom of the well. This can be achieved measuring the sample temperature with the Fine Gauge Thermocouple during steady state and estimating the offset values, as follows:

1. Plug the green Fine Gauge Thermocouple in the port labeled "Reference Temperature" located in the rear panel of the Temperature Control Unit, as illustrated in Figure 4.
2. Insert the Fine Gauge Thermocouple into the chamber by threading the wire through one of the screw holes on the chamber riser.
3. Secure the end of the Fine Gauge Thermocouple to the bottom of Petri dish using some tape making sure to avoid covering the head of the probe (see Figure 44).
4. Fill the Petri dish with distilled water to a level sufficient to fully immerse the Fine Gauge Thermocouple. This is a Reference Well simulating experimental conditions. Place the lid on the Petri dish, and insert it in the incubation chamber. You can also use SENSOR LID-# to close the dish (see the "**Tip**" in paragraph 10).

Note ► Please, monitor the room temperature during the calibration and reduce air drafts and forced convection. If possible divert any draft coming from the air conditioning system in the room, from the incubation system.

5. Wait until the system reaches the steady state (temperature indicator status is green) then approach the oil immersion objective to the bottom of the Petri dish. The Fine Gauge Thermocouple will detect a decrease in temperature because of the contact with the cold lens. You can see the Fine Gauge Thermocouple temperature decreasing in the Status page (see paragraph Figure 41) whether you are working in "Chamber Feedback Mode" or in "Sample Feedback Mode

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If you are working in "Chamber Feedback Mode", verify that the Chamber Calibration has already been completed. Or alternatively, that the factory calibration is appropriate for your laboratory room temperature (i.e. that, even in Chamber Mode, the sample is close to the Set Point temperature). If you are unsure it is recommended to re-check the room temperature and decide whether to re-calibrate the chamber (see 9.2.5.2) and then the Objective Heater.

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6. When the Fine Gauge Thermocouple reads a stable temperature open the "Objective Heater" page as shown in Figure 26 and select "Use Manual" (see Figure 28). Next click

on "+" or "-" on the "Offset" tab (see Figure 28) to adjust the Objective Heater offset value. Okolab recommends starting with an offset value corresponding to the difference between the set point temperature and the actual temperature measured by the Fine Gauge Thermocouple. Press "Set" and wait until the temperature measured by the Fine Gauge Thermocouple is stable. If the temperature measured by the Fine Gauge Thermocouple is now close to the set point, then the Manual Calibration is complete. If the temperature measured by the Fine Gauge Thermocouple is far from the set point, repeat the steps above until the temperature measured by the Fine Gauge Thermocouple reaches the set point temperature

12 Objective Heater Self-Calibration

Before starting the Objective Heater Self-Calibration carefully read the instructions given in this paragraph and in paragraph 9.2.5.3.

The goal of the Self-Calibration procedure is to adjust the Objective Heater temperature by **automatically** varying its offset; while maintaining the sample at the desired temperature (e.g. 37.0 °C) once the oil immersion objective is in contact with the bottom of the well. Before starting the Objective Heater Self-Calibration:

1. Plug the green Fine Gauge Thermocouple in the port labeled "Reference Temperature" located in the rear panel of the Temperature Control Unit, as illustrated in Figure 4.
2. Insert the Fine Gauge Thermocouple into the chamber by threading the wire through one of the screw holes on the chamber riser.
3. Secure the end of the Fine Gauge Thermocouple to the bottom of Petri dish using some tape making sure to avoid covering the head of the probe (see Figure 44).
4. Fill the Petri dish with distilled water to a level sufficient to fully immerse the Fine Gauge Thermocouple. This is a Reference Well simulating experimental conditions. Place the lid on the Petri dish, and insert it in the incubation chamber. You can also use SENSOR LID-# to close the dish (see the "**Tip**" in paragraph 10).

Note ► Please, monitor the room temperature during the calibration and reduce air drafts and forced convection. If possible divert any draft coming from the air conditioning system in the room, from the incubation system.

5. Wait for until the system reaches the steady state (temperature indicator turns green) and then:

A. **Chamber Feedback Mode**:

- Enable the Objective Heater as shown in Figure 26; the window in Figure 29 will open. Follow the instructions on the message: first you have to bring your immersion lens in contact with the Petri dish, next press "CALIBRATE". **Following this sequence of instructions REQUIRED to control the Objective Heater only, without interfering with the incubation chamber**.
- Once you have pressed "Calibrate", the dialog shown in Figure 31 will open. If the Fine Gauge Thermocouple is close to the Set Point Value, press "Start", otherwise you have to calibrate the Chamber Incubator first and then can calibrate the Objective Heater.

Make sure that Chamber Calibration is completed. If the Fine Gauge Thermocouple measures a temperature far from the Set point Value pressing, "Start" will still launch the Self-Calibration procedure. This is NOT advisable because the procedure will raise the offset value of the objective heater.

B. **Sample Feedback Mode**:

- Enable the Objective Heater as shown in Figure 26, the window in Figure 30 will open. Follow the instructions on the message: first press "CALIBRATE", next bring the immersion lens in contact with the petri-dish. **Following this sequence of instructions is REQUIRED to place the Fine Gauge Thermocouple in standby and act on the Objective Heater only**.

The calibration procedure typically takes longer than one hour. While calibrating an icon will appear on the Homepage Temperature tab (see Figure 33) to remind you of the ongoing calibration. Once the calibration is completed the icon will no longer appear.

In the Objective Heater Calibration progress window press "Options" to access "Advanced Calibration Parameters" (see Figure 34). In this window you can adjust Accuracy and Standard Deviation, which will be calculated over 10 minutes or readings. This allows setting more stringent parameters to the objective heater calibration.

Tip ►The higher are these values (therefore the less accurate and precise is the calibration), the less time is taken by the calibration procedure to complete. Use default values to get the most accurate calibration.

When the calibration procedure is completed, the message shown in Figure 35 will appear.

13 Maintenance

To maintain proper Temperature Control Unit operation over time:

- Use a polishing cloth or dry cloth to wipe off dust and dirt.

Before cleaning the unit, pull out the mains plug.

Water must not be entered in the system.

- Never use thinners, benzene, solvents on or near the devices, since these could corrode their surfaces.
- To polish the Stage Incubator and the Humidifying Module, if it is present, you can use distilled water or alcohol
- Verify the status of all cables and if some cable is damaged, contact Okolab to receive assistance

14 Support

14.1 Web Conference For Assistance And Training

Together with your equipment you received a web cam and headset. You can request remote support over the web. Please follow these guidelines to facilitate setting up your web support:

- Webcam installation (instruction and Cdrom included)
- Last Skype® software installed (www.skype.com)
- Register yourself on www.skype.com to have an account (Skype_ID)
- Set the audio and video and test them using Skype.
- Contact our technical support (sibillo@oko-lab.com) by e-mail to take an appointment for web assistance.

14.2 Troubleshooting

Incorrect operations are often mistaken for malfunction. If you think that there is something wrong with a component, see the troubleshooting scheme below. If the problem persists even after troubleshooting as described below, please ask for Okolab support

Symptom	Probable cause	Remedy
Device off	Supply cable disconnected	Properly connect the cable
	Supply cable damaged	Substitute the cable
	Blown fuse	Replace the fuse (see Technical Specifications)
No temperature displayed or "nan"	Thermocouple cable disconnected	Properly connect the cable
	Thermocouple cable damaged	Contact Okolab to receive assistance
	Embedded sensors damaged	Contact Okolab to receive assistance
Chamber Base and Lid cold	Low set point value	Change the set point
	Heater 1/2 and/or cable disconnected	Connect the cable
	Heater 1/2 and/or cable damaged	Contact Okolab to receive assistance
	Heating elements damaged	Contact Okolab to receive assistance
Alarm rings (for specimen temperature feedback)	Chamber Base at high temperature (for specimen temperature feedback)	Quickly shoot down the control unit and properly set the system. Please, check below
Chamber Base at high temperature (for specimen temperature feedback)	Control thermocouples connection	Check the connection scheme
	Damaged thermocouple	Ask Okolab for replacement
	Sensor out of the reference well or chamber open	Make sure that the thermocouple is correctly positioned in the reference well and that the incubating chamber is closed (if working in "Specimen Control Mode)
I check the previous troubleshooting but I cannot solve the problem		Contact Okolab to receive assistance

14.3 Technical support

Please, do not hesitate to contact Okolab should you need any further commercial information or technical support.

Please, check Okolab web site www.oko-lab.com for news, events, new products and general FAQ.

For COMMERCIAL SUPPORT: lanzaro@oko-lab.com

Phone +39 081 806 2624

Fax: +39 081 876 4410

Mobile: +39 348 9680717

For TECHNICAL SUPPORT: sibillo@oko-lab.com

Phone +39 081 806 3470

Mobile: +39 348 9680718

Okolab S.r.l.

Via A. Olivetti, 1 - 80078 Pozzuoli, NA

14.4 ItalyTechnical Specifications

H301-T-UNIT-BL-PLUS – Technical Specifications	
Temperature	Chamber Temperature Range: 25-45°C Sample Temperature range: 3°C above ambient temperature to 45°C Step size: 0.1°C Accuracy:0.1°C in Sample Mode, 0.3°C in Chamber Mode if room temperature remains within ± 1°C
Regime temperature time	about 30 min
Operating Temperature	0°C ~.+55°C
Storage Temperature	-5°C ~ +60°C
Operating Humidity	0-70%
Power Consumption	115V AC 60 Hz Fuse 2.5 AT 250V 5x20 230V AC 50 Hz Fuse 1.25 AT 250 V 5x20 160 W max
Output	Output 1 24 VOC 2.5A Max Current Fuse 2.5 AF 250V 5x20 Output2 24 VOC 1.6 A max Current Fuse 1.6 AF 250V 5x20 Output3 24 VOC 1.6 A Max Current Fuse 1.6 AF 250V 5x20
Weight	6 Kg

15 Figures

WARRANTY

Okolab S.r.l. warrants its products to be free of defects in materials and workmanship for a period of one year starting from invoice date. If the unit malfunctions, it must be returned to the factory for evaluation. If the equipment has to be returned to the factory, please ensure that is carefully and properly packed. Okolab S.r.l. accepts no responsibility for damage due to unsatisfactory packing.

Upon examination of Okolab S.r.l., if the unit is found to be defective, it will be repaired or replaced at no charge. This warranty does not apply to defects resulting from any actions of the purchaser. Okolab S.r.l. neither assumes responsibility for any omissions or errors nor assumes liability for any damages that result from any action of the purchaser that discord from instructions listed in the operation manual. This warranty does not cover or involve any other equipment that may be used along with the Okolab System (i.e. mini-incubators, any gas tank, etc.), whose usage should be considered independent and performed according to their own operational instructions.

Okolab S.r.l. warrants only the parts manufactured by it will as specified and free of defects. Okolab S.r.l. makes no other warranties or representations of any kind whatsoever, express or implied, except that of title, and all implied warranties including any warranty of merchantability and fitness for a particular purpose are hereby disclaimed. LIMITATION OF LIABILITY: the total liability of Okolab S.r.l. shall not exceed the purchase price of the component upon which liability is based. In NO event shall Okolab S.r.l. be liable for consequential, incidental or special damages.