BOLD LINE 3

Top Stage Systems

H303-T

H303-T-H-CO2

H303-T-H-CO2/O2 [0-21]

User Manual

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Index

1 PREFACE

Welcome to the BOLD LINE 3 (BL3) Top Stage Systems Manual. This manual provides comprehensive guidance on the setup, operation, and maintenance of the BL3 Top Stage Systems. It covers essential information to ensure efficient utilization and optimal environmental control within laboratory settings.

Key Features of BL3 Top Stage Systems:

- **Advanced Environmental Control:** Integrated CO2 and Humidity sensors enable precise monitoring and control within the system.
- **Embedded Temperature Sensors:** Continuous temperature feedback enhances control accuracy and stability.
- **Error-Proof System:** Real-time monitoring triggers alarms for deviations from setpoint values, ensuring sample protection.
- **User Interface:** Clear instructions cater to users at all levels, facilitating effective usage.
- **Support:** Our team is available to assist with inquiries or issues.

Components and Configuration: The BL3 Top Stage Systems feature an electrically heated Lid and base. For systems equipped with gas control, a Gas Control Unit is provided, which pairs with the Active Humidity Module (HM-Active) to supply humid gas.

Temperature Range: The BL3 Top Stage Systems control the temperature from 3°C above ambient temperature up to 45°C, ensuring precise environmental conditions for your samples.

Compatibility: The CO2-H-IN-CHAMBER Sensor is compatible with any of the BL3 Chambers, providing seamless integration for advanced environmental monitoring and control.

Control Interface: The BL3 Top Stage Systems are operated via the OKO-TOUCH touch screen control panel. OKO-TOUCH features on-board memory for data logging and a mini-USB port for data download. Data logging is also possible via SMART-BOX, Okolab DATA-LOG software, and compatible third-party imaging software such as LASX 2.0, NIS-Elements, SlideBook, MetaMorph, and MicroManager. A macro for ZEN Black software is available upon request.

The Top Stage systems codes to which this manual applies are:

1. ***H303-T***
2. ***H303-T-H-CO2***
3. ***H303-T-H-CO2/O2 [0-21]***

We recommend carefully reading this manual to familiarize yourself with the functions and operation of the BL3 Top Stage Systems before use. Our team is available to assist with any inquiries or issues you may encounter.

2 REGULATORY COMPLIANCE

2.1 Regulatory Statement

The product complies with the essential requirements of the LVD Directive 2014/35/EU and the EMC Directive 2014/30/EU. The product has been tested and it complies with the requirements of the following standards:

- European standard EN 61010-1: 2010 “Safety requirements for electrical equipment for measurement, control and laboratory use"
- European standard EN 61326-1: 2013 “Electrical equipment for measurement, control and laboratory use – EMC requirements"

The product meets the technical requirement of the Directive 2011/65/EU Restriction of the use of certain hazardous substances (RoHS). The product has been tested according to European Standard EN 50581: 2012 “Technical documentation for the assessment of the electrical and electronic products with respect to the restriction of hazardous substances".

2.2 Waste Electrical and Electronic Equipment Directive (WEEE Directive)

The product must not be disposed as a general household waste. At the end of the product's life, take the product to a collection point designed for recovering and recycling of electrical and electronic devices.

In case of doubt, please return the product to Okolab s.r.l. for proper treatment.

3 SYMBOL DESCRIPTION

This paragraph describes the symbols used in this manual and on the product label.

3.1 Symbols used in this Manual

The following symbols identify important information:

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

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

3.2 Symbols on the Product Label

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

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

This symbol indicates the product production date.

This symbol indicates the Manufacturer data.

This symbol warns you to read the user manual before starting the device.

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

4 SAFETY NOTES

In order to achieve maximum performance and to ensure proper operation of your new equipment, please read carefully the following safety notes and the instructions. If you have any question, please contact OKOLAB.

- The equipment must only be used as intended and as described in this Manual.
- Equipment should be operated only 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 subject to flammable or explosive gases.
- Transport the equipment with care.
- Equipment and its internal parts can be damaged by dropping and by shock.
- 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 Box.
- Do not disassemble any part of the system.
- Do not use a volatile solvent such as paint thinner to clean the instrument, because deformation or discoloration may occur.
- Use a soft, dry cloth to remove stains from the instrument.
- Do not exceed voltage indicated in this manual and on the product label.

- Avoid excessive induction noise, static electricity and magnetic fields.
- Do not expose this instrument to rain or moisture.
- Do NOT go in close contact with or breathe any gas stream whose composition is different from that of ambient air.
- Prevent throttling and kinking of tubing.
- Check tubing time to time for possible material usage.
- Check that all tubing are well inserted into the connectors so they cannot slip off
- This device is not designed for use for medical applications.
- Power cord of unit should be unplugged from electrical outlet when left unused for a long period of time.
- PRESSURIZED GAS. Secure all connections with hose clamps. Never exceed the input pressure limit of 5 barg (72.5 psig). Bleed all lines before disconnecting. Wear safety goggles if needed. If pressure regulators are not within sight and reach, make sure at least one shut off valve is within reach.
- CO2 must be available at 1.4 barg (20.3 psig), with a connector for 6mm OD rigid silicon tube. Gas source must be Standard Purity CO2 (coded as 4.5 that means 99.995 % of CO2) and humidity-free gas.
- When using the Compressed Air option, please ensure that the gas is available at 1.4 barg (20.3 psi)

- N2 must be available at 1.4 barg, with a connector for 6mm OD rigid silicon tube. Gas source must be Standard Purity N2 (coded as 4.5 that means 99.995 % of N2) and humidity-free gas.
- LOW OXYGEN ATMOSPHERES. Never enter a room or enclosure which has a low oxygen atmosphere because of severe danger of suffocation. Only operate in well-ventilated room. A small amount for carbon dioxide gas leaks continuously out of the instrument and should never be allowed to build up in the room.
- Unit should be situated away from heat sources such as open flames, radiators, heat registers, stoves, or other appliances or processes that produce heat.

- 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 to avoid electric shock, fire or malfunction.
- No user serviceable parts inside.
- Unit should never be used where it can fall or be pushed into water
- When removing the CO2-H-IN-CHAMBER-Sensor the device must be disconnected from the power supply and the Sensor must be handled using electrostatic Gloves.

International caution symbol marks this device. It is important to 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, OR LOSSES OR EXPENSES RESULTING FROM ANY DEFECTIVE PRODUCT OR THE USE OF ANY PRODUCT

We reserve the right to make technical upgrades and changes.

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

5 SUPPLIED EQUIPMENT & COMPONENTS COMPATIBILITY

Figure 1 illustrates the components and the different accessories for the Bold Line 3 Top Stage systems.

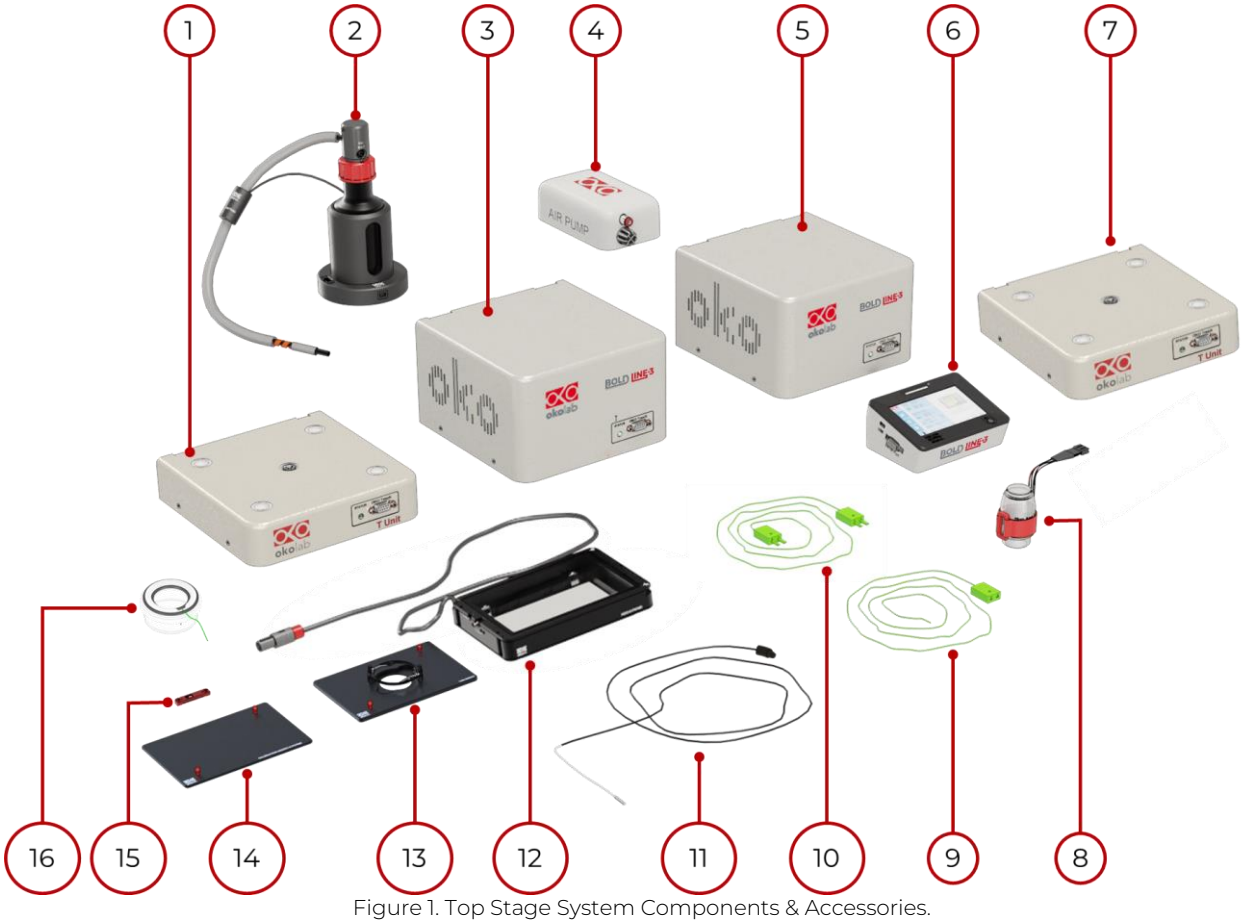

Figure 1. Top Stage System Components & Accessories.

Components

1. **H303-T-UNIT**: A temperature control unit that can operate any H303-Chamber to maintain the required thermal conditions for live cell imaging right on the microscope stage.
2. **HM-ACTIVE-BL3**: Active Humidity Module controls relative humidity in the range of 50-95%.
3. **CO2-UNIT-BL3**: A gas control unit which allows you to control the CO2 concentration in the range of [0-20] %, with the help of embedded sensors.
4. **OKO-AIR-PUMP-BL**: A pump which enables you to use the background air.
5. **CO2-O2 [0-21]-BL3 Unit**: A gas control unit which allows you to control the CO2 concentration in the range of [0-20] % and O2 in the range of [0-18] %, with the help of embedded sensors.
6. **OKO-TOUCH-BL3**: An intuitive and user-friendly touch screen interface which connects to the control unit.

9. **TC**: This is a reference temperature probe that can be utilized for measuring temperatures within the sample and has a female connection port to be used along with the TC-XC.
10. **TC-XC**: This is an extension cable for the TC temperature probe.
11. **TAT**: Also referred to as the NTC thermistor, this is a temperature probe for measuring the ambient temperature.

Note ► *The TC and the TC-XC are collectively referred to as the T Sensor in this document.*

Accessories (Sold Separately)

7. **HM-ACTIVE-STANDALONE-BL3**: A Digital Humidity Controller that controls the Relative Humidity inside the chamber in the range 50-95% by controlling the HM-ACTIVE-BL3.
8. **OBJ-COLLAR**: Also referred to as 'Objective Heater' temperature-controlled heating band that can be placed around the objective lens with the help of the provided Velcro band. The Objective heater is available in 3 different versions:
    A. ***OBJ-COLLAR-1924***- Objective Heater for lenses with diameters ranging from 19 to 24mm
    B. ***OBJ-COLLAR-2532***- Objective Heater for lenses with diameters ranging from 25 to 32mm
    C. ***OBJ-COLLAR-3342***- Objective Heater for lenses with diameters ranging from 33 to 42mm
12. **H303-Chamber**: A micro-environmental chamber with an ITO coated glass, heated base and an embedded Temperature sensor, that fits in the XY stage of the microscope and may be connected to Gas input.
13. **Sample Holder Inserts**: Inserts designed to house different sample holders such as 35mm petri dishes, chamber slides etc.
14. **Calibration Insert**: This insert is provided along with the CO2-H-IN-CHAMBER-Sensor, and must be utilized during the calibration procedure of this sensor.
15. **CO2-H-IN-CHAMBER Sensor**: A miniature CO2 and humidity sensor that can be embedded inside the H303-Chamber, ensuring a real time monitoring of your sample. Continuous CO2 and Humidity feedback enhances control accuracy and assure a total sample protection.
16. **Sensor lid**: This is a an Okolab SENSOR LID to secure the Fine Gauge Thermocouple in place in the Reference Well during calibration or operation in Sample Feedback Mode. This Sensor Lid is available in 3 different versions:
    A. ***SENSOR LID-35-*** To be used in specimen feedback in a 35 mm Petri Dish
    B. ***SENSOR LID-60-*** To be used in specimen feedback in a 60 mm Petri Dish
    C. ***SENSOR LID-GS-*** To be used in specimen feedback in chamber slides and chambered cover glass
17. **DATA-LOG:** This is a software to log Temperature and Concentration data from any OKOLAB device further details on this software can be found in its dedicated user manual.

Note ► *The H303-Chamber, depicted in the image is a **generic model** and the appearance my vary as the chambers are designed to be Microscope Specific.*

Note ► *Information on the **Objective Heater assembly** is explained in Heading 9.3.*

Note ► *The HM-ACTIVE-STANDALONE-BL3 is **only** compatible with the H303-T System.*

Note ► *Please refer to the manual dedicated to HM-ACTIVE-STANDALONE-BL3 controller for instruction relative to its installation and usage*

The following Table 1 details the different components and with which of the Top Stage system codes are they included.

#	Component Name	BL3 Top Stage Code		
		H303-T	H303-T-H-CO2	H303-T-H-CO2/O2 [0-21]
1	H303-T-UNIT	Included	Included	Included
2	HM-ACTIVE-BL3	Not Included	Included	Included
3	CO2-UNIT-BL3	Not Included	Included	Not Included
4	OKO-AIR-PUMP-BL	Not Included	Included	Included
5	CO2/O2 [0-21]-BL3	Not Included	Not Included	Included
6	OKO-TOUCH-BL3	Included	Included	Included
8	TC	Included	Included	Included
9	TC-XC	Included	Included	Included
10	TAT	Included	Included	Included

Table 1. Component Names and Compatibility with Top Stage system codes.

Note ► *The CO2-H-IN-CHAMBER-Sensor is only compatible with the H303-T-H-CO2 and the H303-T-H-CO2/O2 [0-21] system and cannot be purchased as a standalone.*

Included Accessories

The following Table 2 details the different supplied accessories and with which of the BL3 Top Stage system codes are they included.

#	Accessory Name	BOLD LINE 3 Top Stage Systems Commercial Code		
		H303-T	H303-T-H-CO2	H303-T-H-CO2/O2 [0-21]
1	OKO-TOUCH-CABLE	Included	Included	Included
2	OTG-Cable	Included	Included	Included
3	miniUSB-USB Cable	Included	Included	Included
4	Power Supply-Control Units	Included	Included	Included
5	GR-4-M (Gas regulator)	Not Included	Included	Included
6	TUBE-A	Not Included	Included	Included
7	TUBE-B	Not Included	Included	Included
8	TUBE-EY	Not Included	Not Included	Not Included
9	HMCM-2-HEATED TUBE	Not Included	Included	Included

Table 2. Accessories list and with which Top stage systems are they included.

The following list describes the accessories and what they're used for:

1. OKO-TOUCH-CABLE: Cable for connecting OKO-TOUCH-BL3 to the H303-T-UNIT.
2. OTG-Cable: Cable for connecting a USB pen drive to the OKO-TOUCH-BL3.
3. Mini-USB Cable: Cable for connecting OKO-TOUCH-BL3 to the PC.
4. Power Supply-Control Units: Two are provided, one for connecting H303-T-UNIT to the power supply, and the other for connecting CO2-UNIT-BL or the CO2-O2 [0-21]-BL3 to the power supply.
5. GR-4-M (Gas regulator): A gas pressure regulator for regulate gas input from CO2 gas cylinder to the CO2-UNIT-BL or the CO2-O2 [0-21]-BL3.
6. TUBE-A: Tube for gas connection between the GR-4-M to the CO2 gas cylinder; the GR-4-M to the CO2-UNIT-BL or the CO2-O2 [0-21]-BL3 and; 'Gas Output' port on the gas control unit to the input of the H303-Chamber.
7. TUBE-B: Tube containing a PTFE filter, for gas connection between the OKO-AIR-PUMP-BL output & 'Air' Input port on the gas control unit.
8. TUBE-EY: Tube for gas connection between 'Gas Output' port on the gas control unit to the 'Input' port of the HM-ACTIVE-BL3.
9. HMCM-2-HEATED TUBE: Tube for gas connection between 'Output' port of the HM-ACTIVE-BL3 and the H303-Chamber gas Input.

Note ► *The CO2-H-IN-CHAMBER-Sensor is only compatible with the H303-T-H-CO2 and the H303-T-H-CO2/O2 [0-21] system and cannot be purchased as a standalone.*

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6 System Assembly

In order to properly assemble your Top Stage system, please follow the steps as reported on the following quick instructions guides depending on the BL3 Top Stage code you have:

1. H303-T_Quick Instructions.
2. H303-T-H-CO2_Quick Instructions.
3. H303-T-H-CO2/O2 [0-21] _Quick Instructions.

Note ► *If you have the CO2-H-IN-CHAMBER-Sensor, you can refer the CO2-H-IN-CHAMBER-Sensor _Quick Instructions for its installation.*

7 SUPPLIED GAS REQUIREMENTS

Please ensure that the following Gas Supply Requirements are followed for the input to the Gas control Unit:

- CO2 must be available at 1.4 barg (20 psig) at the ***Gas Control Unit's Input Port***. Gas source must be Standard Purity CO2 (coded as 4.5 that means 99.995 % of CO2) and humidity-free gas with a connector for 6mm OD rigid silicon tube.
- N2 (for H303-T-H-CO2/O2 [0-21] system) must be available at 1.4 barg (20 psig) ***Gas Control Unit's Input Port***. Gas source must be Standard Purity N2 (coded as 4.5 that means 99.995 % of N2) and humidity-free gas.

8 SETUP CONFIGURATION & QUICK USAGE

To Once you've successfully setup your Top Stage system by following the detailed instructions provided in the quick assembly guide, you're ready to proceed with configuring your setup and initiating the use of the system.

8.1 Homepage Icons Description

When you're using the OKO-TOUCH-BL3 controller, the homepage will demonstrate the key measurements (relative to your Top Stage system type), Settings Option, and the System Overview option. Figure 2 illustrates a generic representation of the OKO-TOUCH-BL3 Homepages, when used with the H303-T-H-CO2/O2 [0-21] system along with the CO2-H-IN-CHAMBER-Sensor. Figure 2 (a) illustrates the system when it's waiting for chamber feedback and Figure 2 (b) shows when the system is In Chamber Feedback mode.

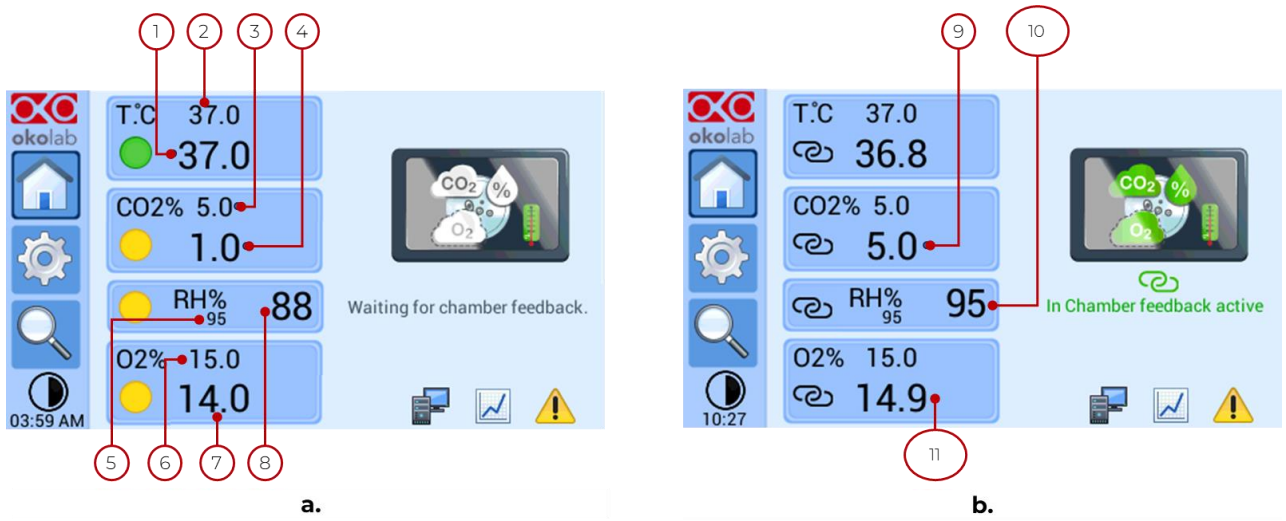


Figure 2. OKO-TOUCH-BL3 Generic Homepage with the CO2-H-IN-CHAMBER Sensor.

Note ► *Only the Parameters pertaining to your Top Stage System Type will be visible to you.*

Chamber Feedback Modes

When using the CO2-H-IN-CHAMBER-Sensor the Homepage will display two of the following messages:

- Waiting for Chamber Feedback: this is the warm up phase of CO2-H-IN-CHAMBER-SENSOR, during this phase CO2 and Humidity feedback are not available and the system works by using the feedback of control unit sensors. The homepage in this state displays the CO2 and Humidity readings of the control unit sensors.

- Chamber Feedback Active: Continuous CO2 and Humidity feedback are now ready. The homepage in this state displays the CO2 and Humidity readings of the CO2-H-IN-CHAMBER-SENSOR. The Chamber Feedback icon is visible in place of the LED icon.

Table 3 provides the description of the different parameters present on the Homepage (parameters 1-11).

Parameter	Description
1	Temperature value read by the Temperature Sensor embedded in the H303-Chamber.
2	Temperature Set Point value.
3	CO2 Set Point value.
4	CO2 concentration read by the Gas Control Unit Sensor.
5	Relative humidity Set Point Value.
6	O2 Set Point value.
7	O2 concentration read by the Gas Control Unit Sensor.
8	Relative humidity read by the HM-ACTIVE-BL3.
9	CO2 concentration read by the CO2-H-IN-CHAMBER-Sensor.
10	Relative humidity read by the CO2-H-IN-CHAMBER-Sensor.
11	O2 concentration inside the H303-Chamber(*see note).

Table 3. Description of different information displayed on the Generic Homepage.

The following Figure 3 illustrates the various icons that can be seen on the OKO-TOUCH-BL3 homepage, their descriptions and the visibility conditions for different Top Stage system types.

ICON NAME	DESCRIPTION	VISIBILITY CONDITION
Status Indicator 	Indicator of the Status of the parameter it is found next to based on its color.	Always, unless the system is in Chamber feedback mode.
Temperature 	Temperature Status indicator based on its color.	Always.
CO2 	CO2 Status Indicator based on its color.	Only Visible when using the CO2-H-IN-CHAMBER Sensor.
O2 	O2 Status Indicator based on its color.	Only Visible when using the CO2-H-IN-CHAMBER Sensor.
Humidity 	Humidity Status Indicator based on its color.	Only Visible when using the CO2-H-IN-CHAMBER Sensor.
Chamber Feedback 	Chamber feedback Active indicator.	Only Visible when the system is in Chamber feedback mode while using the CO2-H-IN-CHAMBER Sensor.
Home 	Home Page icon.	Always.
Settings 	Settings Page Icon.	Always.
OKOLAB Icon 	Okolab Icon which displays the sytem's information such as release date, serial number, software version etc..	Always.
Overview 	Overview Page Icon.	Always.
Minimalislitic 	Minimalistic View Page Icon.	Always.
PC 	Connected to PC Icon.	When the OKO-TOUCH-BL3 is connected to PC.
View Data 	View Data Icon.	Always.
Alarm 	Alarm Icon.	When the system is in Alarm state
Gas Calibration 	Gas Sensor Calibration Icon.	When the Sensors Calibration, O2 zero Reset or the CO2-H-IN-Chamber Sensor calibration is in process.
Temperature Calibration 	Temperature Sensor Calibration Icon.	When the Chamber Calibration (temperature) or the Objective Heater Calibration is in process.

Figure 3. Icons Descriptions & Visibility Condition.

Note ► *The **O2 icon** is represented with a dashed outline O2 is calculated based on O2 measurement of the control unit's sensor and the feedback received from CO2-H IN-CHAMBER Sensor.*

Note ► *The status LED present on the Control unit corresponds to the same colors as that of the Status indicator icon when the system is in Steady or Alarm state.*

The color relative to each icon may represent a Steady state, Transient state or an Alarm state as described below (Figure 4).

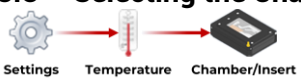
GREEN	The GREEN color indicates that the Setpoint value has been reached and that the system is working properly. **Controller Status: NORMAL**
YELLOW	The YELLOW color indicates that the controller is in transient regime. The Yellow light will appear after the controller is turned on and after any Setpoint change. The system is working properly, it is not in alarm and no action is needed. **Controller Status: TRANSIENT**
ORANGE	The ORANGE color indicates that the current parameter value is not correct and is out of the set tolerance. **Controller Status: ALARM**
RED	The RED color indicates that there is a problem and the system is in Alarm State, this may be due to a broken sensor. Turn the system off, wait for 5 minutes, and turn it back on. If the color is still red, contact Okolab at www.oko-lab.com for support. **Controller Status: ALARM**

Figure 4. Significance of Icons Color during use.

8.2 Setting up Date & Time

In order to set the Date & Time on your system, please navigate to the settings as illustrated above. Setting these parameters will allow you to keep a track of your work when exporting recorded data.

8.3 Selecting the Chamber & Insert



The next step that needs to be undertaken is the selection of the Chamber and the Sample Holder Insert that you'll be utilizing. In order to select the correct chamber and insert please refer the Bill included with the shipping package to verify the correct chamber name.

The following figure illustrates the Chamber & Insert selection page (Figure 5). In order to select the correct Chamber, click on the Chamber being displayed, and choose your Chamber by navigating through the different available options by clicking the **small blue arrow** (labelled as 'See Next'). Once you find the Chamber to be selected, confirm your choice by pressing ***'Next'***, this will take you to the Sample Holder Insert selection page where you have to perform the same steps for selecting the correct Sample Holder Insert.

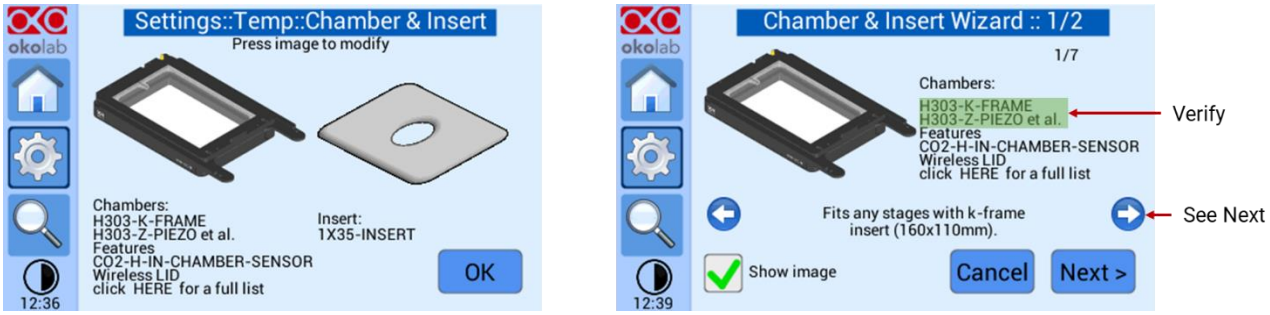


Figure 5. Chamber & Sample Insert Selection.

Note ► *If you have not purchased a sample holder Insert or are not using one, please select the option 'NONE' from the Insert selection page. This is the Default chamber's accommodation for MW Plates.*

Note ► *Please make sure to modify the selected Sample Insert every time you change the Sample Insert to ensure the proper functioning of the system.*

8.4 Setting the Desired set Points

Depending on the Top Stage system you have purchased, you will have access to the setting of parameter's Set Points. The following subsections indicate how to select the desired Temperature, CO2, O2 & Humidity set points.

Note ► *Each time the Set Point value is modified, the system will go into the Transient State and the Led on the OKO-TOUCH-BL3 will turn yellow. During this Transient State the system will not trigger any alarms.*

8.4.1 Temperature (°C) Set Point

On the Homepage click on the Temperature being displayed and select the desired set point within the range of *25°C up to 45°C.*

Note ► *The minimum temperature set point value must be 3°C above the ambient temperature.*

8.4.2 Gas Concentration (%) Set Point

You will have the option to assigning the gas concentrations Set Points only if you possess the ***H303-T-H-CO2*** system or the ***H303-T-H-CO2/O2 [0-21]*** system.

CO2 Concentration Set Point: On the Homepage click on the CO2 concentration being displayed and select the desired set point within the range of *0% up to 20%.*

O2 Concentration Set Point: This function is only available for H303-T-H-CO2/O2 [0-21] system. In order to assign a set point value to the O2 concentration, from the Homepage click on the O2 concentration being displayed and select the desired set point within the range of 0% up to 18%.

8.4.2.1 How to operate without N2 (Only applicable for H303-T-H-CO2/O2 [0-21] systems)

When you need to have Air/CO2 mixtures (therefore in case you don't need to regulate Oxygen too), press displayed O2 value on the homepage and click on "+" sign to increase the set point value until "Air" appears and press Set to save. On the homepage O2 will be replaced by '***AIR'***.

Note ► *When running the system without N2, keep in mind that in this operation mode Oxygen is obviously NO more a parameter that you can regulate, that's why you will see displayed AIR in place of the Setpoint on the Oxygen tab. The Oxygen % value displayed is the current Oxygen % resulting from the fact that Air is composed for c.a. 21% by Oxygen, and from the CO2 % that you have set in Air*

Note ► *When you work with Air and CO2, the N2 supply valve is automatically closed.*

Note ► *For further information on the Gas usage and settings please refer the Chapter 10.*

8.4.3 Humidity (RH%) Set Point

The Humidity Set Point can only be changed only if you possess the ***H303-T-H-CO2*** system or the ***H303-T-H-CO2/O2 [0-21]*** system. In order to modify the Humidity Set Point, from the Homepage click on the RH% being displayed and select the desired set point within the range of *50% up to 95% when working at 37°C.*

8.5 Opening & Closing the Chamber

When operating the Top stage system, you may need to open the H303-Chamber to access the sample for either changing or micro manipulating it. Depending on the type of Chamber you have there are two approaches to Opening/Closing the Chamber:

1. **Wireless Lid Chamber:** If you have a Chamber with a Wireless Lid, on opening the chamber, the system **automatically** displays the chamber open representation on the OKO-TOUCH-BL3 homepage. Besides the '***Chamber Open'*** message and representation, the system pauses its control until the chamber is closed again, this helps in avoiding the system overheating the Lid or the Base of the chamber to recuperate for the heat loss due to an open chamber, the same applies to the paused control of the Gas and Humidity parameters.
2. **Wired Lid Chamber:** If you have a Chamber with a Wired Lid, before opening the chamber, you may simply click on the chamber being displayed and a pop-up message will ask you if would like to open the chamber, to which respond by selecting ***'OK'.*** After you've closed the chamber and would like the system to resume its functioning, please click the Open chamber being

displayed and select ***'OK'*** from the following pop-up message which'll be confirming the closing of the chamber

The Figure 6 illustrates the ***'Chamber Open'*** and ***'Chamber Closed'*** representations.

Figure 6. Chamber Open & Chamber Closed representation.

Note ► *We advise you to avoid opening the chamber during the transient state as this may slow the system's rate of reaching a stable state.*

Note ► *If the chamber is left open for a long period of time the system goes into an Alarm State. Please refer the trouble shooting section for further information.*

8.6 Viewing Data

From the Homepage, if you click on the ***chart icon*** , you can view the graph pertinent to the parameters being worked with your system. The following image (Figure 7) illustrates the view charts page, the different graphs of the values of Temperature, CO2, O2, or Humidity (as in this example) can be viewed by clicking on the parameter on the right.

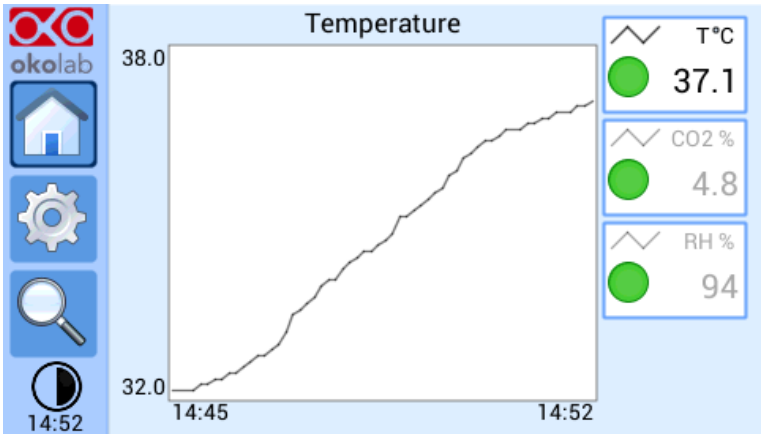


Figure 7. View Charts page.

8.7 System Overview

The Following Figure 8 illustrates the system Overview page which gives you the panorama of systems components and their readings. In order to see the Overview page, from the home screen click on the ***'Overview'*** icon. Please note that only the parameters that are a part of your Top Stage system will be the only ones visible to you during use.

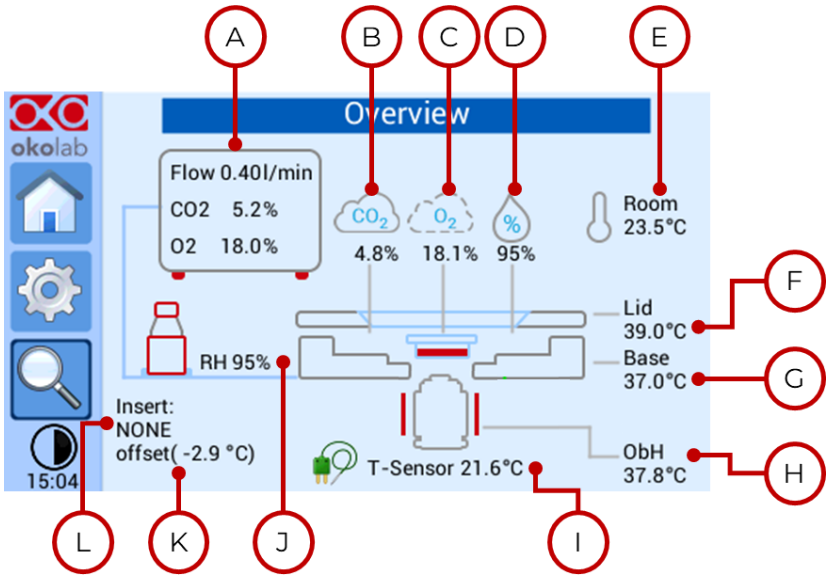


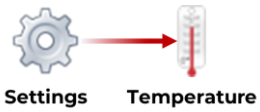
Figure 8. Generic System Overview Page.

The parameters illustrated in the Figure 8 are explained along with their display condition in Table 4.

#	Icon	Display Conditions
A	Gas Control Unit- Gas concentration(s) and Flow Rate.	Displayed only for **H303-T-H-CO2** and **H303-T-H-CO2/O2 [0-21]** systems (O2 is not visible for H303-T-H-CO2 systems).
B	CO2 concentration read by the CO2-H-IN-CHAMBER-Sensor.	Displayed **only when using the CO2-H-IN-CHAMBER Sensor**.
C	*O2 concentration in the chamber (calculated value).	Displayed **only when using the CO2-H-IN-CHAMBER Sensor**.
D	Relative Humidity read by the CO2-H-IN-CHAMBER-Sensor.	Displayed **only when using the CO2-H-IN-CHAMBER Sensor**.
E	Room Temperature read by the NTC Thermistor.	Displayed only if the NTC Thermistor is **connected to the H303-T-UNIT**.
F	Temperature read by the embedded sensor in the H303-Chamber Lid.	Displayed **at all times.**
G	Temperature read by the Temperature Sensor embedded in the H303-Chamber Base.	Displayed **at all times.**
H	Temperature read by the Temperature Sensor embedded in the OBJ-COLLAR.	Displayed **only if the OBJ-COLLAR is connected**
I	Temperature read by the T Sensor.	Displayed **only if the T sensor is connected**.
J	Relative Humidity read by the HM-ACTIVE-BL3.	Displayed only for **H303-T-H-CO2** and **H303-T-H-CO2/O2 [0-21]** systems.
K	Type of the Insert selected by the User	Displayed **at all times.**
L	Temperature Offset value applied on both the Lid and Base of the H303-Chamber	Displayed when working in **CHAMBER MODE.**
*O2 concentration only visible for **H303-T-H-CO2/O2 [0-21]** systems.		

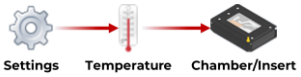
Table 4. Display Conditions for different Icons.

9 TEMPERATURE SETTINGS



This section outlines the temperature settings when operating the Top Stage system. Navigating to this menu you will have access to parameters such as the Chaber/Insert selection, Control Mode selection, Calibrations, advanced settings etc. all of which are explained in the following subheadings.

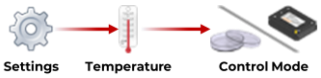
9.1 Chamber/Insert



As explained in the section 8.3, navigating to this menu gives you the option to select the H303-Chaber and the Sample insert being used. The page displays the current Chamber and Insert being used, in order to modify either the selected H303-Chamber or the Sample Insert being used you can simply click on the image displayed.

Note ► *Please note that you must always update the type of the Sample Holder Insert being used if you interchange the Sample Holder Insert.*

9.2 Control Mode



The Temperature Control can run in two different modes, by navigating to this menu, you can choose the one in which you'd like to work.

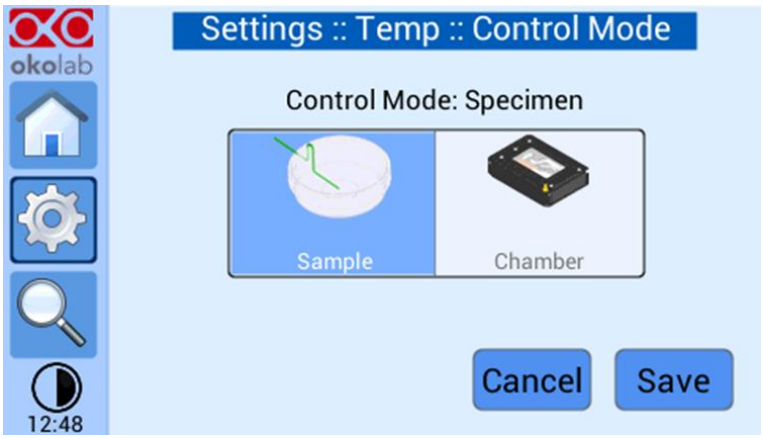


Figure 9. Control Mode selection page.

9.2.1 Chamber Mode

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. This is done by the feedback calculated in coherence to the type of sample insert being used.

The advantage of this solution is that you don't have to manually place the Fine Gauge Thermocouple into a Reference Well. The advantages of this Control Mode are: fast experiment start up and no specific action required in multi user applications. On selecting the ***'Chamber Mode'***, the following screen will give you a message indicating that the system has been calibrated at the factory at a Room Temperature of 23°C. In case your Room Temperature, measured by the NTC thermistor, is different from 23°C (for example 20.3°C) you have 3 options:

1. Use the Current Calibration: this means recalling the last saved calibration settings. Once you calibrate the system these settings will be automatically stored. The system saves only the last calibration performed. Using 'Current Calibration' is ***NOT Recommended*** in any of the following as it will yield inaccurate results:
    D. If the recalled calibration was performed with a different sample adapter than what you intend to use
    E. If the room temperature on the Calibration is significantly different than the current one (i.e. more than 1°C)
    F. If the Current Calibration was performed with a different set point temperature than what you plan on using (for example, the Calibration had set point of 37°C but new experiment requires a set point temperature of 39°C).
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. Choose to Calibrate the system in your lab at your Room Temperature, for maximum of accuracy when using "Chamber Feedback Mode". To do so follow the instructions on paragraph 7 (Chamber Self-Calibration) and then press the "Calibrate" button; the automatic calibration procedure will start.

Note ► *The Calibrations procedure is explained in detail in* ***Section 9.4****.*

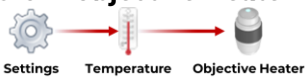
9.2.2 Sample Mode

This In this configuration, every time you run an experiment, you must place the provided T Sensor into a Petri dish (or your cell culture support). Fill the petri dish with distilled water to a level allowing full immersion of the T Sensor. If possible close the petri with its own plastic lid or the Sensor Lid (if you have purchased one) and place it in the appropriate holder. In case of Multiwell plates, you may assign a well to have the T sensor placed within it (this well will be referred to as the Reference Well). 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 the Room Temperature variations. Advantages of Sample Feedback Control Mode are:

- Direct monitoring and active control of sample temperature and,
- Independence from room temperature variations.

- The temperatures of the Lid and the Base are controlled based on the feedback of the sample so this is more accurate.

9.3 Objective Heater



The Objective Heater can be connected around your objective lens by the following the steps illustrated in Figure 10. The Objective Heater wire is then connected to the port labelled 'Obj Heater behind the H303-T-Unit.

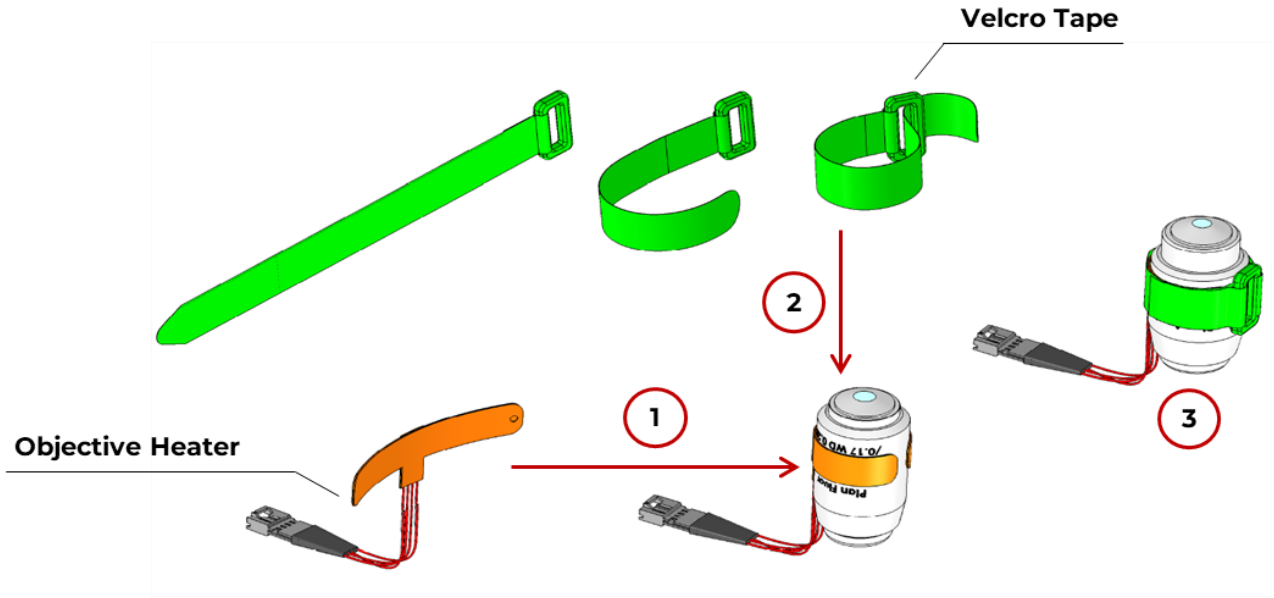
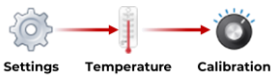


Figure 10. Fixing the Objective Heater.

By navigating to this menu, you can select the option to Enable or Disable the Objective Heater. Furthermore, you have the option to select the desired ***'Ramp Rate'***; by clicking on this option, you can choose to set the rate at which the Objective heater will be heated in order to reach the desired set point temperature of the sample.

Note ► *Please note that you have the option to set the **'Ramp Rate'** value to the maximum but this is advisable when the objective heater is still not in contact with the cell culture support as this may induce a thermal shock in your specimen.*

9.4 Calibration



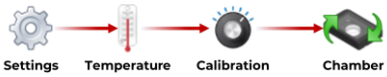
This section offers detailed instructions for calibrating the Chamber or the Objective heater. Please note that calibrations depend on:

- Temperature Set Point;
- Room Temperature;

- The type of Sample Holder Insert in use.

When the Calibration process is ongoing on the homepage, you'll be able to see the temperature calibration icon , clicking on this icon will navigate you back to the calibration page and display the progress bar of the procedure.

9.4.1 Chamber Calibration



This subheading explains the procedure for performing the calibration of the H303-Chamber, this procedure calibrates the embedded Temperature sensors within the Chamber Lid and Base in order to adjust the factory offset allowing you to achieve further accuracy of your temperature Set Point in the sample. You have the option to perform either the ***'Factory'*** reset of the chamber's calibration or perform a new calibration by selecting the ***'Calibrate'*** option.

In order to proceed with the Chamber calibration you will first need to attach the tip of the T Sensor to the bottom of a petri dish or the cell culture holder which you use along with the Sample Holder Insert that you've purchased, making sure that it is placed as centrally as possible (see Figure 11)

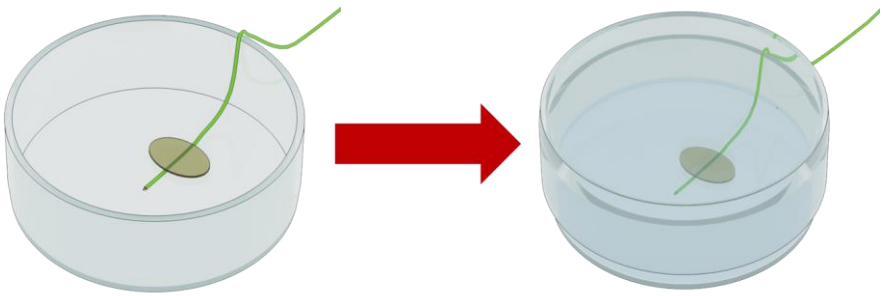


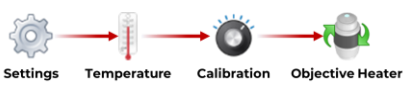
Figure 11. Petri Dish with T sensor placed centrally, filled with water and lid closed.

After placing the T sensor with in the petri dish, fill it with water and place it within the chamber (where the sample will be placed) with its top lid closed, and click on the ***'Calibrate'*** option on the screen.

Note ► *Before starting the Calibration, make sure you've set the correct working Set Point as the calibration will be performed at that Temperature Set Point.*

Note ► The Calibration procedure may take between 1—2 hours for completion.

9.4.2 Objective Heater Calibration



On navigating to this menu, you will have the option to select between the ***'Factory'*** reset, ***'Manual'*** offset insertion, or the performing the system's ***'Calibration'*** process.

Manual Offset Insertion: For inserting the offset manually, you can simply click on the ***'Manual'*** option, **go into Oil Contact** of the Objective lens with the Petri dish and wait for the T sensor reading **to stabilize**. Once

the temperature read by the T sensor has stabilized, insert the difference of the Set-Point temperature and the Temperature read by the T Sensor.

OFFSET (°C) = Set Point Temperature (°C) – Temperature read by the T Sensor (°C)

Calibration: For performing the Calibration procedure instead, you can do so with the help of the provided T-Sensor. The process is similar to that of the Chamber calibration i.e., you will have to attach the tip of the T-Sensor to the bottom of the cell culture support (as illustrated previously in Figure 11).

Note ► *Before proceed with the calibration of the Objective Heater, make sure that the chamber calibration (***'Self Calibration'** has already been performed, so that the T Sensor's reading is close to the Temperature Set Point.

Proceed with the calibration process by clicking on the ***'Calibrate'*** option **and then** go into Oil Contact of the Objective Lens with the Petri dish.

Note ► *This feature is only available if you have purchased the Objective Heater accessory and is connected to the H303-T-UNIT control unit.*

Note ► *Similar to the Chamber Calibration, before starting the Calibration, make sure you've set the correct working Set Point as the calibration will be performed at that Temperature Set Point.*

9.5 Advanced Temperature Settings (Temperature Cycles)

By clicking on this option, you can access the advanced temperature settings, this section allows you to set the temperature cycles.

In this menu, you can choose to load an existing gas cycle or setup a new one Figure 16Figure 12(a).

A temperature cycle is assigning two temperature set points to the system with a duration of time for which the set points will be adhered to before switching to the other set point. In order to setup a new temperature cycle, click on ***'New'***, this will navigate you to the page for selecting the SP1 and SP2 (Set Point 1 & Set Point 2 Figure 12 (b)), after which on clicking the ***'Next'*** option you can select the duration of each Set Point and the number of cycles you would like to impose (Figure 12 (c)). The page following this shows the summary of the temperature cycle setup page (Figure 12 (d)) with an option to either save the temperature cycle or proceed directly to the next step. The page allows you to save the temperature cycle as one of the configurations, the options greyed are empty configurations can be selected to save this new temperature cycle (Figure 12 (e)). The final page displays the option to select the time at which the temperature cycle should begin (Figure 12 (f)).

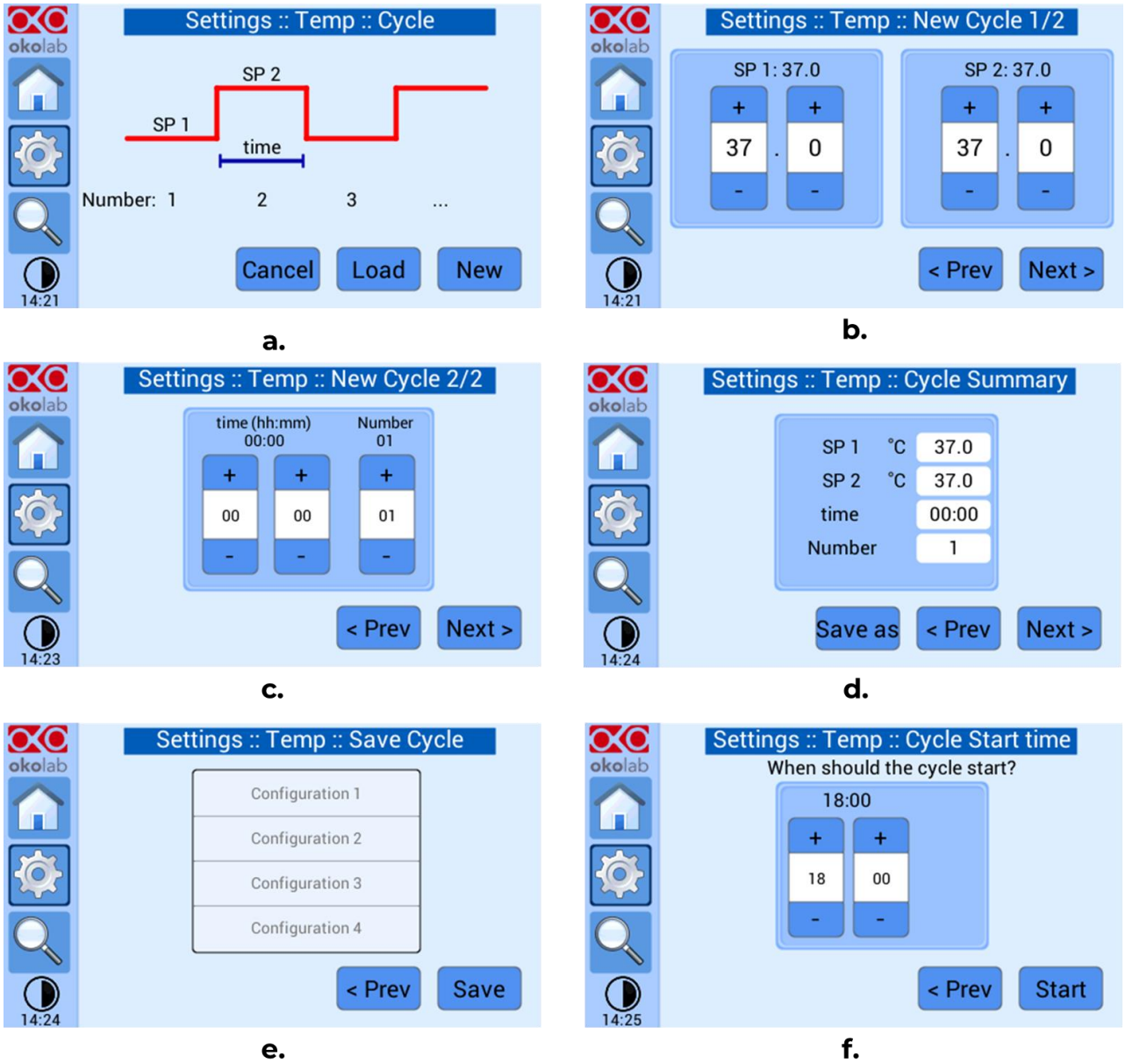


Figure 12. Temperature Cycles Setup.

10 GAS SETTINGS (NOT applicable for H303-T systems)

This section is dedicated for users with the gas control configuration, specifically with the H303-T-H-CO2 and the H303-T-H-CO2/O2 [0-21] systems.

10.1 Gas Pause

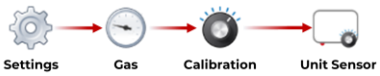
In order to pause the gas supply, simply click on the ***'Gas Pause'*** option, and on the pop-up window that appears select yes. In order to restart the gas supply, navigate to the same path and click on the ***'Gas Pause'*** option and the pop-up will ask you to confirm restarting the gas supply.

10.2 Calibration

In order to calibrate the gas sensor pertinent to the Gas control Unit or the CO2-H-IN-CHAMBER-Sensor, please navigate to the calibration page. These calibration procedures may be useful to ensure further accuracy of the systems functioning. The Unit Sensor calibration option allows you calibrate the Gas Control Unit's embedded sensors by utilizing an external gas sensor, whereas the In Chamber Sensor calibration option allows you to calibrate the CO2-H-IN-CHAMBER-Sensor by using the Gas Control Unit's sensors as a reference.

When the Calibration process is ongoing on the homepage, you'll be able to see the gas calibration icon  , clicking on this icon will navigate you back to the calibration page and display the progress bar of the procedure.

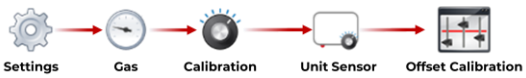
10.2.1 Unit Sensor Calibration



In this section, you will have access to the ***'Offset' calibration*** menu, the ***'Sensor' calibration*** menu and the ***'Factory Reset'*** menu.

Note ► *If you have the **H303-T-H-CO2/O2 [0-21]** system you will also have access to the **'O2 Zero Reset'** option.*

10.2.1.1 OFFSET Calibration



In this section, you can perform a calibration of the Unit sensor by utilizing an external meter. In order to proceed with the Offset calibration, please connect the gas controller's output tube (TUBE-A) to the inlet port of the external gas measurement meter. When the readings from the external meter reach a stable value, please click on the ***'Adj CO2'*** and manually enter the value measured by the external meter, once done, the system will automatically the offset which will be imposed to calibrate the unit sensor (Figure 13).

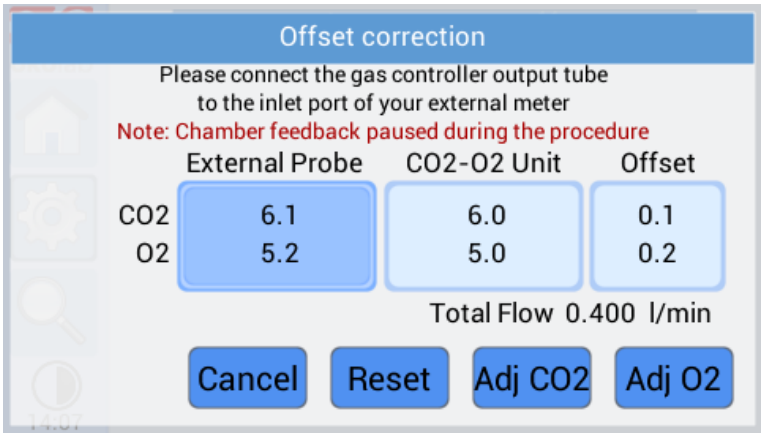


Figure 13. Gas Offset Calibration.

Note ► *The option to adjust the O2 value is available for* ***H303-T-H-CO2/O2 [0-21]-*** *system.*

Note ► *After performing the* ***Offset Calibration*** *please perform an* ***IN-Chamber Sensor Calibration*** *(section 10.2.2)*

10.2.1.2 SENSORS Calibration

In this section, you can perform a calibration of the Unit sensor by utilizing ***Certified Calibration*** gas. On navigating to this section, you will have access to the following options:

A. Gas Setting: Clicking on this option you'll be navigated to the page where you have to insert the information relative to the ***Certified Calibration*** gas being utilized. Please enter the correct CO2 and O2 concentrations as reported on the cylinder. Enable/Disable the *'Add Zero Calibration'* depending on your requirement (the zero calibration for both CO2 & O2 is performed utilizing the N2 gas).

B. Start Calibration: Once you have assigned the desired gas settings, click on the *'Start Calibration'* icon and follow the on-screen instructions.

C. View Calibration: Selecting this option will allow you to view the latest calibration details of the Unit sensor. Details such as the latest CO2 Span Correction and the CO2 Zero Offset values and if they were custom or factory performed. The same parameters for O2 are also visible if you possess the *H303-T-H-CO2/O2 system.*

Note ► *After performing the* ***Sensors Calibration*** *please perform an* ***IN-Chamber Sensor Calibration*** *(section 10.2.2)*

10.2.1.3 O2 ZERO RESET

This option is only available if you have the H303-T-H-CO2/O2 [0-21] system, and will allow you to perform a zero reset of the O2 using **N2 connected** to the **CO2/O2 [0-21]-UNIT**. In order to do so, click on this option and a pop-up message will warn you that the procedure requires the system to stop the control ask you to confirm if you'd like to proceed.

Note ► *The system will alert you monthly to perform this O2 ZERO RESET procedure.*

10.2.1.4 FACTORY RESET

This option will allow you to perform a factory reset of the sensors of the Control Unit sensor, it will remove all the offset calibrations imposed with the help of an external meter. On selecting this option, a pop-up message will ask you to confirm this selection as this cannot be undone.

10.2.2 IN-Chamber Sensor Calibration

The calibration of the CO2-H-IN-CHAMBER-Sensor can be performed by navigating to this menu. In this section, you can perform a calibration of the Unit sensor by utilizing ***the utilizing the gas supply already connected to the Gas Control Unit***. Please note that in order to proceed with this procedure you will have to utilize the provided '***Calibration Insert'.***

Note ► *The system will alert you monthly to perform this calibration procedure.*

Note ► *This calibration procedure is suggested to be performed after performing the* ***Offset Calibration*** *or the* ***Sensors Calibration.***

In this section, you will have access to the following options:

A. Gas Setting CO_2: In order to perform the calibration of the IN-Chamber sensor you'll need to insert the settings of the gas (CO2) to which the sensor will be calibrated. Depending on the CO2 concentration Set Point you work with, you have the option to perform either a single point calibration or a 2 Point calibration.

The single point calibration is advisable when you always work with the same Set Point value for the CO2 concentration. If in case you work with different CO2 concentration Set Points, performing a 2 Point calibration (with the maximum and minimum values of your working Set points as the CO2 Point 1& CO2 Point 2) is advisable.

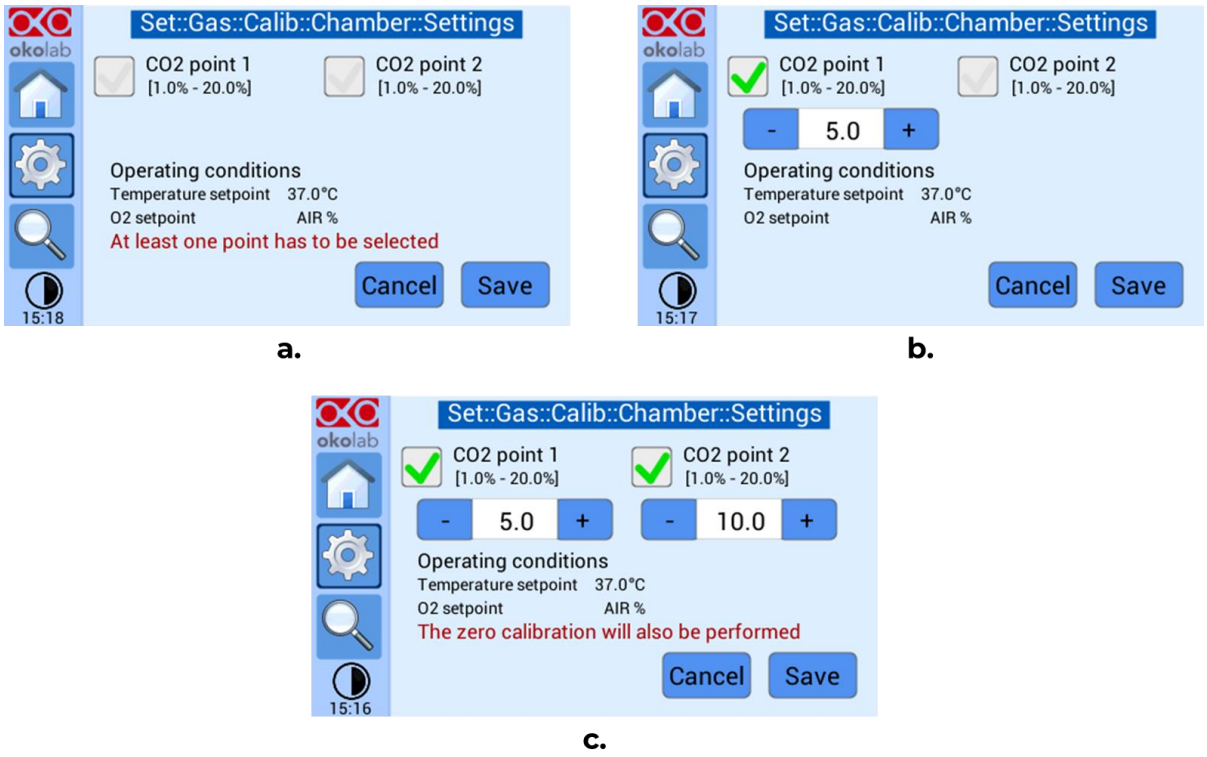


Figure 14. CO2-H-IN-CHAMBER Calibration Gas Settings.

As illustrated in Figure 14(a) at least one CO2 point for calibration must be selected. If you perform the calibration just for a single point the second CO2 point must be unflagged and no zero point calibration will be performed (Figure 14(b)).Lastly, if you perform 2 point calibration the zero calibration will be performed automatically (Figure 14(c)).

B. Start Calibration : Once you have assigned the desired gas settings, click on the ***'Start Calibration'*** icon and follow the on-screen instructions.

C. View Calibration : Selecting this option will allow you to view the latest calibration details of the IN-Chamber sensor. Specifically details such as the *'Last Calibration Date'*, *'Calibration Results'* and the *'Operating Conditions'* during which the calibration was performed.

D. Factory Reset : To perform the factory reset of the CO2-H-IN-Chamber sensor click on the ***'Factory Reset' option.*** A pop-up window will ask you to confirm this action, please select ok to confirm.

10.3 Advanced Settings

You can access the advanced gas settings by clicking on the ***'Advanced Settings'*** icon. Under this section you can find Gas Flowrates settings, the Air source settings, and the Gas cycles settings.

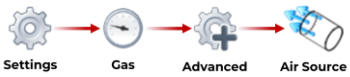
10.3.1 Gas Flowrates Setting

By navigating to this menu, you can modify the flowrates of Air, CO2 & O2 (only in case of H303-T-H-CO2/O2 [0-21] system) or the total flowrate.

Note ► *Please keep in mind that only when you run the system without N2, total gas flowrate can be set to 0.1 l/min. If you were using this setting and change mode to AIR/N2/CO2 mode, the flowrate set point will be forced to the new minimum which is 0.2 Liters for minute.*

Note ► *When using the **CO2-H-IN-CHAMBER SENSOR** the **minimum value** of the flowrate that can be set is 0.2 l/min.*

10.3.2 Air Source Setting

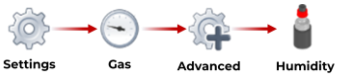


By navigating to *the **'Air Source'*** menu, you can choose desired air source between a Compressed Air option or using the **OKO-AIR-PUMP-BL** provided along with your system.

Note ► *When using the Compressed Air option, please ensure that the gas is available at 1.4 barg (20 psi).*

On selecting the **OKO-AIR-PUMP-BL** two more options are displayed on the screen namely:
USAGE: On clicking on the '***Usage'*** option, you can view the total number of hours for which the pump has been utilized. If you have replaced the pump please click on the ***'Reset'*** option to reset the pump life to zero.
ADVANCED: On clicking on the '***Adv'*** option, you can increase or decrease the Air pump Speed offset (also referred to as 'duty').

10.3.3 Humidity Setting



The Humidity Settings are only displayed when the RH Set Point value is 95%. By navigating to this option, you can regulate the humidity by choosing between a *'Less Humid'* or a *'More Humid'* option and also activate/Deactivate the *'Humidity Status Led'*.

As illustrated in Figure 15, you can modify the humidity value to more or less humid by moving the regulator left or right. Each bar on the regulation bar corresponds to 1% of humidity.

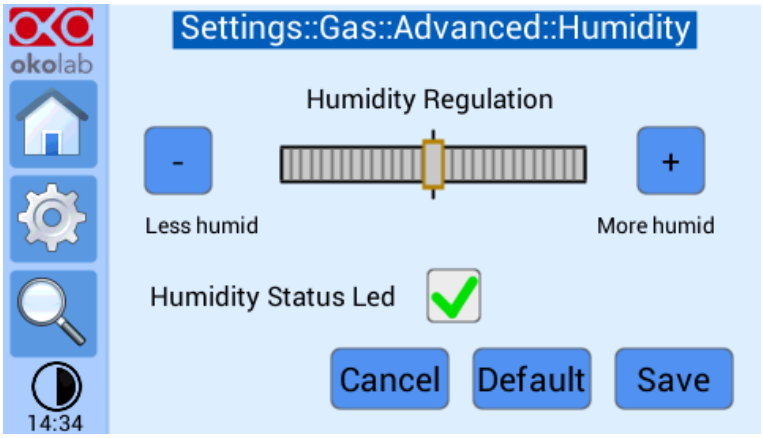
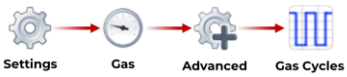


Figure 15. Humidity Regulation.

If your chamber has a humidity setpoint of 95% but see rapid evaporation of the sample medium (maybe due to constant opening closing of the chamber) you can select to provide more humidity to the chamber by moving the selector bar to the right and vice versa for the other way round.

10.3.4 Gas Cycles Setting



In this menu, you can choose to load an existing gas cycle or setup a new one Figure 16 (a).

A gas cycle is assigning two gas set points to the system with a duration of time for which the set points will be adhered to before switching to the other set point. In order to setup a new gas cycle, click on ***'New'***, this will navigate you to the page for selecting the CO2 SP1 and SP2 (Set Point 1 & Set Point 2 Figure 16 (b)), after which on clicking the ***'Next'*** option you can select the duration of each Set Point and the number of cycles you would like to impose (Figure 16 (c)). The page following this shows the summary of the gas cycle setup page (Figure 16 (d)) with an option to either save the gas cycle or proceed directly to the next step. The page allows you to save the gas cycle as one of the configurations, the options greyed are empty configurations can be selected to save this new gas cycle (Figure 16 (e)). The final page displays the option to select the time at which the gas cycle should begin (Figure 16 (f)).

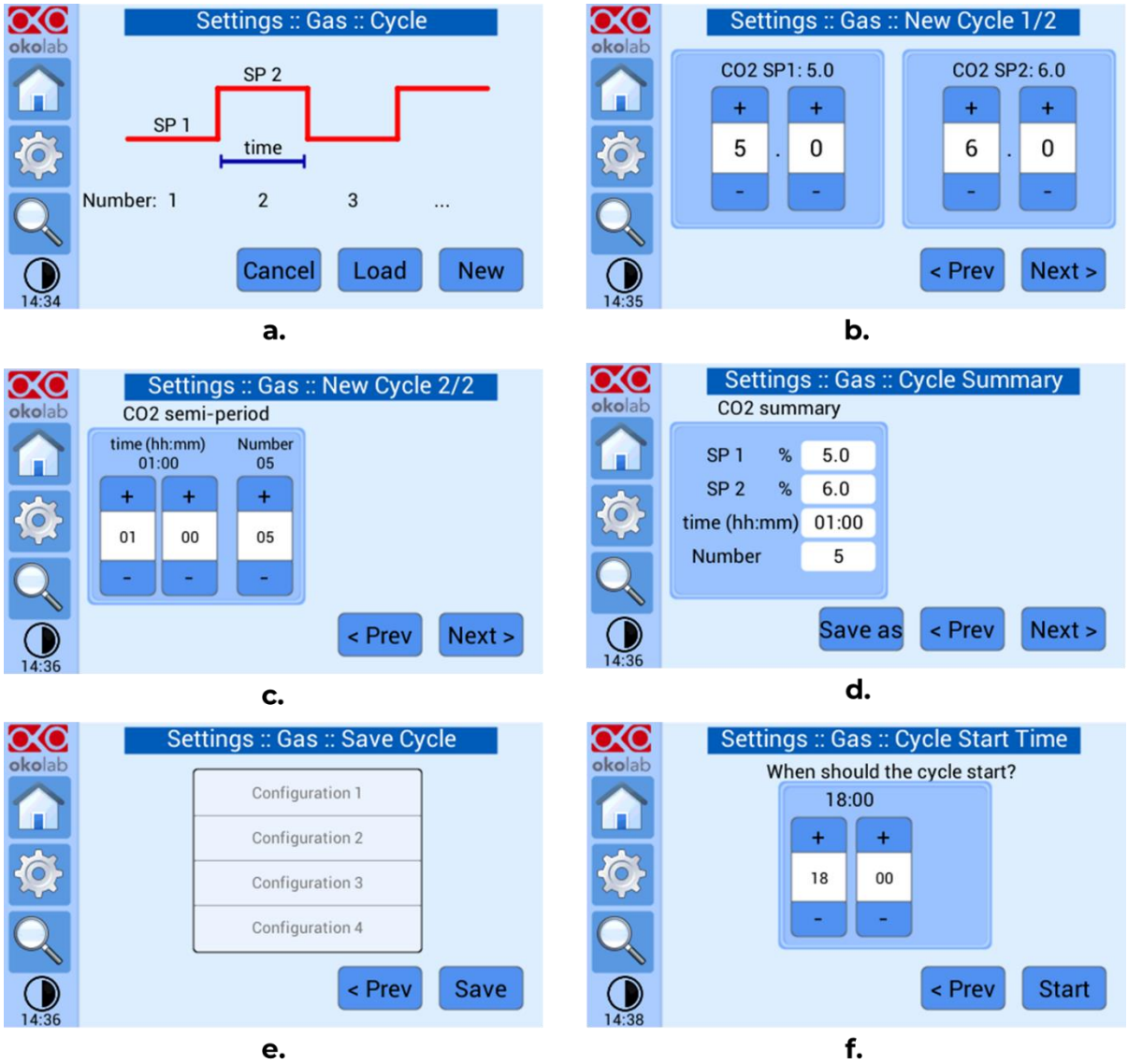


Figure 16. Gas Cycles Setup (a-f).

11 ALARM SETUP

This section details the Alarm setting for different variable being worked with. When the system goes into an Alarm state the **homepage** of the OKO-TOUCH-BL3 displays the '***Alarm***' icon ⚠ on the bottom left. The Status LED of the OKO-TOUCH-BL3 as illustrated in Figure 17 (a) starts **to change color.** On the other hand, the Status LED of the Control unit, as illustrated in Figure 17 (b), starts to **blink** corresponding to the Status indicator on the home page.

Figure 17. Status LED: (a) On the OKO-TOUCH-BL3; (b) On the Control Unit (CO2-UNIT-BL3).

Figure 18 (a & b) shows an example of an alarm Message that is displayed on clicking the '***Alarm***' icon ⚠ on the bottom left homepage.

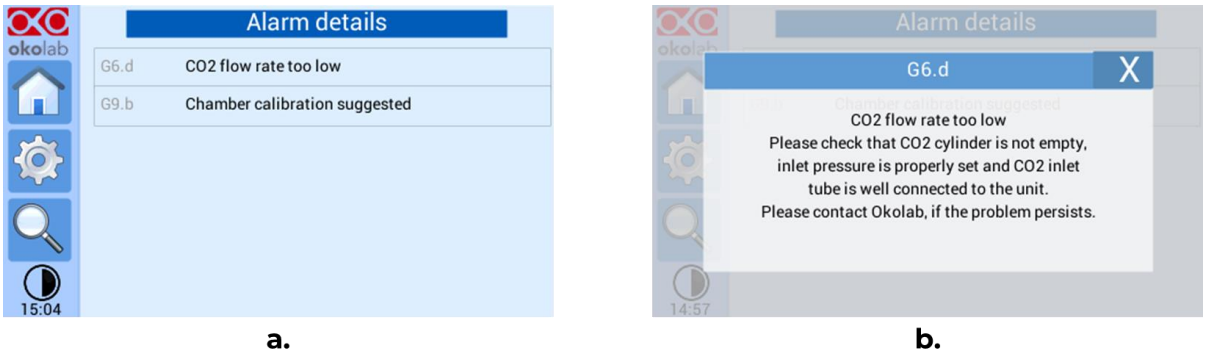


Figure 18. Alarm Message.

The following sub headings detail the alarm setup procedures for the Temperature, Gas and Humidity.

11.1 Temperature Alarm

In this section, you can setup the value of the deviation (tolerance), in °C, from the temperature set point and the time for which the system has to read the measurement out of tolerance value, before going into an alarm state (Figure 19).

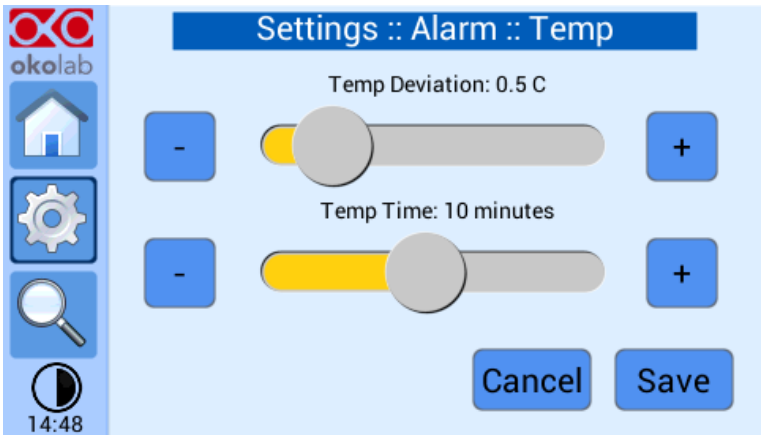


Figure 19. Temperature Alarm Settings Page.

11.2 Gas Alarm

The Gas alarm settings not available for H303-T systems. In this section, you can setup the value of the deviation (tolerance), in % of concentration, from the gas set point and the time for which the system has to read the measurement out of tolerance value, before going into an alarm state (Figure 20).

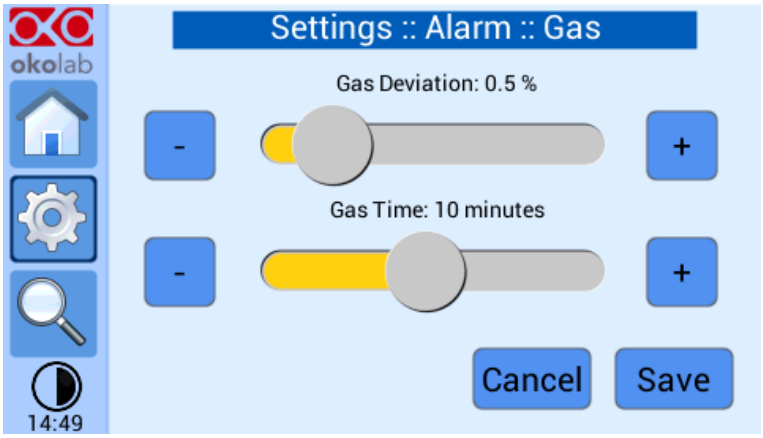


Figure 20. Gas Alarm Settings Page.

11.3 Humidity Alarm

The Humidity alarm settings not available for H303-T systems. From this menu, you can setup the value of the deviation (tolerance), in % of humidity, from the humidity set point and the time for which the system has to read the measurement out of tolerance value, before going into an alarm state (Figure 21).

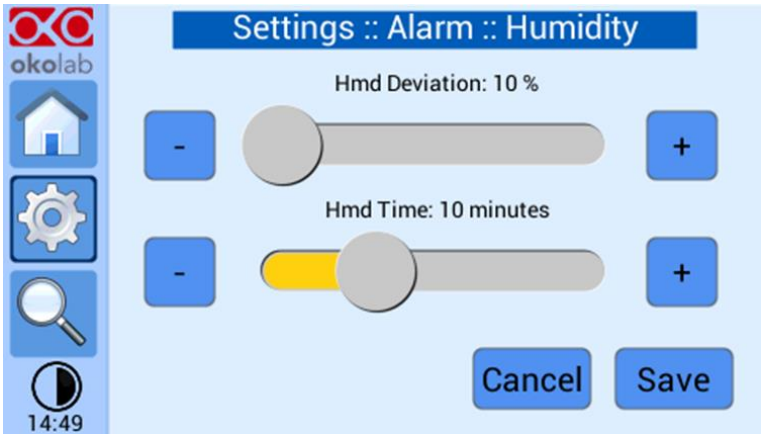


Figure 21. Humidity Alarm Settings Page.

12 SYSTEM STATUS

For quick visualization of the system's status, you can navigate to this menu and view the details pertinent to the system's functioning. The figure below illustrates the status pages for Temperature (Figure 22 (a)), Gas (Figure 22 (b)), Humidity (Figure 22 (c)) and the CO2-H-IN-CHAMBER-Sensor (Figure 22 (d)).

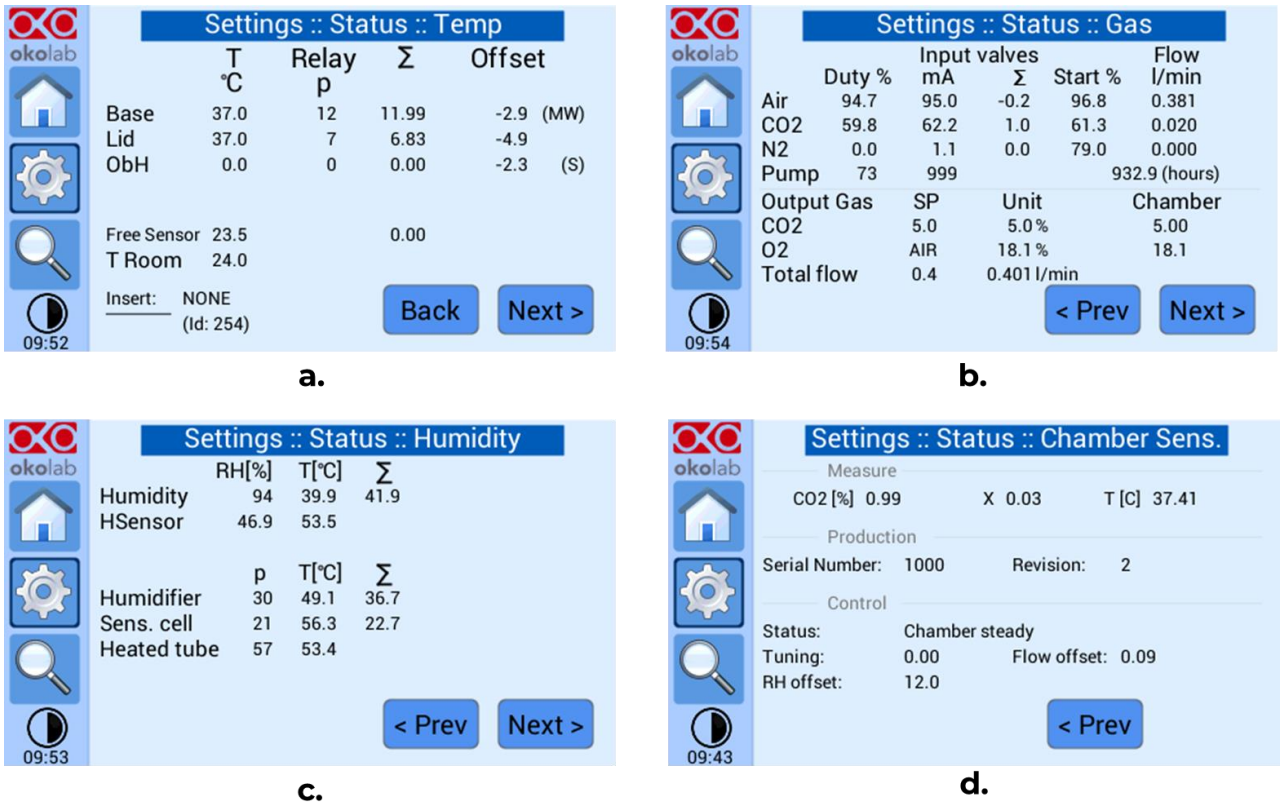


Figure 22. Status Pages for different parameters (a-d).

On the homepage, clicking on the ***OKOLAB*** icon on the top left of the screen will allow you to view system details such as the release date, serial number, software version etc. as illustrated in Figure 23.

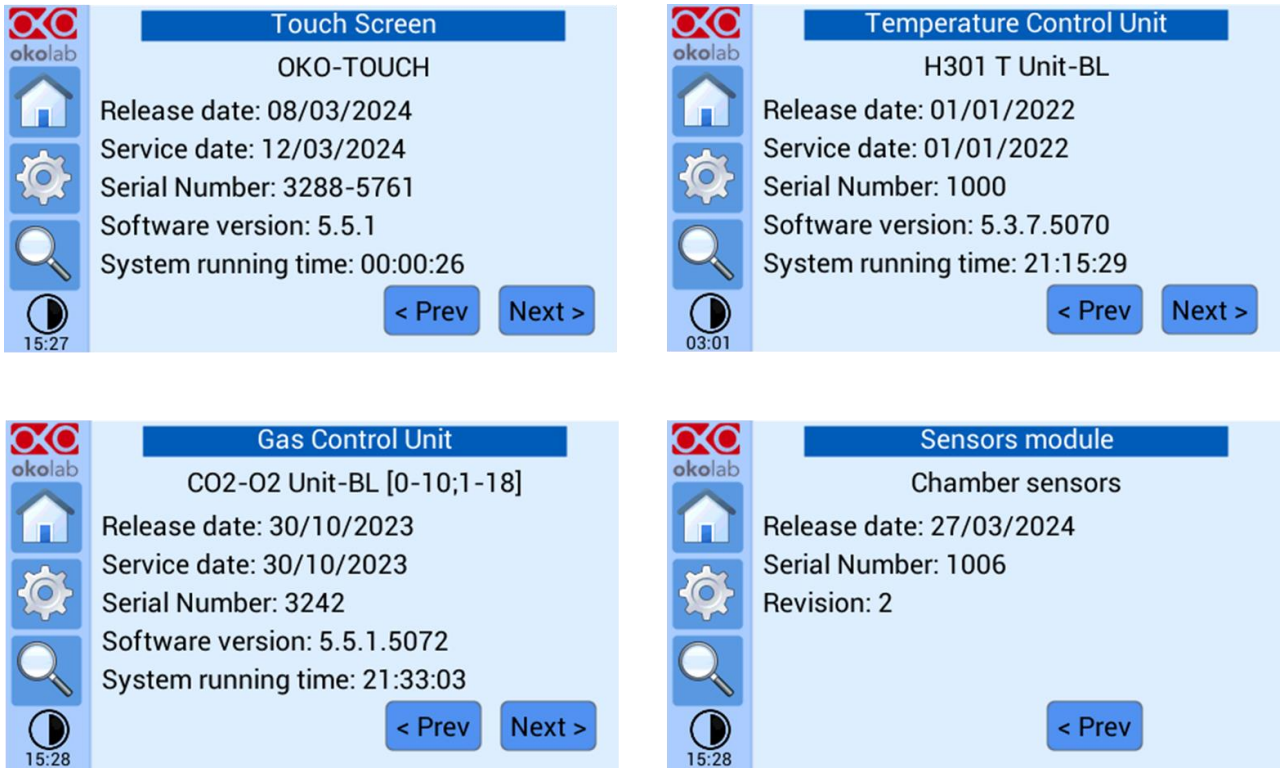


Figure 23. System Information for different devices of the Enclosure

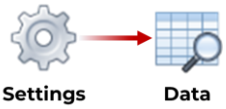
Note ► *These details such as the serial number, status pages, will be requested by the OKOLAB Technical Support team during assistance sessions. If you find yourself in need for a technical assistance, please attach images of the Status page for each of the parameters available to you relative to the Top Stage system that you possess.*

13 SYSTEM SETTINGS

In this menu the following system settings are available:

- **Visual Effects :** From this page you can set the Top LED to be enabled as per your requirement, and select between setting the High contrast color as 'White' or 'Dynamic'.
- **Brightness :** From this page you can set the brightness of the OKO-TOUCH-BL3's screen.
- **Date & Time :** From this page you can set the Date & Time on the OKO-TOUCH-BL3. Please note that we suggest to do this during the first setup of your system.
- **Touch Options :** From this page you can set the Touch Sound as enabled/disabled and also manipulate the buzzer frequency as you prefer.

14 DATA SETTINGS



This section covers the settings pertinent to the Data being worked with using the Top Stage system. In this menu you will be able to access the following settings:

Data Logging Settings

In the data Logging settings menu, you can choose between logging the data directly on to an external USB Pen Drive or to the device's internal memory.

Saving data to External USB Drive: To start logging the data directly to your external USB Pen Drive and check the box to enable the ***'USB Drive'*** option. The USB Pen Drive must be connected to the OKO-TOUCH-BL3 via the provided OTG cable (as shown in Figure 24).

Figure 24. OTG Cable port on the OKO-TOUCH-BL3 for attaching the USB Pen Drive.

Saving data to Internal Memory: To start logging the data to the device's internal memory, enable the ***'Internal Memory'*** option. By clicking on the screwdriver icon , you can view the **start date** from which the logging has been active, the **Available Memory** and the **Latest Download date**. Besides you can also choose to ***'ERASE'*** the data in the device or download it to a USB Pen Drive by clicking on ***'To USB'*** option and transfer data to a USB Pen Drive connected to OKO-TOUCH-BL3.

On this page you also have access to the following functions:

- Set the Time Interval for data logging, this can be done by clicking on the ***edit*** icon and manually entering the time interval on the pop-up menu.
- Select whether you'd like your data to be grouped by ***'Day', 'Week' or 'Month'.***
- Lastly, select the ***'File name suffix'*** that you'd like to set to the logged data by clicking on the ***keyboard*** icon.

Data Chart Settings

In the data Chart settings menu, you can set the length (in terms of time duration) of the chart history.

15 TOUCH SCREEN CALIBRATION

Keep pressed the ON/OFF button on OKO TOUCH for 7 seconds to start the Touch Screen Calibration. While holding the button, the pop-up message 'Keep the button pressed for 8 seconds to calibrate the touch. Once the calibration starts, tap blue calibration dots until the calibration is complete.

Figure 25. Performing the Touch Calibration.

16 TROUBLESHOOTING

Please refer to the following Alarms list for the troubleshooting.

Alarm Code	Alarm Name	Remedy
Temperature Alarms		
T1.a	**Temperature far from setpoint**	Please ensure that the Chamber is closed and the samples Insert is placed correctly; If the problem persists, contact OKOLAB Technical Support team for further assistance.
T4.a	**Base sensor error**	Please ensure that the Chamber cable is properly connected to the H303-T-Unit rear panel; If the problem persists, contact OKOLAB Technical Support team for further assistance.
T4.b	**Sample sensor error**	Please ensure that the T Sensor cable is properly connected to the H303-T-Unit rear panel; If the problem persists, contact OKOLAB Technical Support team for further assistance.
T4.c	**Lid sensor error**	Please ensure that the Chamber cable is properly connected to the H303-T-Unit rear panel; If the problem persists, contact OKOLAB Technical Support team for further assistance.
T4.d	**Objective sensor error**	Please ensure that the Objective Heater Cable is properly connected to the H303-T-Unit rear panel; If the problem persists, contact OKOLAB Technical Support team for further assistance.
T4.f	**Room sensor error**	Please ensure that the room temperature sensor is properly connected to the H303-T-Unit rear panel; If the problem persists, contact OKOLAB Technical Support team for further assistance.
T6.a	**Base relay not working**	Contact OKOLAB Technical Support team for further assistance.

T6.c	**Lid relay not working**	Contact OKOLAB Technical Support team for further assistance.
T6.d	**Objective relay not working**	Contact OKOLAB Technical Support team for further assistance.
T9	**Setpoint invalid near room temperature**	The Set Point temperature must be at least 3°C above the room temperature; Contact OKOLAB Technical Support team for further assistance.
T11	**Chamber open for a long time**	Incubator opened for more than 5 minutes Ignore this warning if the opening is intended. Close the incubator to cancel this alert; Contact OKOLAB Technical Support team if the problem persists.
Gas Alarms		
G1.a	**CO2 far from setpoint**	Please ensure that the gas tubes are properly connected; Please ensure that the CO2 supply cylinder isn't empty and the inlet pressure is properly set. If the problem persists, please contact OKOLAB Technical Support team for further assistance.
G1.b	**O2 far from setpoint**	Please ensure that the gas tubes are properly connected; Please ensure that the N2 supply cylinder isn't empty and the inlet pressure is properly set; Please ensure that the air pump is connected properly to the gas control unit; If the problem persists, please contact OKOLAB Technical Support team for further assistance.
G1.c	**CO2 in Chamber far from setpoint**	Please ensure that the gas tubes are properly connected; Please ensure that the CO2 supply cylinder isn't empty; Please ensure that the Samples Insert doesn't have any vacant slots If the problem persists, please contact OKOLAB Technical Support team for further assistance.

G1e	**Air Flow far from setpoint**	Please try increasing the air pump duty offset or check the pressure of the air cylinder If the problem persists, please contact OKOLAB Technical Support team for further assistance.
G4.a	**CO2 sensor error**	Contact OKOLAB Technical Support team for further assistance.
G4.b	**O2 sensor error**	Contact OKOLAB Technical Support team for further assistance.
G4.c	**CO2-H-IN-CHAMBER SENSOR error**	Please check that sensor is properly installed in the chamber and that the chamber is well connected; If the problem persists, please contact OKOLAB Technical Support team for further assistance.
G6.d	**CO2 flow rate too low**	Please ensure that the gas tubes are properly connected; Please ensure that the CO2 supply cylinder isn't empty and the inlet pressure is properly set; If the problem persists, please contact OKOLAB Technical Support team for further assistance.
G6.e	**Air flow rate too low**	Please ensure that the gas tubes are properly connected; Please check that Air pump is connected to the unit, is active and is actually vibrating; If the problem persists, please contact OKOLAB Technical Support team for further assistance.
G6.f	**N2 flow rate too low**	Please ensure that the gas tubes are properly connected; Please ensure that the N2 supply isn't empty or improperly connected; If the problem persists, please contact OKOLAB Technical Support team for further assistance.
G7.a **G7.b**	**Low CO2 detected**	Please ensure that the gas tubes are properly connected; Please verify that chamber is correctly closed, all tubes are properly connected and sample holders are correctly placed;

		If the problem persists, please contact OKOLAB Technical Support team for further assistance.
G8	**Air pump lifetime expired**	Please contact OKOLAB Technical Support team for a replacement of the pump.
G9.a	**O2 Zero reset suggested**	Please perform the O2 Zero Reset.
G9.b	**Chamber calibration suggested**	Please perform the Chamber Calibration.
G10.a	**CO2 calibration failed**	Please re-perform the calibration procedure; Ensure the proper gas connections. If the problem persists, please contact OKOLAB Technical Support team for further assistance.
G10.b	**O2 calibration failed**	Please re-perform the calibration procedure; Ensure that the N2 gas supply is not empty. If the problem persists, please contact OKOLAB Technical Support team for further assistance.
G10.c	**CO2 in chamber calibration failed**	Please re-perform the calibration procedure; Ensure that the calibration insert is placed in the chamber during the calibration procedure; Please ensure that the CO2 supply isn't empty; If the problem persists, please contact OKOLAB Technical Support team for further assistance.
G12.a	**CO2 drift too high**	Please re-perform the calibration procedure; If the problem persists, please contact OKOLAB Technical Support team for further assistance.
G12.b	**Chamber leakage too high**	Please verify that chamber is correctly closed, all tubes are properly connected and sample holders are correctly placed. If the problem persists, please contact OKOLAB Technical Support team for further assistance.

Humidity Alarms		
H1.a	**Humidity far from setpoint**	Low humidity read by HM-Active sensing cell, please refill the HM-Active bottle and verify that all tubes are properly connected; If the problem persists, contact OKOLAB Technical Support team for further assistance.
H1.b	**Humidity far from setpoint**	Please ensure that the chamber is properly closed and all tubes are properly connected and sample holders are correctly placed; If the problem persists, contact OKOLAB Technical Support team for further assistance.
H4.a	**Humidity sensor error**	Check that the sensing cell power cable is well connected to the humidifier; If the problem persists, contact OKOLAB Technical Support team for further assistance
H4.b	**HM-ACTIVE humidifier sensor error**	Contact OKOLAB Technical Support team for further assistance.
H4.c	**Sensing Cell Temperature sensor error**	Please ensure that the Sensing Cell cable is properly connected to the base of the HM-ACTIVE-BL3; If the problem persists, contact OKOLAB Technical Support team for further assistance.
H4.d	**Tube T sensor error**	Please ensure that the heated tube's cable is properly connected to the base of the HM-ACTIVE-BL3; If the problem persists, contact OKOLAB Technical Support team for further assistance.
H14	**Water Bottle Empty**	Refill the water bottle up to the indicated water level; If the problem persists, contact OKOLAB Technical Support team for further assistance.

17 CLEANING & MAINTENANCE

17.1 Cleaning

The following subheadings explain the cleaning of the different components.

17.1.1 Control Units Cleaning

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

---

Before cleaning the unit, disconnect all the electrical connections.
Water must not enter in the system.

17.1.2 Chamber Cleaning

In order to keep the Chamber clean, please follow the steps below:

4. Turn the system off and pull the mains plug out the socket
5. Wait till the chamber cools down.
6. To clean the body of the chamber, wipe with a soft micro-fiber cloth. For stubborn smudges, you can damp the soft micro-fiber cloth with ethyl alcohol (product code UN1170). Do not put any liquid directly on the chamber. While cleaning the glass lid, do not apply strong force to the surface of the glass lid because it can be damaged.

17.1.3 HM-ACTIVE-BL3 Cleaning

In order to keep the HM-ACTIVE-BL3 clean please follow the steps below:

1. Turn the system off and pull the mains plug out the socket.
2. Disconnect the HM-ACTIVE-BL3 from the H303-T-UNIT.
3. Disconnect the Sensing cell from the Base.
4. Wait the bottle cools down.
5. Empty the bottle.
6. Wipe the exterior of the bottle with a 70% alcohol solution.
7. Only if needed rinse the bottle with the above-mentioned solution and wait till the bottle's interior is completely dry before reusing it.

When the system is switched off by the OKO-TOUCH-BL3, the sensing cell still remains ON to avoid the water from condensing on it.
When you disconnect the system from the power supply, or when the HM-ACTIVE-BL3 is not being utilized, we advise you to remove the sensing cell from the bottle to avoid water condensation.

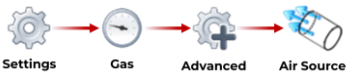
17.1.4 CO2-H-IN-CHAMBER SENSOR Cleaning

The maintenance of the CO2-H-IN-CHAMBER Sensor must be performed when suggested by the Okolab Technical Support team. The process involves the substitution of the white membrane, every six months, present within the sensor. Detailed instructions can be found in the dedicated instructions manual.

When handling (maintenance) the CO2-H-IN-CHAMBER Sensor please use electrostatic gloves at all times.

17.1.5 OKO-AIR-PUMP-BL Cleaning

The OKO-AIR-PUMP-BL has a lifetime of 6000 hours. When the maintenance of the embedded pump is due the system will display the alarm code **G8- "Please contact Okolab to replace OKO-AIR-PUMP-BL."** On the homepage advising you to change the pump. Contact Okolab Technical Support Team for instructions on the pump replacement procedure.

Note ► *After replacing the pump, please navigate to* 

and click on the ***'Pump' > 'Usage' >*** *and click on* ***'Reset'****.*

17.2 Maintenance

The following table reports the list of the spare parts and when they should be replaced.

Spare Parts List	Suggested Replacment Time
Pump	At end of its Lifetime. (when you see the alarm code G8)
CO2 Valve	If suggested by Okolab Technical Support Team.
CO2 Sensor	At end of its Lifetime.
Air Valve Mass flow sensor	If suggested by Okolab Technical Support Team.
O2 Sensor	At end of its Lifetime.
CO2-H-IN-CHAMBER Sensor White Membrane	Every 12 months.
HM-ACTIVE Glass Bottle	If Damaged.

Hydrophobic PTFE (for OKO-AIR-PUMP-BL)	Every 12 months.

18 TECHNICAL SPECIFICATIONS

H303-T system - Technical Specifications	
Measurement Range	3°C above ambient temperature to 45°C
Accuracy	± 0.1°C
Step size	0.1 °C
Power Consumption	
Control Unit – Supply Voltage	24 VDC
Power Consumption	100 W max
External Environment Requirements – Indoor Use	
Temperature Range	18 – 30 °C (23 °C suggested)
Storage Temperature	-5 – 60 °C
Relative Humidity Range	0 - 70%

H303-T-H-CO2 system - Technical Specifications	
Temperature Control	
Measurement Range	3°C above ambient temperature to 45°C
Accuracy	± 0.1°C
Step size	0.1 °C
CO2 Control	
Measurement Range	0-20%
Accuracy	± 0.1%
Step size	0.1%
Output Flowrate	0 – 0.4 l/min
Humidity Control	
Measurement Range	• 89 – 95% @25°C • 51 – 95% @35°C • 26 – 95% @50°C
Step Size	1.0%
Accuracy	± 3.0%
Expected Lifetime	
CO2 Sensor	5 years
OKO-AIR -PUMP-BL	6000h
Gas Input Requirements	
CO2 Inlet Pressure	1.4 barg (20 psig)
CO2 Gas Purity	99.995%
Gas Connectors	6.0 OD Push to fit connector
Power Consumption	
Control Unit – Supply Voltage	24 VDC
Power Consumption	180 W max
External Environment Requirements – Indoor Use	

Temperature Range	18 – 30 °C (23 °C suggested)
Storage Temperature	-5 – 60 °C
Relative Humidity Range	0 - 70%

H303-T-H-CO2/O2 [0-21] system - Technical Specifications	
Temperature Control	
Measurement Range	3°C above ambient temperature to 45°C
Accuracy	± 0.1°C
Step size	0.1 °C
CO2/O2 Control	
CO2 Measurement Range	0-20%
CO2 Accuracy	± 0.1%
CO2 Step size	0.1%
O2 Measurement Range	0-21% [1]
O2 Accuracy	± 0.1%
O2 Step size	0.1%
Output Flowrate range	0 – 0.4 l/min
[1] Operating range may decrease to 0-10% after 2 years of operation	
Gas Input Requirements	
CO2 Inlet Pressure	1.4 barg (20 psig)
CO2 Gas Purity	99.995%
N2 Gas Purity	99.995%
N2 Inlet Pressure	1.4 barg (20 psig)
Gas Connectors	6.0 OD Push to fit connector
Humidity Control	
Measurement Range	• 89 – 95% @25°C • 51 – 95% @35°C • 26 – 95% @50°C
Step Size	1.0%
Accuracy	± 3.0%
Expected Lifetime	
CO2 Sensor	5 years
O2 Sensor	3 years
OKO-AIR -PUMP-BL	6000h
Power Consumption	
Control Unit – Supply Voltage	24 VDC
Power Consumption	180 W max
External Environment Requirements – Indoor Use	
Temperature Range	18 – 30 °C (23 °C suggested)
Storage Temperature	-5 – 60 °C
Relative Humidity Range	0 - 70%

CO2-H-IN-CHAMBER Sensor - Technical Specifications	
Expected Lifetime	5 years
CO2 Measurement Range	0-20%
CO2 Measurement Accuracy	± 0.1%
Humidity Measurement Range	50-99%
Humidity Measurement Accuracy	± 3.0%

19 FIGURES LIST

20 TABLES LIST

21 SUPPORT

To contact one of our engineers please send an email to the technical support address listed below or contact us through the live chat on www.oko-lab.com. You can request a remote support session anytime.

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

For TECHNICAL SUPPORT: support@oko-lab.com
Phone +39 081 806 3470
Fax: +39 081 876 4410
Mobile: +39 348 96807 18

For COMMERCIAL SUPPORT WORLDWIDE: lanzaro@oko-lab.com
Phone +39 081 806 2624
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Mobile: +39 348 96807 17

For COMMERCIAL SUPPORT US&CANADA: usa@oko-lab.com

For COMMERCIAL SUPPORT CHINA: tong@oko-lab.cn

WARRANTY

Okolab S.r.l. warrants “Bold Line 3 Top Stage systems" to be free of defects in materials and workmanship for a period of three years starting from invoice date. If the units malfunction, they 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. 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. Components which wear are not warranted. Okolab S.r.l. neither assumes responsibility for any omissions or errors nor assumes liability for any damage that may results from improper use of its products in accordance with information provided by Okolab S.r.l. Okolab S.r.l. warrants only the parts manufactured by Okolab S.r.l to be 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 damage.