CO2 O2 UNIT BL-CP2
Tri-Gas Mixer with Controlled Pressure

Manual

Vers. 06.14
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The CO2/O2 Unit BL–CP2 is a Digital CO2/O2 Controller that generates Air/CO2 or N2/CO2/O2 mixtures of desired concentration by mixing together Air, CO2 and Nitrogen. Gas output has constant pressure up to 1.5 bar. CO2 and O2 concentration can be adjusted within 0-10%. Input requirements: compressed air, CO2. Add N2 for tri-gas mixtures. The CO2/O2 Unit BL–CP2 will be called ’Tri-Gas Mixer’ from here on.

The Tri-Gas Mixer can be used along with Mini-Incubators (for IVF application) as well as with microscope incubators or perfusion chambers (for Live Cell Microscopy) and for all applications where a gas mixture of CO2 in Air is required.

The Tri-Gas Mixer eliminates the need for premixed Air/CO2 and N2/O2/CO2 tanks and can supply the desired gas mixture to several equipment. The Tri-Gas Mixer can supply up to 1.5 L/min of mixed gas. Different gas lines can be set up to provide different flow rate values for each equipment, as long as the sum of flow rates of all gas lines does NOT exceed 1.5 L/min.

The Unit includes three main parts: a Control Unit (Tri-Gas Mixer), a Touch Screen user interface and a Pressurized Tank. The Tri-Gas Mixer is operated via the Touch Screen.

The time required to reach the steady state depends on the volume of the tank in use and on the spilled gas flow rate. Once the steady state is reached (i.e. gas set-point values are reached as displayed on the Touch Screen), the device can be used uninterruptedly.

The mixing accuracy of the Tri-Gas Mixer can be manually fine tuned with any external CO2 and O2 percentage reader. The Tri-Gas Mixer can also be used with the Okolab CO2/O2 Analyzer. To automatically calibrate the Tri-Gas Mixer connect it to the CO2/O2 Analyzer using the provided serial cable. Gas concentration feedback from the CO2/O2 Analyzer will be used to adjust each gas flow rate compensating for gas composition deviation from the set point.

The Tri-Gas Mixer can be also equipped with the Okolab Junction Box. The Junction Box is a delivery gas unit ensuring continuity of gas supply. It automatically switches to a backup gas supply (Span Gas) in case any of the input gas sources runs low/out. The Junction Box also allows performing the Zero and Span Calibration of the CO2/O2 Gas Analyzer sensors.

Deviation from normal operation is immediately detected triggering a sound alarm.

Should any electrical or mechanical fault occur while using this device, stop using it until checked by an authorized service agent.

Before use please read this manual and familiarize yourself with the functions and the operation of the device.

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1 Okolab S.r.l recommends that the gases used with the Tri-Gas Mixer have a purity of 99.99%. Otherwise, presence of impurities in the gas tanks alters the CO2/O2 compositions obtained downstream of the Tri-Gas Mixer (See Application note 1). In order to be sure of the gases purity, you can check the composition of each tank with an external meter.)
3 Symbols descriptions

3.1.1 Symbols in this manual and on the product:
The following symbols identify important information:

![Warning Symbol] CAUTION or WARNING: This symbol warns about the risk of electrical shock.

![Warning Symbol] CAUTION or WARNING or IMPORTANT: This symbol warns about circumstances or practices that can affect the instrument’s functionality and must refer to accompanying documents.

Tip ► Provides helpful suggestions.

Note ► Provides important information for successful setup and use the instrument.

3.1.2 Symbols on the product label:

![CE Marking] CE MARKING: This symbol indicates a product’s compliance with EU legislation.

![Disposal Symbol] 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. Technically qualified personnel should only operate equipment. 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. 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 Tri Gas Mixer and Gas Analyzer and on the top panel of the Junction Box.

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 tubing time to time for possible material usage. Check the tubes are well fixed to their own connectors so they cannot slip off This device is not designed for use under medical conditions Install safety valves and adequate pressure regulators on gas lines before the Tri-Gas Mixer input connectors.
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.

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.

5 AC section wiring diagram

The Figure 1 shows the section in alternate current (ac) intended for connecting power supplies (see C in Figure 1) in parallel to the distribution grid of low voltage phase. It also includes on-off control switch (see A in Figure 1) of energy.

A. **On-Off Control Switch**, 8500 Rocker Switcher-Miniature DPST
B. **Power Connector**, OM-SP8210263
C. **Power supplies**, TDK-Lambda-ZWS30-12 and ZWS5-5.
Figure 1 AC section wiring diagram
6 Equipment and Connections

Tri-Gas Mixer can be provided with different optional devices allowing setting up several configurations depending on user needs.

The Tri-Gas Mixer combines Carbon Dioxide, Nitrogen and Air. By controlling flow rate of each gas the Tri-Gas Mixer generates a gas mixture of controlled composition within the range 0-10% of CO₂ and Oxygen in Nitrogen. Main components of this configuration are: ‘Tri-Gas Mixer’ and ‘Pressurized Tank’.

The system can also be equipped with an external CO₂/O₂ Analyzer monitoring the output gas composition. The Analyzer adjusts the Tri-Gas Mixer working parameters fine-tuning system accuracy and repeatability.

The system complete with all optional devices consists of the Tri-Gas Mixer, the CO₂/O₂ Analyzer and the Junction Box. The Junction Box is a delivery gas unit adding the ability to switch the input gas to the Span Gas tank (backup gas source) ensuring continuity of gas supply. It also allows for automatic calibration for Zero and Span set points of the CO₂/O₂ Analyzer sensors. The Junction Box together with the CO₂/O₂ Analyzer allows fine-tuning of the Tri-Gas Mixer working conditions. A system configuration including Junction Box and CO₂/O₂ Analyzer also allows for ‘Autotuning’ (see section 10.4.3.). During ‘Autotuning’ the Gas Analyzer automatically opens its input valve for a few minutes every 4 hours and analyzes CO₂/O₂ ensuring correct composition. Data from the Analyzer feeds back to the Tri-Gas Mixer and automatically fine tunes output gas composition.

The following pages list the supplied accessories for each system configuration.


1. Tri-Gas Mixer
2. A Touch Screen with magnetic pad
3. A Pressurized Tank
4. Tank Support
5. Push in to fit connectors with ¼” NPT male thread with an O-ring for 6mm O.D. rigid tubing (x4). Install a connector into the output port of each tank’s regulator. Next, insert the 6mm O.D. tubing into the ‘push in to fit’ end of the connector
6. 10 meter long 6mm O.D. white Teflon tube (x3). Use white Teflon tube to connect the output ports of Air, Nitrogen and Carbon Dioxide tanks to the corresponding input ports (Air, N₂, CO₂) on the rear panel of the Tri-Gas Mixer
7. Pressure gauge with filter, regulator and assembly stirrup for CO₂, Air and N₂ (x3). Install pressure gauge between the pure CO₂, Air, and N₂ tanks pressure regulators and the Tri-Gas Mixer input ports
8. 2.0 barg end of scale Pressure Gauge with regulator and assembly stirrup (x1). This pressure gauge regulates the mixed gas pressure output from ‘Pressurized Tank’. Set
the pressure within the range 0-1.5 barg, i.e. ambient pressure up to 1.5 bar above the ambient pressure.

**Note** ▶ BARG (Bar Gauge: pressure above ambient)

9. **1.5 meter long yellow polyurethane tubing (x1).** Use yellow tubing to connect the Tri-Gas Mixer Control Unit gas output port to the 'Pressurized Tank' input port.

10. **1.5 meter long blue polyurethane tubing (x1).** Use blue tubing to connect the 'Pressurized Tank' output port to the pressure gauge described at point 8.

11. **Manifold with 1 input and 3 output ports fitting 6 mm O.D. Tubing (x1).** Use the Manifold to connect pressure gauge of the output gas to the equipment downstream. Manifolds with the following configurations are available upon request: 1) with 3 NPTF threaded holes, 2) with 1/8” Threaded holes, 3) with 3 Swagelok connectors SS-400-1-4RT. Please contact Okolab if the configuration you need is not listed.

12. **1.5 meter long blue polyurethane tubing (x1).** Use blue tubing to connect the output port of the pressure gauge to the input port of the manifold.

13. **Mini USB cable (x1).** Use mini USB cable to connect the Touch Screen to the Tri-Gas Mixer.

14. **Power cord (x1).** Use power cord with Tri-Gas Mixer. The Tri-Gas Mixer Unit can be powered at a voltage within the range 110-230VAC.

15. **Volatile Organic Compounds (VOC) Filter.** Optional


When the optional Okolab CO2/O2 Concentration Analyzer is selected, the following items will be included:

1. **Tri-Gas Mixer**
2. **A Touch Screen with magnetic pad**
3. **A Pressurized Tank**
4. **Tank Support**
5. **Push in to fit connectors with ¼” NPT male thread with an O-ring for 6mm O.D. rigid tubing (x4).** Install a connector into the output port of each tank’s regulator. Next, insert the 6mm O.D. tubing into the ‘push in to fit’ end of the connector.
6. **10 meter long 6mm O.D. white Teflon tube (x3).** Use white Teflon tube to connect the output ports of Air, Nitrogen and Carbon Dioxide tanks to corresponding input ports (Air, N2, CO2) on the rear panel of the Tri-Gas Mixer.
7. **Pressure gauge with filter, regulator and assembly stirrup for CO2, Air and N2 (x3).** Install pressure gauge between the pure CO2, Air, and N2 tanks pressure regulators and the Tri-Gas Mixer input ports.
8. **1.5 meter long yellow polyurethane tubing (x1).** Use yellow tubing to connect the Tri-Gas Mixer gas output port to the Pressurized Tank input port.
9. **Tee Connector.** Use the Tee Connector to split pressurized gas output from the Pressurized Tank to feed A) pressure gauge for output gas and B) pressure gauge for CO2/O2 Analyzer. (See page 35, Figure 26)

10. **1.5 meter long blue polyurethane tubing (x1).** Use blue tubing to connect the Pressurized Tank output port to the Tee Connector input port

11. **Okolab CO2/O2 Analyzer**

12. **2.0 barg end of scale Pressure Gauge with regulator and assembly stirrup (x2).** (See page 35, Figure 26)

   A. Use one pressure gauge to regulate the mixed gas pressure output from ‘Pressurized Tank’. Set the pressure within the range 0-1.5 barg i.e. ambient pressure up to 1.5 bar above the ambient pressure.

   **Note ► BARG (Bar Gauge: pressure above ambient)**

   B. Use one pressure gauge to regulate the mixed gas pressure input to the CO2/O2 Analyzer

13. **Manifold with 1 input port and 3 output ports fitting 6 mm O.D. Tubing (x1).** Use the Manifold to connect pressure gauge of the output gas to the equipment downstream. Manifolds with the following configurations are available upon request: 1) with 3 NPTF threaded holes, 2) with 1/8” Threaded holes, 3) with 3 Swagelok connectors SS-400-1-4RT. Additional configurations are available upon request

14. **1.5 meter long Blue polyurethane tubing (x3).** Use blue tubing to connect:

   A. Tee Connector output port to the input of the Pressure Gauge with regulator

   B. Tee Connector output port to the input port of the CO2/O2 Analyzer

   C. Output port of the Pressure Gauge with regulator to the input port of the Manifold

15. **1.5 meters long, 6mm OD Transparent Polyurethane tube with in-line valve connector (x1).** Use Polyurethane tube to connect the output port of the Pressure Gauge and the ‘Gas Input’ port located on the side of the CO2/O2 Analyzer

16. **Mini USB cable (x1) to connect the Touch Screen to the Tri-Gas Mixer Control Unit**

17. **Power cord (x1) to power Tri-Gas Mixer Control Unit.** The Tri-Gas Mixer Unit can be powered at a voltage within the range 110-230VAC

18. **RS232 Serial Cable (x1).** To connect the Gas Analyzer to Tri-Gas Mixer

19. **5VDC Power adapter (x1).** To power the CO2/O2 Gas Analyzer

20. **Volatile Organic Compounds (VOC) Filter.** Optional

### Configuration 3. Tri-Gas Mixer with CO2/O2 Analyzer and Junction Box.

**Equipment supplied and Tubing Specs.**

When the Junction Box is integrated in the Tri-Gas Mixer system, the following items will be included:
1. **Tri-Gas Mixer**
2. **A Touch Screen with magnetic pad**
3. **A Pressurized Tank**
4. **Tank Support**
5. **Push in to fit connectors with ¼” NPT male thread with an O-ring for 6mm O.D. rigid tubing (x4).** Screw a connector into the output port of each tank’s regulator. Next, insert the 6mm O.D. tubing into the ‘push in to fit’ end of the connector
6. **White Teflon tube, 6mm O.D., 10 meter long (x3).** Use white Teflon tube to connect the output ports of Air, Nitrogen, Carbon Dioxide and Span Gas tanks to corresponding connectors (Air In, N2 In, CO2 In and Span Gas In) on the left panel of the Junction Box
7. **1.5 meter long 6mm O.D White Teflon tubing (x3).** Use white Teflon tubing to connect output ports of Air, Nitrogen and Carbon Dioxide on the bottom panel of the Junction Box to corresponding input ports for Air, N2, and CO2 located on the rear panel of the Tri-Gas Mixer
8. **Pressure Gauge with regulator and assembly stirrup for CO₂, Air, N₂ and Span Gas (x4).** Install a pressure gauge between the pure CO₂, Air, N₂ and Span Gas tanks pressure regulators and the Junction Box
9. **1.5 meter long yellow polyurethane tubing (x1)** Use yellow polyurethane tubing to connect the output port of the Tri-Gas Mixer to the TOP input port of the Pressurized Tank
10. **1.5 meter long blue polyurethane tubing (x1).** Use blue tubing to connect the Pressurized Tank BOTTOM output port to the Junction Box input port labeled ‘To Tank’
11. **Okolab CO₂/O₂ Analyzer**
12. **2.0 barg end of scale Pressure Gauge with regulator and assembly stirrup (x2).**
   A. Use one pressure gauge to regulate the mixed gas pressure output from ‘Junction Box’. (see Figure 39).
   B. Install the second pressure gauge between the Junction box output and the input to the CO₂/O₂ Analyzer (see Figure 39).
   Set both (A and B) within the range 0-1.5 barg*. i.e. ambient pressure up to 1.5 bar above the ambient pressure.
   **Note ►** BARG (Bar Gauge: pressure above ambient)
13. **Manifold with 1 input port and 3 output ports fitting 6 mm O.D. Tubing (x1).** Use the Manifold to connect pressure gauge of the output gas to the equipment downstream. Manifolds with the following configurations are available upon request: 1) with 3 NPTF threaded holes, 2) with 1/8” Threaded holes, 3) with 3 Swagelok connectors SS-400-1-4RT. Additional configurations are available upon request.
14. **1.5 meter long blue polyurethane tubing (x1).** Use blue tubing to connect the output port of Junction Box Gas (labeled: ‘Gas Output’) to the input port of the Pressure Gauge (A. Point 12)
15. **1.5 meter long blue polyurethane tubing (x1)**. Use blue tubing, to connect the output port of the Pressure Gauge to the input connector of the Manifold.

16. **1.5 meter long blue polyurethane tubing (x1)**. Use blue tubing to connect the Junction Box port labeled ‘To Gas Analyzer’ to the Pressure Gauge input port (B. point 12) (see Figure 39).

17. **1.5 meters long, 6mm OD Transparent Polyurethane tube with in-line valve connector (x1)**. Use Polyurethane tube to connect the output port of the Pressure Gauge and the “Gas Input” port located on the side of the CO2/O2 Analyzer.

18. **Mini USB cable (x1)** to connect the Touch Screen to the Tri-Gas Mixer Control Unit.

19. **Power cord (x1)**, to power Tri-Gas Mixer Control Unit. The Tri-Gas Mixer Unit can be powered at a voltage within the range 110-230VAC.

20. **RS232 Serial Cable (x2)** To connect:
   A. The Tri-Gas Mixer to the Junction Box
   B. To connect the CO2/O2 Gas Analyzer to the Junction Box

21. **5VDC Power adapter** to connect the CO2/O2 Gas Analyzer

22. **Junction Box**. Delivery gas unit

23. **Volatile Organic Compounds (VOC) Filter**. Optional

### 6.4 Equipment supplied in case of integrated Smartbox

When the Smartbox has been chosen as an optional, the following items will be included:

1. **Smartbox**.
2. **#1 Mini USB cable**, to connect the Smartbox to the Tri-Gas Mixer Control Unit.

The Smartbox can be included in each configuration described in the previous paragraphs.

### 6.5 Additional Equipment Required BUT NOT SUPPLIED

- Air tank or compressed air with safety valve/pressure gauge regulator (degree of filtration 20 µm and condensate drain) accepting rigid tubing 6 mm OD.
- CO2 tank with safety valve/pressure gauge regulator accepting rigid tubing 6 mm OD
  - **Note**: ¼ NPT male connector to 6mm OD push-in fitting connector adapters are included. Gas supply must be high-purity (medical grade) and humidity-free.
- Nitrogen tank with safety valve/pressure gauge regulator accepting rigid tubing 6 mm OD
  - **Note**: ¼ NPT male connector to 6mm OD push-in fitting connector adapters are included. Gas supply must be high-purity (medical grade) and humidity-free. N2 is OPTIONAL: REQUIRED ONLY FOR TRI-GAS MIXTURE.
- Span Gas tank (i.e. backup gas supply). Prevents interruption of gas supply to downstream equipment. **REQUIRED with Junction Box ONLY**. Gas source must be high-purity (medical grade) and humidity-free.
- **Live Cell Microscopy Applications** require one gas flow meter per microscope incubator. Each flow meter regulates the gas mixture flow rate supplied to the individual microscope.
incubator. Place a flow meter after each Tee used to split the main gas line and before the microscope incubator.

**Note** ► If possible position the flow meter within reach of the microscope incubator.

**Gas Flow Meter Selection** Important considerations for gas flow meter selection:

1) Gas flow meter must be calibrated for Air
2) Max value of the scale of each flow meter should not be more than twice the value set for that gas line (e.g. if gas line must be operated at 0.5 L/min Max value of scale not to exceed 1.0 L/Min). **IMPORTANT: adding together the gas flow rate sent to each scope MUST not exceed a total of 1.5L/min.** Select flow meters with appropriate scale accordingly.
3) High full scale reading error of the gas flow meter has to be less than 5%.

**Okolab can supply appropriate flow meters upon request. See Application note 3 for an example of a total system for Live cell Microscopy**

Tubing connecting the Tri-Gas Mixer to each flow meter/microscope incubator are also NOT supplied. Tubing should be Teflon 6mm O.D. Please contact Okolab for further information about tubing.

**For IVF Applications**: braided connecting hoses to connect the Manifold to Mini-incubators **See Application note 3 for an example of a total system for IVF**

### 7 IMPORTANT NOTES

1. **THE TRI-GAS MIXER WORKS AT 2 BARG (MAXIMUM PRESSURE INSIDE THE TANK IS 3 BARG), THIS VALUE IS FIXED AND CANNOT BE CHANGED. HOWEVER, PRESSURE OUTGOING FROM THE TRI-GASMIXER CAN BE REGULATED, FROM 0 TO 1.5 BARG THROUGH THE PRESSURE GAUGE PLACED AFTER THE TANK.**

2. **REGULATE THE PRESSURE AFTER PURE GAS TANKS (AIR/NITROGEN/CO₂ tanks) TO 4.2 BARG USING THE PRESSURE GAUGE INCLUDED IN THE PACKAGE (DO NOT EXCEED THE PRESSURE OF 5 BARG!). BARG MEANS BAR GAUGE, THAT IS THE PRESSURE ABOVE THE AMBIENT. 4.2 BARG MEANS 4.2 BAR ABOVE THE AMBIENT PRESSURE OR 5.2 ABSOLUTE PRESSURE AT SEA LEVEL.**

3. **IMPORTANT CONNECTION:** Connect TOP port of the Okolab ‘PRESSURIZED TANK’ to REAR SIDE OF THE TRI-GAS MIXER. Connect BOTTOM port of Okolab “PRESSURIZED TANK” to PRESSURE GAUGE. FOR YOUR CONVENIENCE TUBING AND CONNECTORS ARE COLOR-CODED. INSTALL MATCHING COLORS (See Figure 9)

4. **THE TRI-GAS MIXER CAN SUPPLY DESIRED GAS TO MULTIPLE EQUIPMENT UP TO A TOTAL FLOWRATE OF MAXIMUM 1.5 LITERS PER MINUTE.
8 Illustration of the Main Units.

8.1 Tri-Gas Mixer

1. Power Input.
2. Pure CO₂ Push to fit Input Connector for 6 mm O.D. rigid tubing.
3. Pure Air Push to fit Input Connector for 6 mm O.D. rigid tubing.
4. Pure N₂ or Air (depending on the Configuration) Push to fit Input Connector for 6 mm O.D. rigid tubing.
5. Gas Purge Port.
6. Purging Valve. It allows emptying the tank. Gas will purge from the Purge Output port.
7. Gas output.
8. External Alarm Contact closure.
10. RS232 9 pin D-Sub Connector.
11. Power Switch.

Figure 2: Tri-Gas Mixer Rear Panel Overview.
8.2 CO2/O2 Analyzer

1. Pin to Ground.
2. RS232 9 pins D-Sub Connector.
4. 5VDC Input Power plug.
5. Gas input Connector with O-ring.
6. Regulating Valve. It allows regulating the gas flow rate to the Sensors inside the CO2/O2 Analyzer.
7. Power Switch.
8. Mini USB connector.

8.3 Junction Box

Figure 3: CO2/O2 Analyzer Rear and Lateral Panel Overview.

Figure 4: Junction Box Left, Bottom and Right Panel Overview.

This Pin must be grounded to make the system electrically stable.
1. Span Gas Push to fit Input Connector for 6 mm O.D. rigid tubing.
2. Pure CO₂ Push to fit Input Connector for 6 mm O.D. rigid tubing.
3. Pure Air Push to fit Input Connector for 6 mm O.D. rigid tubing.
4. Pure N₂ Push to fit Input Connector for 6 mm O.D. rigid tubing.
5. Pure N₂ Push to fit Output Connector for 6 mm O.D. rigid tubing.
6. Pure Air Push to fit Output Connector for 6 mm O.D. rigid tubing.
7. Pure CO₂ Push to fit Output Connector for 6 mm O.D. rigid tubing.
8. To Tank Push to fit Output Connector for 6 mm O.D. rigid tubing.
9. RS232 9 pin D-Sub Connector to be connected to the CO2/O2 Analyzer RS232 Connector.
10. RS232 9 pin D-Sub Connector to be connected to the Tri-Gas Mixer RS232 Connector.
12. Gas port to be connected to the Pressure Gauge of the CO2/O2 Analyzer.

### Smartbox

1. Power Led.
2. Status Led.
3. Micro USB Connector to connect the Smartbox to the PC.
4. USB ports, mainly used to connect the Smartbox to the Control Unit and to the Web Cam.
5. 5-pole bus connector used to power up the Smartbox when the Control Unit is on.
6. Ethernet port to connect the Smartbox to your router.

### Pressure Gauge set up and use

Follow the arrow on the Pressure Gauge for the correct Gas In-Out (See Figure 6. below). On the bottom of the unit there is an automatic purging valve. Close it before operating the Pressure Gauge. To close the purging valve -looking at the regulator vertically from the top knob- rotate it counterclockwise. Now you can operate the Pressure Gauge. To close the gauge, pull the knob up and rotate it counterclockwise. Push the knob down to lock it. Open
valve on gas supply tank. Adjust Pressure Gauge by pulling the knob up and rotating it clockwise until the pressure reaches the desired value.

![Image of a pressure gauge]

Figure 6: Pressure Gauge Installation. Input and Output gas ports.


The Tri-Gas Mixer can be coupled with optional devices allowing setting up different configurations for user needs.

Configuration 1 illustrates how to install and use the Tri-Gas Mixer without any optional device.

### 9.1 Configuration 1: Tri-Gas Mixer. Installation guide.

The following instructions will guide you through the installation. IMPORTANT: Please read carefully all instructions and safety notes.

1. Install 6 mm O.D. white Teflon tubing between the ’gas out’ side of the Pressure Gauges (see Figure 8) and the Tri-Gas Mixer gas inputs. Make sure to push tubes securely all the way into connectors avoiding any gas leak. If the regulator of pure gases tanks does not accept 6 mm O.D. tubing, install the provided ¼ NPT male connector with 6mm O.D. push-in adapter. To remove tubing from push-in connectors push the black or red rubber ring while pulling the tubing. If tubing doesn’t easily come out, do not force it, simply make sure the black ring is properly pushed.
Before disconnecting any of the polyurethane tubing connected to tanks, Tri-Gas Mixer, Junction Box or Gas Analyzer, make sure there is no residual pressure by adjusting the pressure gauges upstream of the system and the tank manometer.

2. Figure 8 shows the connections between the CO2, Air and Nitrogen tanks and the respective connectors on the rear panel of the Tri-Gas Mixer.

3. Connect the Pressurized Tank to the Tri-Gas Mixer and to the downstream Pressure Gauge with two Polyurethane tubing, blue and yellow, as illustrated in Figure 9.
Figure 9: Pressurized Tank connection to the Control Unit and to the Pressure Gauge.

**Tip** The tank is equipped with a safety valve (AirTek, with direct exhaust, for air, inert gases, and saturated steam. Set at 6 bar, Thread G1/4) as shown in Figure 10. The Safety Valve opens only if the tank pressure exceeds 6 bar.

Figure 10. Pressurized Tank Safety Valve

IMPORTANT PAY EXTRA CARE IN THE FOLLOWING CONNECTION and its ORIENTATION. Connect the **TOP** port of the ‘Pressurized Tank’ to the **Tri-Gas Mixer**. Connect the **BOTTOM** port of the ‘Pressurized Tank’ to the **downstream Pressure Gauge**. Tubing and connectors involved in the Tri-Gas Mixer – Pressurized Tank-Pressure Gauge connections are color-coded. Match colors (blue into blue, yellow into yellow). Push tubing securely all the way into connectors to avoid any gas leak. To remove tubing from these push-in connectors remember to keep pushed the black ring. The Pressure Gauge has to be installed between the Pressurized Tank and the incubators.
4. If your system is equipped also with VOC Filter (for example Oosafe Filter AS-851585), install it between the Pressure Gauge downstream of the Pressurized Tank and the Manifold as shown in Figure 11. In order to install the VOC Filter in the correct orientation, follow the gas flow direction indicated on the filter itself.

![VOC Filter Installation](image)

*Figure 11. VOC Filter Installation.*

5. Connect the Power cord to the Tri-Gas Mixer

6. Place the Touch Screen on the Tri-Gas Mixer and connect them together through the mini USB cable as illustrated in Figure 12. The Touch Screen case has a magnetic base for secure placement on top of the Tri-Gas Mixer (See Figure 14).

![Oko Touch Connection](image)

*Figure 12: Oko Touch Connection.*

7. Connecting the Manifold. An aluminum manifold with 3 gas output ports is provided. Connect the Pressure Gauge (downstream of the Pressure Tank) to the input side of the manifold. There are 3 independent gas output ports on the provided Manifold. Manifold can be requested in different configuration, as indicated on page 12 paragraph 6.1
8. Use 6mm O.D Teflon tubing (NOT PROVIDED) to connect the output ports of the Manifold with the input ports of the incubators.

9. Turn the Control Unit on (rocker switch is located on the rear panel). A green LED light on the front of the Tri-Gas Mixer will turn on and the screen shown in Figure 13 will appear on the Touch Screen for a few seconds.

Figure 13: Screenshot at Startup.

Figure 14 shows the configuration on the system.

Figure 14: Final tubing and Cabling when the configuration comprises only the Tri-Gas Mixer.

**Tip** Note the Purging Valve and the Screw Terminal on the rear side of the Tri-Gas Mixer. The Purging Valve allows emptying the ‘Pressurized Tank’ in case of maintenance operations. During normal operation of the system this valve must be closed by rotating the valve knob clockwise until it no longer turns (see Figure 15 panel A label (1) and panel C). When you need
to empty the ‘Pressurized Tank’, open the valve by rotating the knob counterclockwise. Next, you can set the output gas flow rate at the desired value by rotating the O-ring (see Figure 15 panel A label (2)) Directional arrows on the unit indicate the open/close direction of rotation.

![Diagram showing Purging Valve](image)

**Figure 15. How to use the needle valve on the rear panel.**

The Screw Terminal is a contact closure alarm activating in case of irregular operation of the Tri-Gas Mixer. Use it only with suitable alarm transmitters activated by means of contact closure. Safety extra low voltage powered alarm equipment, not exceeding stated contact rating might be connected to the external alarm terminals. Refer to technical specification for alarm contact rating.

![Diagram showing Alarm Contact Closure Screw Terminal](image)

**Figure 16: Zoom sketches on the Alarm Contact Closure Screw Terminal and Purging Valve on the rear panel of the Control Unit.**

### 9.2 Configuration 1: System Set Up And Operation.

1. Close Purging Valve on the rear panel of the Pressurized Tank
2. Set output pressure at the pure gas tanks at 5 barg (i.e. 5 bar above the ambient pressure or 6 bar of absolute pressure at sea level.
3. Set pressure of each gas input to the Tri-Gas Mixer at 4.2 barg using the pressure gauges with regulators provided. IMPORTANT: **Do not exceed 5 barg.**
4. Make sure that all tubing is properly installed as described in paragraph 9.1 above.
5. Close the output gas pressure gauge by rotating its knob counterclockwise.
6. Close all the valves of the downstream equipment.
7. Switch the unit on.
8. Set the desired gas mix composition using the touch screen Home page.
9. Using the touch screen input the gas tank volume in use.
10. Wait until the Tri-Gas Mixer fills the Pressurized Tank and the pressure reaches to 2.5 or 3 barg depending on tank volume.

11. Make sure that the valves of the downstream equipment are closed.

12. Set output gas pressure regulator at the desired value within the range 0-1.5 barg.

13. Go to the equipment and regulate their valves to consume no more than 1.1 L/min or 1.5 L/min depending on the tank in use.

14. Wait until the indicators on Home page screen become green (see 9.3.1 Figure 17 below). When indicators are green desired set point for CO₂% and O₂% has been reached.

9.3 Configuration 1: Unit operation through the Touch Screen.

9.3.1 Home page.

![Home page of the Tri-Gas Mixer Touch Screen Display.](image)

The Touch Screen is pre-set at 6% CO₂ and 5% O₂. Once turned on the Unit will operate to reach the pre-set values.

9.3.2 Changing Set Points.
To change set points, simply touch the tab of the gas to be adjusted. For instance to change CO₂, touch the CO₂ tab. The CO₂ Set point page will open. Increase or decrease the
concentration set point by clicking on + and – to reach the desired concentration. Press “Set” to save the new set point or “Cancel” to undo.

9.3.3 Configuration settings

The first time you turn the system on, it is very important to verify that you are using the right configuration parameters for your device. Press the button with ‘gear’ icon to get to the ‘Settings’ page. Now press ‘Configuration’ (wrench and screw driver icon). The Configuration page will open with the following options: ‘Tank size’, ‘Control Mode’ and ‘Junction Box’

9.3.4 Setting Correct Tank Size.

This screen allows you to select the size of the Okolab ‘Pressurized Tank’ provided. The ‘Pressurized Tank’ size determines the maximum flow output from the Control Unit. 20 L and 8.5 L tanks, have a maximum output flow rate of 1,5 L/min. A 4.5 L tank, has a maximum output flow rate of 1,1 L/min.
9.3.5 Control Mode Set Up: Tri-Gas Mixer or CO₂ in Air.

From the ‘Configuration’ page press ‘Control Mode’. The ‘Gas mode’ page will open. This page allows you to select the control mode you want to run the system in depending on your gas input. ‘CO₂ in Air’: If you are using a gas input of 100%CO₂ and compressed Air select CO₂ by pressing on it. The system will control CO₂ levels.

‘Tri-Gas-Mixer’ mode: If you are using a gas input of 100% N₂, 100% CO₂ and compressed Air select CO₂/O₂ by pressing on it. In this ‘Tri-Gas Mixer’ mode the system controls both Oxygen and CO₂ levels.

IMPORTANT: Make sure to connect Air, Nitrogen and CO₂ supply to the respective Input ports located on the back of the Tri-Gas Mixer. NOTE: If your input is 100% CO₂ and Air connect Air supply to N2 input port. Hence, the Air Input will remain unused.

When changing ‘Gas mode’, please keep note the following points:
The system will need some time to purge the Pressurized Tank and reach the new steady state.

When you work in ‘CO₂ in Air’, then Oxygen will no longer be a parameter that you can regulate. The screen will show ‘AIR’ in place of the set point on the Oxygen tab. The Oxygen % value displayed is a calculated one by keeping into account the approx. concentration of O₂ in air, (c.a. 21%), and the desired output concentration. The CO₂ % displayed is the set point (See Figure 22)

![Figure 22: Tri-Gas Mixer Homepage when CO₂ in Air Mode is active.](image)

9.3.6 How To Disable The Junction Box From The System.

When the Tri-Gas Mixer configuration does not include the Junction Box, you must confirm its absence from the system.

![Figure 23: Junction Box Connection Procedure.](image)

From the ‘Configuration’ page press ‘Junction Box’. The ‘Junction Box’ page will open. Press the button labeled ‘Connected’ to gray it out. Check that all the others checkboxes are disabled/grayed out. These features can only be activated when the Junction Box is connected to the Tri-Gas mixer.
9.3.7 Using An External Gas Sensor To Calibrate The Tri-Gas Mixer.
In order to fine-tune the accuracy of the gas composition an external CO2 Sensor/Analyzer and/or an external Oxygen Sensor/Analyzer (not provided) can be used to manually calibrate the Tri-Gas Mixer.

Connect the external gas Analyzer to one of the output ports of the manifold installed in line with the system (see Figure 24). Follow the steps shown in Figure 25.

![Figure 24: Tubing connections for the Tri Gas Mixer Calibration without CO2 O2 Analyzer.](image)

The CO2 or O2 Meter will read the same gas levels that are going into downstream equipment. Wait for the external meter to steadily read CO2 and/or Oxygen levels. This usually takes 10-15 minutes. When you are sure values being read are at steady state click on the “Adjust” Tab. Once you have pressed “Adjust”, the system will calibrate itself in accordance with value read from the external meter, hence calculating and applying offset values. After clicking “Adjust”, the offset applied by the system will be displayed on the calibration page of the given gas. Now you can click on the “Cancel” icon to go back to the Main page.

**IMPORTANT:** Make sure that the external Gas Analyzer has been recently calibrated and its accuracy is rated higher than the Tri-Gas Mixer accuracy.
Figure 25: Calibration Procedure, via touch, without CO2/O2 Analyzer. In this example: before calibration, the CO₂ set point is 6.0% and the external meter reads 6.1%; after calibration CO₂ set point and read value by the external meter are equal and the CO₂ Offset is 0.1.

The Tri-Gas Mixer can be provided with the CO2/O2 Analyzer as an optional device. The CO2/O2 Analyzer allows measuring the actual gas composition and adjusting the Tri-Gas Mixer working condition to correct for deviations from the set point.

The following paragraphs illustrate how to install and use the Tri-Gas Mixer with the CO2/O2 Analyzer.


1. Use 6 mm O.D. white Teflon tubing between the ‘gas out’ side of the Pressure Gauges (connected to the pure Air, pure CO2 and pure Nitrogen tanks) and the Tri-Gas Mixer gas inputs (see Figure 8). Push tubes securely all the way in to avoid any gas leak. If the regulators of the pure gas tanks do not accept 6 mm O.D. tubing, install ¼ NPT male connector with 6mm O.D. push-in fitting adapter provided with the system. To remove tubes from push-in connectors press the black or red ring in while pulling the tube. This action doesn’t require any particular force. If tubing doesn’t easily come out, it means that the black ring isn’t being properly pushed in.

2. Figure 8 shows the connections between CO2, Air and Nitrogen tanks and the respective input ports on the rear panel of the Tri-Gas Mixer.

3. Use the yellow polyurethane tubing to connect the ‘Gas Output’ Port located on the rear panel of the Tri-Gas Mixer to the TOP port of the tank. Use the blue polyurethane tubing to connect the BOTTOM port of the tank to the Tee Connector input port. Use the provided tubing to connect the output ports of the Tee Connector to a Pressure Gauge each. Use the transparent polyurethane rigid tube 6 mm O.D to connect one Pressure Gauge to the input of the CO2/O2 Analyzer with connector with in-line valve. Use the provided blue polyurethane tubing to connect the other Pressure Gauge to the output gas manifold. (See Figure 26).
4. If your system is equipped also with VOC Filter (for example Oosafe Filter AS-851585), install it between the pressure gauge downstream of the Pressurized Tank and the manifold as shown in Figure 11. In order to install the VOC Filter in the correct orientation, follow the gas flow direction indicated on the filter itself.

5. Connect the RS232 serial cable between CO2/O2 Analyzer and the Tri-Gas Mixer and connect the 5VDC power feeder (for example, GS15A-1P1J, code 721-2089 on www.rswww.com) to the power input jack on the rear panel of the CO2/O2 Gas Analyzer, as shown in Figure 27 below.
Figure 28 shows the final configuration of the system with integrated CO2/O2 Analyzer.

![Figure 28: Final tubing configuration with integrated CO2/O2 Analyzer.](image)

### 10.2 Configuration 2: Tri-Gas Mixer with the CO2/O2 Analyzer. System Set Up And Operation.

1. Close Purging Valve on the rear panel of the Tri-Gas Mixer.
2. Set output pressure at the pure gas tanks at 5 barg (i.e. 5 bar above the ambient pressure or 6 bar of absolute pressure at sea level).
3. Set pressure of each gas input to the Tri-Gas Mixer at 4.2 barg using the pressure gauges with regulators provided. **IMPORTANT: Do not exceed 5 barg.**
4. Make sure that all tubing is properly installed as described in `paragraph 10.1` above.
5. Close the output gas pressure gauge downstream to the Pressurized Tank and upstream of the manifold by rotating its knob counterclockwise.
6. Close all the valves of the downstream equipment.
7. Switch the unit on.
8. Input set point gas composition on Home Page of touch screen with Tri-Gas Mixer connected (see Figure 30).
9. Select the size of the Okolab ‘Pressurized Tank’ provided as described on paragraph 9.3.4 on page 30.
10. Wait until the Tri-Gas Mixer fills the ‘Pressurized Tank’ and the pressure reaches 2.5 or 3 barg depending on tank volume.
11. Check that all the valves of the downstream equipment are closed.
12. Set the pressure regulator in point 5 at the desired value within the range 0-1.5 barg.
13. Set the pressure regulator of the CO2/O2 Analyzer to 0.5 barg.
14. Switch the Gas Analyzer on.

15. Use the Needle Valve on the rear panel of the CO2/O2 Analyzer to set the gas flow rate to a value within the range 35-50 cc/m. Actual gas flow rate will appear on the touch screen home page of the CO2/O2 Analyzer (see Figure 30 ‘Gas Meter Measured Values’)

Tip ► To regulate the gas flow rate at the CO2/O2 Analyzer, turn the needle valve knob on the rear panel of the unit (see Figure 29 panel a. label (1) and directional arrow in panel b.) counterclockwise. Set gas flow rate to a value between 35 and 50 cc/m. You can then rotate the O-ring (see Figure 29 panel a. label (2)) clockwise until it stops. This will lock the needle valve and the selected flow rate (See Figure 29 panel b.)

![Figure 29. How to use the needle valve on the rear panel.](image)

16. Go to the equipment and regulate their valves to consume no more than 1.1 L/min or 1.5 L/min depending on the tank in use.

17. Wait until the system reaches the steady state and the indicator on the Touch Screen Home Page turns green.
10.3 Home Page With CO2/O2 Analyzer Connected To The Tri-Gas Mixer

![Image of a home page screen with CO2/O2 Analyzer connected to the Tri-Gas Mixer]

Figure 30: Overview of the home page screen when the CO2/O2 Analyzer is connected to the Tri-Gas Mixer.

The Touch Screen is pre-set at 6% CO₂ and 5% O₂. Once turned on the Unit will operate to reach the pre-set values.

When the CO2/O2 Analyzer is connected, its readout will appear on the Touch Screen Home Page, as shown in Figure 30 ‘Gas Meter Measured Values’.

10.4 Tuning Settings With The CO2/O2 Analyzer

10.4.1 Manual Tuning

With the CO2/O2 Analyzer connected to the Tri-Gas Mixer (see Figure 28) the latter can be automatically calibrated as follows:

![Image of tuning settings with CO2/O2 Analyzer]

Figure 31: Single Tuning. Step 1.
Press “Adjust”. A warning message will appear on the screen. If the input flow to the Analyzer is higher than 30 ml/min (minimum allowed flow), press “Ok” to perform the tuning. The gas analyzer will compare its CO$_2$ and O$_2$ readings with the set points of the Tri-Gas Mixer. The Analyzer will automatically calculate and save ‘offsets’ hence calibrating the Tri-Gas Mixer. The page will be refreshed, showing current offsets applied by the system.

### 10.4.2 Tuning Parameters Reset

To reset the tuning parameters to the default factory setting, follow the steps in Figure 33. It is recommended to reset the parameters to the factory default before any manual tuning.

### 10.4.3 Automatic Tuning

To periodically and automatically repeat the calibration described above press ‘Auto Tuning’ on panel ‘Calibration” (See Figure 34)
11 Configuration 3: Tri-Gas Mixer With The CO2/O2 Analyzer And Junction Box. Installation And User Guide.

Tri-Gas Mixer can be provided with the CO2/O2 Analyzer and the Junction Box as optional devices.

11.1 Setting-Up The System With Integrated Junction Box (See Figure 35)

1. Use 6 mm O.D. white Teflon tubing between the ‘gas out’ side of the Pressure Gauges (connected to the pure Air, pure CO₂ and pure Nitrogen tanks) and the Junction Box gas inputs, as shown in Figure 35. Push tubes securely all the way into connectors to avoid any gas leak. If the regulators of pure gas tanks do not accept 6 mm O.D. tubing, install the ¼ NPT male connector with 6mm O.D. push-in fitting adapter provided with the system. To remove tubes from these push-in connectors keep the black ring pushed in while pulling the tubing. If tubing doesn’t easily come out, it means that the black ring isn’t being properly pushed-in.

2. Use the 1.5 meter long, 6 mm O.D. white Teflon tubing provided connect the N₂, Air and CO₂ input on the Junction Box and to the corresponding input onto the Tri-Gas Mixer (see Figure 35).

3. Connect the ‘Pressurized Tank’ to the Tri-Gas Mixer and to the Junction Box with the blue and yellow Polyurethane tubing as illustrated in Figure 35. IMPORTANT: Connect Tri-Gas Mixer to the TOP port of the ‘Pressurized Tank’. Connect the Junction Box to the BOTTOM port of the ‘Pressurized Tank’. Tubing and connectors are color-coded for easy installation. Push tubing securely all the way into connectors to avoid any gas leak. To remove tubing from push-in connectors push the black or red rubber ring while pulling the tubing. If tubing doesn’t easily come out, do not force it, and simply make sure the black ring is properly pushed. Figure 35 shows connections listed above.
4. Use 6 mm O.D. blue Polyurethane tubing to connect, the two output ports of the Junction Box, “Gas Output” and “To Gas Analyzer”, to the Pressure Gauges with regulator provided. Use the transparent tubing with in-line valve to connect one of Pressure Gauges to the CO2/O2 Analyzer. Connect the other Pressure Gauge to the output gas manifold (see Figure 36).

5. If your system is equipped also with VOC Filter (for example Oosafe Filter AS-851585), install it between the Pressure Gauge downstream of the Junction Box and the manifold as
shown in Figure 11. In order to install the VOC Filter in the correct orientation, follow the gas flow direction indicated on the filter itself.

6. **Serial connection between Tri-Gas-Mixer and Junction Box.** Insert the RS232 serial cable provided into the serial ports of the Tri-Gas Mixer (labeled ‘External Gas Meter’) and Junction Box (labeled ‘To Gas Mixer’).

![Serial connection diagram]

*Figure 37: Cable Connection between the Junction Box the Tri-Gas Mixer and the CO2/O2 Analyzer through two RS232 Serial Cables.*

7. **Serial connection between CO2/O2 Analyzer and Junction Box.** Insert the RS232 serial cable provided into the serial ports of the CO2/O2 Analyzer (labeled ‘Gas Mixer Communication Port’) and Junction Box (labeled ‘To Gas Analyzer’).

8. Place the Touch Screen on the Tri-Gas Mixer and use the mini USB cable to connect them as illustrated in Figure 12.

9. Insert power cord into ‘power input’ of Tri-Gas Mixer.

10. Insert the 5VDC power adapter into the power input jack on the rear panel of the CO2/O2 Gas Analyzer.

**Note** ► Figure 38 shows the location for wall mounting anchors on the Junction Box. Make sure to use wall anchors appropriate for your wall type.
Figure 38: Junction Box Wall Installation.

Figure 39 shows the final configuration of the system.

Figure 39: Final tubing configuration with integrated CO2 O2 Analyzer and Junction Box.

12 Smartbox: Installation

The Tri-Gas Mixer can be equipped with the Smartbox, an integrated web server system. In this case the system can be controlled through a Web browser running on your PC, notebook or smartphone.

Install the Tri-Gas Mixer as shown in paragraphs 9, 10 or 11:
Okolab recommends installing the Smartbox when the Tri-Gas Mixer is switched off

1. Connect the Smartbox to the Tri-Gas Mixer with the 5-pole cable-less connector AND with the Mini USB cable.
2. Connect a network cable from Smartbox Ethernet port to your local router. DHCP service must be enabled on your router. Okolab Smartbox is equipped with a standard Ethernet RJ45 socket, which can be connected through any commercially available network cable. If no Internet connection is available, only local access is possible, either through the PC or router.
3. To monitor the equipment/lab remotely connect the webcam to the Smartbox using the USB cable.
4. Connect the Smartbox to the PC with the Mini USB Cable. Connection to the PC is optional and has to be done only if you have an Okolab BL products’ compatible software.

Figure 40. SmartBox Installation.
13 Operating The System Using The Touch Screen Control

13.1 Touch Screen Home Page With CO2/O2 Analyzer And Junction Box Connection To The Tri-Gas Mixer.

![Image of the home page screen with connected CO2/O2 Analyzer.](image)

*Figure 41: Overview of the home page screen with connected CO2/O2 Analyzer.*

The Touch Screen is pre-set at 6% CO₂ and 5% O₂. Once turned on the Unit will operate to reach the pre-set values.

When the CO2/O2 Analyzer is connected, its readout will appear on the Touch Screen Home Page, as shown in Figure 41 ‘Gas Meter Measured Values’.

13.2 Junction Box Settings

When the Junction Box is installed and connected it must be configured as shown in Figure 42 below.
From the ‘Configuration’ page press ‘Junction Box’. The ‘Junction Box’ page will open. Press the button labeled ‘Connected’ to activate it. Activate the features ‘Span on Alarm’, and ‘Low Gas’ to switch gas source from Okolab Tri-Gas Mixer to the connected Span Gas Tank (backup gas). This may be necessary if the unit is not properly working or if one of the gas supply runs low/empty. By pressing ‘Span Now’ the system will immediately switch gas source to Span Gas (Backup gas source). By pressing ‘Span on Alarm’ the system will switch to Span Gas source only when an alarm is triggered (see Figure 43).

Select ‘Low Gas’ to reduce the amount of gas diverted to the CO2/O2 Analyzer during the Auto-tuning procedure, i.e. in the closed loop operation mode (this option can be used only if CO2/O2 Analyzer is integrated in your system). IMPORTANT Press ‘Save’ to save your selection.
Figure 44: Choose of the Low Gas Consumption during the Auto-tuning procedure.

13.2.1 Calibration with Span Gas, with CO2/O2 Analyzer when Junction Box is connected to the Tri-Gas Mixer.

If the Tri-Gas Mixer is also connected to the Junction Box, the Analyzer calibration procedure is completely automatic. Press ‘Calibration’ in the Settings page. The ‘Calibration’ page will open with the following options: ‘View’, ‘Span Setting’, ‘Start’ and ‘Factory Reset’ (see Figure 45).

Figure 45: How to reach the Calibration page and Calibration page with connected Junction Box.

13.2.2 ‘View’, ‘Span Settings’, ‘Start’, ‘Factory Reset’

View: Selecting ‘View’ opens a screen with the off sets of the last calibration performed.
Figure 46: Calibration Offset Overview of the CO2/O2 Analyzer.

**Span Setting:** Before calibrating the Span Gas set the correct parameters of your span gas tank in Span Settings page. Select the gas that you want to calibrate by checking the correct button (CO₂ or O₂ or both) (see Figure 47).

Figure 47: How to set the CO₂ and O₂ concentrations of the reference gas during the Span Calibration.
**Start:** Press ‘Start’ to start the calibration. Figure 48 shows the panels that will open.

![Self-Calibration and Self Calibration page](image)

Figure 48: How to initiate a Self-Calibration and Self Calibration page.

In the **Self Calibration** page a progress bar will appear. Pressing ‘Abort’ will stop the calibration.

**Factory Reset:**
To reset to the Factory Setting press ‘Factory Reset’ on the ‘Calibration’ panel. All custom calibration will be erased (Figure 49)

![Factory Reset](image)

Figure 49: How to reset the offset stored after a calibration procedure.
14 Color Indicators On Home Page

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 indicator will also appear each time you change set points for CO₂ or O₂.

An ORANGE indicator means that the current gas concentration is not correct and its value is out of the set tolerance (see section “ALARMS”). Verify that all cables are correctly connected. Check all tubing for gas leaks and pressure in gas supply tanks.

A RED indicator means that the problem is persisting and the system is not able to correct it. Most commonly this is related to gas leaks or gas source(s) running low. If these are correct there may be a problem with the unit itself. Turn the system off, wait 5 minutes and turn it back on. If the problem persists contact Okolab at http://www.okolab.com/onlineSupport.page
15 General Settings Of The Tri-Gas Mixer

15.1 Changing Set Points
To change set points, simply touch the tab of the gas to be adjusted. For instance to change CO₂, touch the CO₂ tab. The CO₂ Set point page will open. Increase or decrease the concentration set point by clicking on + and − to reach the desired concentration. Press “Set” to save the new set point or “Cancel” to undo (see Figure 50).

![Figure 50: Description of the change in Set Point CO₂ and O₂ concentrations.]

15.2 Touch Screen Orientation, Screen Saver, Alarms Settings And Status Page
Figure 51 shows how to access Settings.

![Figure 51: How to enter in Touch Screen Settings.]

15.2.1 Touch Screen: Rotate The Display And Change Images Orientation.
The first time you turn on the system, set the screen orientation.
The default screen setting is ‘desktop’ orientation. Pressing ‘Rotate’ will rotate the screen by 180°.

**Screen Saver**: the screen saver appears after 20 minutes of inactivity of the touch screen. The screen saver shows gas concentration on a black background. Press ‘Screen Saver’ to enable or disable this feature. To go back to the main page press ‘Home’

**Touch screen calibration**: In this page you can calibrate the touch screen, touch the Touch Calibration tab and follow the instructions that appear on the screen. The calibration will align touch screen's coordinates to the display.
15.2.2 Alarm Settings
Press ‘Alarms’ on the ‘Settings’ page. Adjust values from the desired set points beyond which you want an alarm to be triggered.

![Alarms Settings](image)

Figure 54: How to reach the Alarm page and Alarms Settings.

Figure 54 shows the following example: In case the gas concentration is 0.5 % or more from the set point (e.g. if set point is 6.0% and the gas concentration is ≤ 5.5% or ≥6.5 %) for 10 minutes or longer a sound alarm will be triggered. Press ‘Buzzer’ to enable or disable the sound of the alarm.

15.2.3 Status page
This page shows the Status of the Tri-Gas Mixer. ‘Mode’ indicates the Control Mode of the system. The first eight rows duplicate the information shown on the homepage. In the lower part of the page Air, CO₂ and N₂ valve duty are listed.

![Status](image)

Figure 55: Status Page of the Tri-Gas Mixer only.

15.2.4 System Information page
This page shows the Gas Control Unit version in use.
15.2.5 Configuration settings

The first time you turn the system on, select the correct configuration parameters for your device. In the Configuration page you can select ‘Tank Size’, ‘Control Mode’ and ‘Junction Box Connection’.

15.2.6 Setting Correct Tank Size

Select the ‘Tank Size’. This screen allows you to select the size of the Okolab ‘Pressurized Tank’ provided. The ‘Pressurized Tank’ size determines the maximum flow output from the Control Unit. 20 L and 8.5 L tanks, have a maximum output flow rate of 1,5 L/min. A 4.5 L tank, has a maximum output flow rate of 1,1 L/min.
15.2.7 Setting Up Control Mode: Tri-Gas Mixer or CO₂ in Air.

From the ‘Configuration’ page press ‘Control Mode’. The ‘Gas mode’ page will open. This page allows you to select the control mode you want to run the system in depending on your gas input. ‘CO₂ in Air’ mode: If you are using a gas input of 100%CO₂ and compressed Air select CO₂ by pressing on it. The system will control CO₂ levels.

‘TRI-GAS MIXER’ mode: If you are using a gas input of 100% N₂, 100% CO₂ and compressed Air select CO₂/O₂ by pressing on it. In this ‘Tri-Gas Mixer’ mode the system controls both Oxygen and CO₂ levels.

**IMPORTANT:** Make sure to connect Air, Nitrogen and CO₂ supply to the respective Input ports located on the back of the Control Unit. **NOTE:** If your input is 100% CO₂ and Air connect Air supply to N₂ input port. Hence, the Air Input will remain unused.

When changing ‘Gas mode’, please keep note the following points:

- The system will need some time to purge the tank and reach the new steady state.
When you work in ‘CO₂ in Air’ mode, then Oxygen will no longer be a parameter that you can regulate. The screen will show ‘AIR’ in place of the set point on the Oxygen tab. The Oxygen % value displayed is a calculated one by keeping into account the approx. concentration of O₂ in air, (c.a. 21%), and the desired output concentration. (The CO₂ % displayed is the set point (see Figure 60).

![Tri-Gas Mixer homepage when CO₂ in Air Mode is active.](image)

**Figure 60: Tri-Gas Mixer homepage when CO₂ in Air Mode is active.**

## 16 Unit Operation Through CO₂/O₂ Analyzer Touch Screen Control

### 16.1 Home page

![CO₂/O₂ Analyzer home page screen.](image)

*Figure 61: CO₂/O₂ Analyzer home page screen.*

The meaning of color indicators is described on page 53.
16.2 **General Settings**

16.2.1 **Set Point change**
When the CO2/O2 Analyzer is connected to the Tri-Gas Mixer, the set points appearing on its home page are the same selected from the Tri-Gas Mixer. It is not possible to change set points from the Gas Analyzer. When the CO2/O2 Gas Analyzer is used as a stand-alone device measuring gas composition then it can also be used to change set points as follows: simply touch the tab of the gas to be adjusted. For instance to change CO2, touch the CO2 tab. The CO2 Set point page will open. Increase or decrease the concentration set point by clicking on + and – to reach the desired concentration. Press “Set” to save the new set point or “Cancel” to undo (see Figure 1).

![Figure 62: Description of the change in Set Point CO2 and O2 concentrations.](image)

16.2.2 **Touch screen settings**
See paragraph 15.2 “Touch Screen Orientation, Screen Saver, Alarms Settings And Status Page” on page 50.

16.2.3 **Alarms settings**
See paragraph 15.2.2 “Alarm Settings” on page 52.

16.2.4 **Calibration Of The CO2/O2 Analyzer Without Junction Box Connected To The Tri-Gas Mixer**
Calibration setting allows doing a manual calibration of the instrument by using an external Nitrogen supply for the Zero Calibration and an external Span Gas supply for the Span Gas Calibration.
Running the Zero Calibration:

1. Use polyurethane tubing to connect the Nitrogen supply to the Pressure Gauge.
2. Use polyurethane tubing with an in-line valve to connect the Pressure Gauge to the input port of the Gas Analyzer.
3. Adjust the pressure of the pure Nitrogen tanks to 1.5 barg. That means 1.5 bar above the ambient pressure or 2.5 bar of absolute pressure at sea level.

4. Adjust the pressure of gas entering the Gas Analyzer at 0.5/1 barg with the Pressure Gauge.

![Pressure Gauge Connections to the Gas Analyzer and N₂ tank.](image)

Figure 63: Pressure Gauge Connections to the Gas Analyzer and N₂ tank.

5. Adjust the gas flow rate of the CO₂/O₂ Analyzer to a value within the range 35-50 ccm by using the Needle Valve (see Figure 64) on the rear panel of the Analyzer.

![Needle Valve on the rear panel of the CO2/O2Analyzer.](image)

Figure 64: Needle Valve on the rear panel of the CO2/O2Analyzer.

6. Switch the Gas Analyzer on and start the calibration following the instructions shown in Figure 65.
The Gas Analyzer will read gas levels that are entering and it will compare them with zero reference, applying an offset. Wait for the external Gas meter reach a steady state this usually takes 10-15 minutes. When you’re sure values being read are at steady state click on the “Adjust” Tab. After pressing “Adjust”, the page will refresh displaying the new offset value that the system is now applying. Repeat the same procedure to perform O₂ Zero Calibration.

To run the Span Gas Calibration replace the Nitrogen supply with the external Span Gas supply and follow the steps shown in the following Figure.
The Gas Analyzer will read gas levels that are entering and it will compare them with the values on the Reference tank. Wait for the external Gas meter to reach a steady state this usually takes 10-15 minutes. When you’re sure values being read are at steady state click on the “Adjust” Tab. After pressing “Adjust”, the page will refresh displaying the new offset value that the system is now applying. Repeat the same procedure to calibrate the O₂.

16.2.5 Status page
The ‘Status’ Page of the CO₂/O₂ Analyzer shows the actual values of CO₂ and O₂ concentrations, as well as the gas flow rate.
17 Maintenance

Tri-Gas Mixer care:

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

Before cleaning the unit pull out the mains plug.

Keep system away from water.

- Never use thinners, benzene, solvents on or near the devices, since these could corrode their surfaces.
- Control the output gas CO₂/O₂ compositions, at least once a year, (See Application Note 2)
- Verify the status of all hoses and replace those damaged.
- Check for leaks at the tank under pressure.
- Check that there are no leaks in the tank and the tank pressure gauge measures correctly.
  The pressure indicated by the tank pressure gauge should be equal to the one shown on the home page of the CP2 touch screen.
- After about 3 years Okolab Srl recommends replacing the safety valve on the tank (CEE Approved Valve, 97/23 Ped, set at 6 Bar, Thread G1 / 4).
- After 2 years, disconnect all polyurethane tubing, 2 cm off the ends and reconnect them.
Before disconnecting any of the polyurethane tubing from the tank, the Tri-Gas Mixer, the Junction Box or the Gas Analyzer, make sure that there is no residual gas pressure. Check pressure gauges upstream of the system and on the tank manometer.

- After five years, Okolab Srl recommends a complete revision directly at the factory. During the revision, our specialized staff will do: replacing of all the tubing inside the unit, a re-calibration of the unit by mass flow meter and verification of accuracy and operation.

18 Application Note

18.1 Application note 1

If the gases sent to the Tri-Gas Mixer don’t have a purity of 99.99%, the output gas composition will be different from the expected. The presence of impurities in the gas sources alters the CO₂/O₂ compositions obtained downstream of the Tri-Gas Mixer.

In particular, the O₂ material balance, in proper working conditions, is (see the logical scheme shown in Figure 68: Logical diagram of the system)

\[ 20.96/100 \times F_{\text{Air}} = \chi_{\text{O}_2} \times F_{\text{tot}} \]

where the left side of the equation is O₂ content in the Air.

![Logical diagram of the system](image_url)

The internal software of the Tri-Gas Mixer regulates the Air, N₂ and CO₂ flow rate values for each set output gas composition. Hence using pure gas sources ensures that the \( \chi_{\text{O}_2} \) value will correspond to the set point.
But, if your Nitrogen tank contains a small O₂ percentage, for example 4.0%, the O₂ material balance becomes:

\[
\frac{4}{100}F_{N2} + \frac{20.96}{100}F_{Air} = x'O_2 \cdot F_{tot}
\]

Hence \(x'O_2\) will be different from \(xO_2 = xO_2\) set point.

In order to prevent this error Okolab S.r.l recommends checking the composition of each tank with an external meter before connecting them to the Tri-Gas Mixer.

### 18.2 Application Note 2

To double check the CO₂/O₂ concentrations of the Tri-Gas Mixer, the gas output can be checked with a gas meter downstream of the Tri-Gas Mixer. If your system configuration is Configuration 2 or Configuration 3, you can use the Gas Analyzer after its calibration as reported in paragraph 16.2.4. If your system configuration is the Configuration 1, you can use any other gas meter previously calibrated.

Follow the steps listed below:

1. Reset the tuning parameters via Tri-Gas Mixer touch in order to set to zero any correction, see paragraph 10.4.2 ‘Tuning Parameters Reset’
2. Wait for the Tri-Gas Mixer to reach the desired compositions (e.g. 6% CO₂ and 5% O₂) in the Pressurized Tank and make sure that the indicators on the CP2 touch home page are green.
3. Verify the pressurized gas compositions in the tank using the Gas Analyzer or a manual gas meter:
   - If the difference between readout of the gas meter and the Set Point compositions is less than 0.3%, the Tri-Gas Mixer is working within specs. You can further calibrate it to bring the error to zero (See paragraph 9.3.7 for Configuration 1, see paragraph 10.4.1 for Configuration 2 or Configuration 3)
   - If the difference between readout of the gas meter and the Set Point compositions is higher than 0.3%, the Tri-Gas Mixer is showing an error due to the elapsed working time called “annual drift of the sensors”. In this case, you can also cancel the error by performing a manual calibration (see paragraph 9.3.7 for Configuration 1, see paragraph 10.4.1 for Configuration 2 or Configuration 3) but only if the error is less than 1.5%.
   - If the difference between the readout of the gas meter and the Set Point compositions is higher than 1.5%, the Tri-Gas Mixer has some problems. In this case, contact Okolab Srl.
18.3 Application note 3

18.3.1 Complete System For Live Cell Microscopy Applications.

A. Pressure gauge with fine regulator suitable for pure CO₂ tanks. Full Scale of 8/116 (barg/PSIG) with Push-To Connect fittings for tubing of 6 mm O.D.

B. Devices and accessories provided by Okolab.

C. Pressure gauge with fine regulator suitable for pressurized Nitrogen or Air tanks. Full Scale of 8/116 (barg/PSIG) with Push-To Connect fittings for tubing of 6 mm O.D.

D. Pressure gauge with filter and fine regulator. Full Scale of 8/116 (barg/PSIG) with Push-To Connect fittings for tubing of 6 mm O.D. Provided by Okolab.

E. Tubing provided by Okolab.

F. Pressure gauge with fine regulator. Full Scale 2.5/36.2 (barg/PSIG) with Push-To Connect fittings for tubing of 6 mm O.D. Provided by Okolab.

G. Push To Connect T Cross Type Connector for 6 mm O.D. tubing.

H. Manual ball valve with Push-To Connect Inlet and Outlet for 6 mm O.D. Tubing.

I. Tubing made by PTFE for fixed installation. 4 mm I.D. – 6 mm O.D.

Figure 69: Tubing Connections for Live Cell Microscopy applications, Configuration 1.
18.3.2 Complete System for IVF Applications.

Figure 70: Tubing Connections for IVF applications. Configuration 1.

The Figure 70 shows the same tubing and accessories listed in previous paragraph but in IVF Applications, after the pressure gauge (Point F in Figure 70), there is a manifold (Point L in Figure 70) splitting the pressurized output gas flows among several users.

19 Support

19.1 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 CD-ROM 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 set an appointment for web assistance.
19.2 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.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Probable cause</th>
<th>Remedy</th>
</tr>
</thead>
<tbody>
<tr>
<td>The little led on the front of the Control Unit is off</td>
<td>Power plug is disconnected from outlet</td>
<td>Insert plug securely into outlet</td>
</tr>
<tr>
<td></td>
<td>Blown fuse</td>
<td>Replace the fuse, 1A-Time Lag Fuses</td>
</tr>
<tr>
<td>No N₂, CO₂ and/or Air flow rate stability or the valves icons on the Homepage are red</td>
<td>Pressure drop</td>
<td>Check reservoir and/or compressed line</td>
</tr>
<tr>
<td>No N₂, CO₂ and/or Air flow streams</td>
<td>Regulators closed</td>
<td>Open regulators</td>
</tr>
<tr>
<td>Acoustic alarm sounds</td>
<td>The current CO₂ and O₂ values are far from CO₂ and O₂ set</td>
<td>Check the CO₂, Air and N₂ supplies</td>
</tr>
<tr>
<td>The little led on the front of the Tri-Gas Mixer is green but the Oko-Touch is off</td>
<td>Mini USB cable is disconnected</td>
<td>Check the connection between Control Unit and the Oko-Touch</td>
</tr>
<tr>
<td>Gas leak on the rear of the control box</td>
<td>Not properly inserted tubes</td>
<td>Strongly push the tube into the Swift-Fit connector</td>
</tr>
<tr>
<td>The tank never reaches to 3 bar</td>
<td>Not properly inserted tubes or output flow rate is too high</td>
<td>Strongly push the tube into the Swift-Fit connector or reduce the output flow rate</td>
</tr>
<tr>
<td>I check the previous troubleshooting but I cannot solve the problem</td>
<td>Contact Okolab to receive assistance</td>
<td></td>
</tr>
</tbody>
</table>
19.3 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
Italy
## Tri-Gas Mixer with Controlled Pressure – Technical Specifications

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
</table>
| **CO₂**                    | Range: 0-10%  
                          | Step size: 0.1%  
                          | Accuracy: 0.3% (0.1% when the CO₂/O₂ Analyzer is connected to the Tri-Gas Mixer)  
                          | Recommended Calibration Interval: 1 year |
| **O₂**                     | Range: 0-10%  
                          | Step size: 0.1%  
                          | Accuracy: 0.3% (0.1% when the CO₂/O₂ Analyzer is connected to the Tri-Gas Mixer)  
                          | Recommended Calibration Interval: 1 year |
| Output pressure            | 3 bar                                                          |
| Input Gas                  | CO₂, Air, Nitrogen, Span Gas                                               |
| Max Gas output Flow rate   | 1.1 L/min with a tank volume of 4.5 L  
                          | 1.5 L/min with a tank volume more than 4.5 L                              |
| Operating Temperature      | 0°C ~ +55°C                                                               |
| Storage Temperature        | -5°C ~ +60°C                                                              |
| Operating Humidity         | 0-70%                                                                      |
| Power Consumption          | 115/230V AC 50/60 Hz; Fuse 1AT 250V 5x20; 37 W max                         |
| Weight                     | 5 Kg                                                                        |
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**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.
21 Glossary

**Autotuning**: automatic and periodic calibration routine, it can be enabled and disabled by the Tri-Gas Mixer touch screen in the Configuration2 and Configuration3 of the system.

**Tuning**: automatic calibration routine which can be run by the Tri-Gas Mixer touch screen in the Configuration2 and Configuration3 of the system.