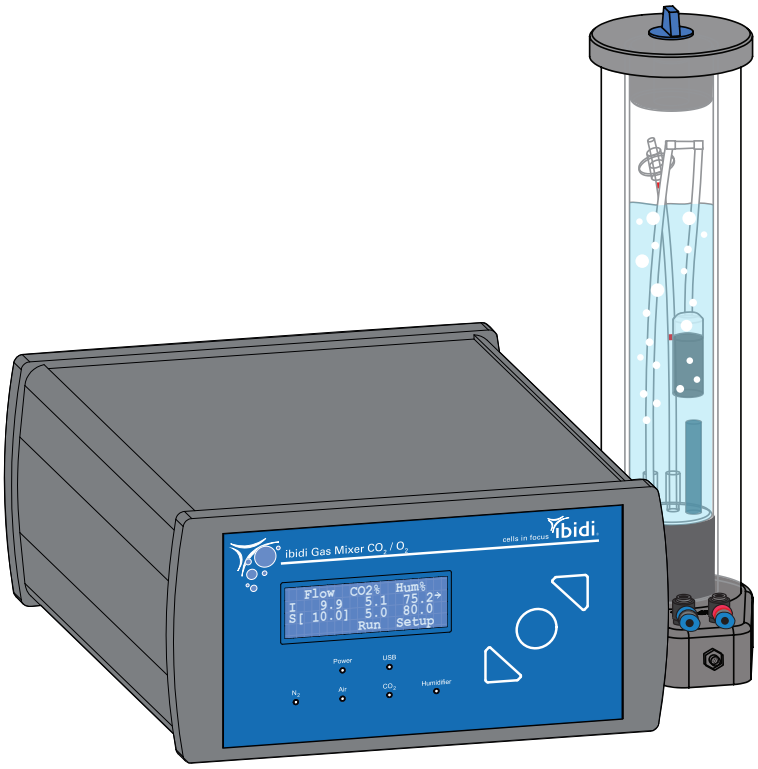
ibidi
cells in focus

Instruction Manual
ibidi Gas Incubation System

Version 2.2



11920 ibidi Gas Incubation System for CO_2

11922 ibidi Gas Incubation System for CO_2 and O_2

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Contents

1 Preamble

1.1 Introduction

This manual is your guide to using the Gas Incubation System for cell culture experiments on an optical microscope. It instructs first-time users how to use the instrument, and serves as a reference for experienced users.

Before using the Gas Incubation System, please read this instruction manual carefully and make sure that the contents are fully understood. This manual should be easily accessible to the operator at all times during instrument operation. If this manual gets lost, order a replacement from www.ibidi.com.

To ensure safe operation, the Gas Incubation System must only be operated with the supplied components and according to the instruction manual.

1.2 Safety Symbols

Note that the signal words **WARNING**, **CAUTION** and **NOTE** have specific meanings in this manual. Do not proceed beyond a signal word until you have performed the indicated actions.

WARNING! A potentially hazardous situation which, if not avoided, could result in serious injury or even death. Warning messages in the text are displayed in a gray shaded box.

CAUTION A potentially hazardous situation which, if not avoided, could result in minor or moderate injury. It is also used to alert against damaging the equipment or the instrument.

NOTE Additional information to help achieve optimal instrument and assay performance.

Symbols on the product identification label and back panel of the device:

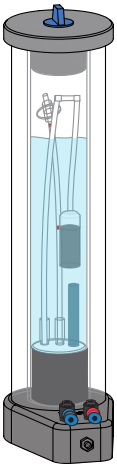
CE Marking: This symbol indicates the product's compliance with EU legislation.

This label is positioned on the back of the device and prompts you to read the manual before using the device.

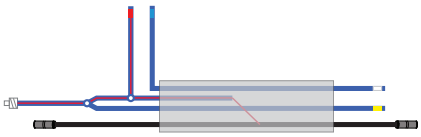
Product disposal: The symbol indicates that this product must be recycled/disposed of separately from other household waste. See page 13 for details.

1.3 Nomenclature

Gas Mixer



Humidifying Column



Heated Tubing Package

1.4 Specifications

Table 1 – Specifications of the Gas Incubation System

Electrical Specifications Power Supply	
Protection class	I
International protection marking (IEC 60529)	IP 20
Overvoltage category	II
External power supply	AC 100-240 V, 50/60 Hz, 1.4 A
Input line voltage Gas Mixer	DC 24 V, 5 A, 120 W
Output voltage (to Humidifying Column)	DC 24 V, max. 3.6 A

Operating Conditions	
Operating site	Indoor use only
Operating temperature	18-30°C/64-86°F
Operating humidity	max. 80% relative humidity (RH)
Operating altitude	max. 2000 m (atmospheric pressure 800–1060 hPa/11.6–15.4 psi)
Storage Conditions	-5-50°C/23-122°F, humidity <60% relative humidity (RH)

Outer Dimensions and Characteristics of the Components	
Gas Mixer	90 × 170 × 230 mm^3 2230 g/4.9 lbs
Humidifying Column	70 × 110 × 300 mm^3 660/710/790 g /1.45/1.57/1.74 lbs (empty/min/max)
Heated Tubing Package	0.6 m (between Controller and Column)

Table 1 – (continued)

	1.5 m (between Column and Heated Lid) 82 g/0.18 lbs
Cable for Humidity Sensor	1.5 m
USB cable	1.8 m
Power supply cable	2.0 m (power supply to wall) 1.2 m (power supply to device)

Gas Connections	
Gas tubing material	Polyurethane, hydrolysis resistant
Gas tubing connections	Push-in fittings
Gas Mixer input	ID 4 mm OD 6 mm
Gas Mixer output and Humidifying Column connection	ID 2.5 mm OD 4 mm
Connection to the Heated Lid	Male Luer Lock

Gas Input Requirements	
Compressed air	Purity class: ISO 8573-1:2010 [1:2:1] 1 bar/14.5 psi optimum (0.8–1.2 bar / 11.6–17.4 psi)
Carbon dioxide (CO_2)	Purity: >99% CO_2 1 bar/14.5 psi optimum (0.8–1.2 bar / 11.6–17.4 psi)
Nitrogen (N_2)*	Purity: >99% N_2 1 bar/14.5 psi optimum (0.8–1.2 bar / 11.6–17.4 psi)

*only for the CO_2/O_2 version

CO_2 Control	
Control range	0-15%
Accuracy	±(0.2%vol. + 3% of reading) e.g. ±0.35% at 5% CO_2 concentration
Resolution	0.1%
Self-calibration	At least once per week the Gas Mixer must be either turned off for five minutes or the CO_2 concentration must be set to 0% for five minutes This is necessary for the sensor's onboard automatic calibration routine, which needs an atmospheric CO_2 measurement to combat sensor drift.

O_2 Control*	
Control range	1-21% (Note: Settings below 1% are less accurate and a typical minimum value in an ibidi Heating Chamber is 0.5% O_2)
Accuracy	±0.2% (typical) ±0.5% (max.)
Resolution	0.1%
Heat-up time	Five minutes before proper operation of sensor

Table 1 – (continued)

CO_2 cross sensitivity	The O_2 sensor has some cross–sensitivity to CO_2 gas at 0% O_2 concentrations. This manifests as an offset in the measured O_2 concentration, with typical values shown in the table below:

CO_2 Conc.	Set O_2 Conc.	Displayed O_2 Conc.	Actual O_2 Conc.
0%	0%	0.1%	0.1%
5%	0%	0.5%	0.1%
10%	0%	0.6%	0.1%

Lifetime	Sensor lifetime is three years under continuous operation.

*only for the CO_2/O_2 version

Humidity Control	
Humidity sensor type	Sensirion SHT31
Control range	20–99% (lower limit dependent on environment and gas supply, min. set humidity is 0%)
Sensor accuracy	±2.5% (max.)
Resolution	0.1%

Humidifying Column Temperature Control*	
Control range	Ambient water temperature to 55°C (min set temp 0°C)
Accuracy	±2°C
Resolution	0.1%

*only controllable on the Gas Mixer, not in IncuControl

Flow Control Mixed Gas	
Control range	0–20 l/h
Accuracy	±25%
Resolution	0.1%

Humidifying Column	
Liquid	Ultra-pure water
Minimum volume	50 ml
Maximum volume	130 ml
Refilling	80 ml (approximately after 7 days at 10 l/h)

1.5 Disclaimer

- ibidi shall not be held liable, either directly or indirectly, for any damage incurred as a result of product use.
- The contents of this manual are subject to change without notice for product improvement.
- This manual is considered complete and accurate at publication.
- This manual does not guarantee the validity of any patent rights or other rights.
- If an ibidi software program doesn't function properly, this may be caused by a conflict from another program operating on the computer. In this case, take corrective action by uninstalling the conflicting product(s).
- ibidi is a registered trademark of ibidi GmbH in Germany and other countries.

1.6 Safety Considerations

WARNING!

- Only operate the Gas Incubation System with the supplied components.
- Only use the cables and plugs delivered with the system. The power plug of the control unit must be inserted in an outlet with a ground (earth) contact.
- Do not replace detachable power cables by power cables with inadequate specifications. By violating these instructions you risk electric shock and fire.
- Only use extension cables that have a protective ground wire.
- Do not operate the Gas Incubation System under conditions that pose a risk of explosion, implosion, or the release of gases. Only operate the Gas Incubation System with aqueous solutions.
- Do not operate a damaged Gas Incubation System. If the housing seems damaged or something is rattling inside the controller, contact the ibidi service hotline for repair.
- Some accessible parts of the Gas Incubation System (Humidifying Column base, water inside the column, and Heated Tubing Package) can reach temperatures up to 55°C/131°F (60°C/140°F the water inside the column). Avoid touching the temperature-controlled parts of the system when hot.

CAUTION

- Ensure that the external power supply is easily accessible. The Gas Incubation System must be installed in a manner such that none of its components hinders access to the external power supply.
- Immediately replace damaged cords, plugs, or cables to avoid risk of personal injury or damage to the instrument.

- Only ibidi technical staff and technical staff instructed by ibidi are permitted to open and service the Gas Incubation System.
- The external power supply should not be brought into contact with moisture. If the housing is damaged, the external power supply should not be used.
- Avoid strong magnetic fields and sources of high frequency. The Gas Incubation System might not function properly when located near a strong magnetic field or high frequency source.
- Avoid vibrations from vacuum pumps, centrifuges, electric motors, processing equipment, and machine tools.
- Avoid dust and corrosive gas. Do not install the Gas Incubation System where it could be exposed to high levels of dust or to outside air or ventilation outlets.
- Install the Gas Incubation System in a horizontal and stable position, such as a table, bench, or desk upon which the instrument is installed.
- Install the Gas Incubation System in a location that enables easy access for maintenance.
- Do not place heavy objects on the instrument.
- The Gas Mixer needs a gas input of 1 bar/14.5 psi. Only operate the Gas Mixer with the pressure indicated in the specifications on page 7.

1.7 Regulatory Statement

The Gas Incubation System has been designed, produced and tested in compliance with the European standard DIN EN 61010-1 (IEC 61010-1, ”Safety requirements for electrical equipment for measurement, control and laboratory use”). Furthermore it meets the IEC 61326-1 (”Electrical equipment for measurement, control and laboratory use - EMC requirements”) and CISPR 11 (”International Standard for electromagnetic emissions (disturbances) from Industrial, Scientific and Medical (ISM) Equipment”) standards .

The device carries the CE mark.

The Gas Incubation System meets the Low Voltage Directive 2014/35/EU and the EMC Directive 2014/30/EC.

1.8 Limited Warranty

Products manufactured by ibidi, unless otherwise specified, are warrantied for a period of one year from the date of shipment to be free of defects in materials and workmanship. If any defects in the product are found during this warranty period, ibidi will repair or replace the defective part(s) or product free of charge.

This warranty does not apply to defects resulting from the following:

1. Improper or inadequate installation.
2. Improper or inadequate operation, maintenance, adjustment, or calibration.
3. Unauthorized modification or misuse.
4. Use of unauthorized tubing or fluidic connectors.
5. Use of consumables, disposables, and parts not supplied by an authorized ibidi distributor.
6. Corrosion due to the use of improper solvents, samples, or due to surrounding gases.
7. Accidents beyond ibidi's control, including natural disasters.

This warranty does not cover consumables, such as cell culture chambers and dishes, tubes, fluidic connectors, reagents etc.

The warranty for all parts supplied and repairs provided under this warranty expires on the warranty expiration date of the original product.

1.9 Transporting the Gas Incubation System

The weight of the Gas Mixer is approx. 2.2 kg/4.9 lbs. The weight of the Humidifying Column is approx. 0.66 kg/1.5 lbs. Moving the devices during operation will pose a risk of personal injury or damage to the instrument.

For transport, switch off the Gas Mixer, close all gas input lines and then disconnect all cables and tubing from the controller and peripheral components. Leave the instrument to cool down for approximately five minutes. Carry the devices carefully and avoid mechanical shocks.

WARNING!

Close all gas input lines before disconnecting the gas tubing from the Gas Mixer!

1.10 Repairing the Gas Incubation System

For inquiries concerning repair service, contact the ibidi service personnel and provide the model name and serial number of your system.

ibidi GmbH
Service Hotline: service@ibidi.com

CAUTION

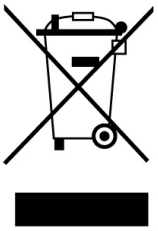
Do not try to repair the Gas Incubation System by yourself. Disassembly of the Gas Incubation System is not allowed. Disassembly poses a risk of personal injury or damage to the devices. Contact ibidi service personnel if there is a need to disassemble a device.

1.11 Waste Disposal – WEEE/RoHS Compliance Statement

The European Union (EU) has enacted two directives, the first on product recycling (Waste Electrical and Electronic Equipment, WEEE) and the second on limiting the use of certain substances (Restriction on the use of Hazardous Substances, RoHS).

1.11.1 EU Directive WEEE

The Gas Incubation System must be disposed of in compliance with the WEEE Directive 2012/19/EC.



This symbol on the product is in accordance with the European Union's Waste Electrical and Electronic Equipment (WEEE) Directive. The symbol indicates that this product must be recycled/disposed of separately from other household waste. It is the end user's responsibility to dispose of this product by taking it to a designated WEEE collection facility for the proper collection and recycling of the waste equipment. The separate collection and recycling of waste equipment will help to conserve natural resources and protect human health and the environment. For more information about recycling, please contact your local environmental office, an electrical/electronic waste disposal company or distributor where you purchased the product.

1.11.2 EU Directive RoHS

Two Categories of products covered by the WEEE Directive are currently exempt from the RoHS Directive – Category 8, medical devices (with the exception of implanted or infected products) and Category 9, monitoring and control instruments.

All of our products fall into either Category 8 or 9, and are currently exempt from the RoHS Directive. Nevertheless, the Gas Incubation System meets the requirements set forth in the RoHS Directive 2011/65/EC.

2 Intended Use

The Gas Incubation System provides defined gas control for live cell imaging experiments. It is intended to be used with stage top incubators and can be applied on various microscopes. The Gas Incubation System mixes the required gas ratios in the output and actively humidifies this mixed gas output before the gas enters the incubation chamber.

Two versions of the Gas Incubation System are available: the ibidi Gas Incubation System for CO_2 and the ibidi Gas Incubation System for CO_2 and O_2. Both versions can control the CO_2 concentration and humidity. The O_2 version can additionally downregulate the amount of O_2 in the gas mixture to create hypoxia.

The ibidi Gas Incubation System can be combined with the Heating System, providing a fully controlled stage top chamber.

3 Principle

Physiological Conditions in Live Cell Imaging

Whereas the Heating System[1] controls the temperature of the incubation chamber, the Gas Incubation System provides a controlled atmosphere of gas (CO_2 and optionally O_2) as well as a defined humidity in the emitted gas. The humidified atmosphere prevents the samples with living cells from evaporation of the culture medium.

The gas stream is flushed continuously through the stage top incubator and evades through the gaps of the incubation chamber.

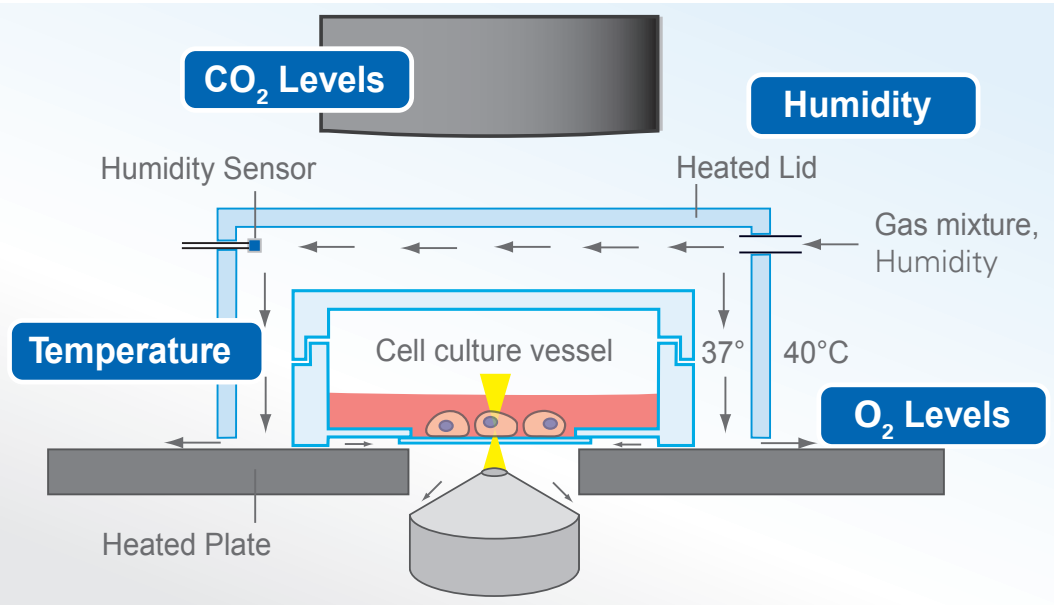


Figure 1 – Schematic cross-sectional view of the Gas Incubation System combined with the Heating System.

[1] Details about the Heating System are given in the Heating System instructions.

4 Equipment

Both versions of the Gas Incubation System are delivered with the complete set of cables and tubing needed for the operation of their system.

4.1 Components of the Gas Incubation System

An overview of the different Gas Incubation System versions is given in this section. Table 2 lists all available options of the Gas Incubation System and Table 3 shows all included components.

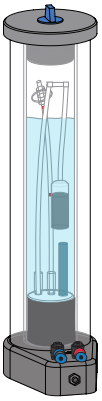
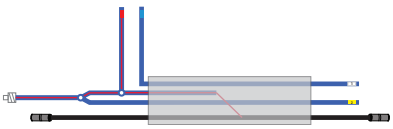
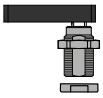
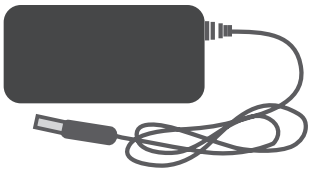
Table 2 – Overview of the available Gas Mixer Systems

Cat. No.	Description	Components
11920	Gas Incubation System for CO_2	Gas Mixer CO_2 Humidifying Column Heated Tubing Package Gas Tubing for CO_2 and Air Humidity sensor Humidity sensor cable USB cable Power cable USB flash drive Wrench for humidity sensor
11922	Gas Incubation System for CO_2 and O_2	Gas Mixer CO_2/O_2 Additional to all parts of #11920: Gas Tubing for N_2 Dead end tubing

Table 3 – Overview of the components of the ibidi Gas Incubation System for CO_2/O_2 and ibidi Gas Incubation System for CO_2. The parts that are only available in the CO_2/O_2 version are marked with an asterisk (*). The drawings are not to scale.

Component Name	Drawing
Gas Mixer	

Table 3 – (continued)

Component Name	Drawing
Humidifying Column	
Heated Tubing Package: cables and tubing to connect the gas outlet of the Gas Mixer, gas ports of the Humidifying Column and the gas inlet of the Heated Lid	
Gas tubing for CO_2, Air and N_2*	
Dead end tubing to close the N_2 gas inlet*	
Humidity sensor with counter nut to fix on the Heated Lid	
Humidity sensor cable: Cable to connect the Gas Mixer to the humidity sensor	
USB cable to connect the Gas Mixer with a computer	
External power supply for the Gas Mixer	
Country specific power cord to connect the external power supply to the wall socket	
USB flash drive with IncuControl software	
Wrench to fix the humidity sensor on the Heated Lid	

*only for the CO_2/O_2 Version

4.2 Gas Mixer CO_2 or CO_2/O_2

The Gas Mixer is the gas mixing and control unit of the ibidi Gas Incubation System. There are two versions of the Gas Mixer controlling either the amount of CO_2 or the amount of both CO_2 and O_2. Using the CO_2/O_2 version, hypoxia conditions can be created by replacing O_2 with nitrogen (N_2).

In addition to the gas mixture, the humidity within the incubation chamber is controlled by a feedback loop using a humidity sensor located in the incubation chamber. The humidity is increased by streaming the gas through a water column if the atmosphere is too dry.

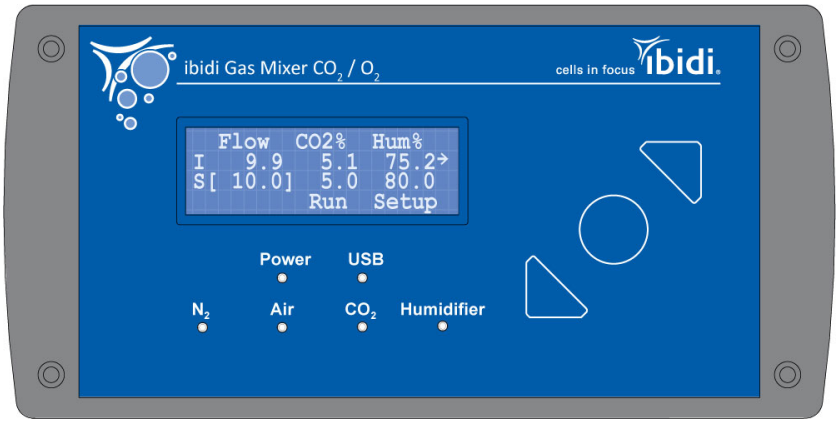


Figure 2 – Front view of the Gas Mixer.

The LEDs on the front indicate the status of the channels: connection to the power supply, connection to the USB, gas control, and humidity control (Figure 2).

Table 4 – Control LEDs of the Gas Mixer

LED Mode	Significance
LED off	Channel inactive
LED on	Channel active
LED fast blinking	Channel error

On the back of the Gas Mixer, all connections for cables and tubing are provided (Figure 3). In the CO_2 version, the N_2 port is closed inside the instrument and thus non-functional.

The Gas Incubation System takes as input pure CO_2 gas and pressurized air, as well as pure N_2 gas (only Gas Mixer CO_2/O_2). Pressurized air can be supplied by the lab gas line or a small air pump or compressor. Purity information is given on page 8.

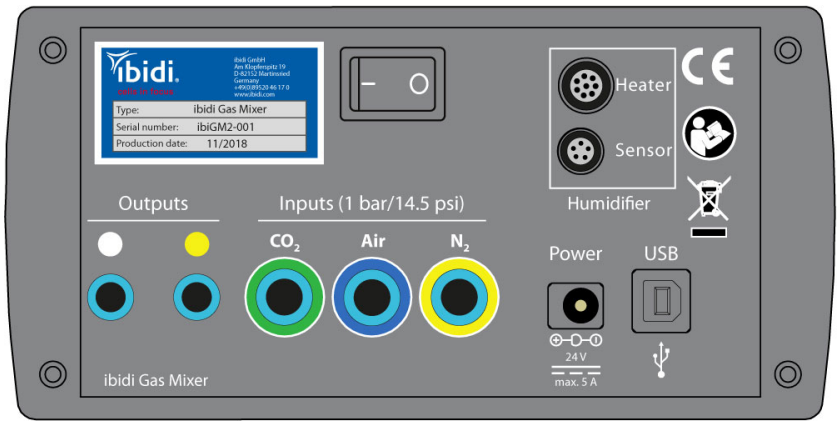


Figure 3 – Back view of the Gas Mixer.

4.3 Humidifying Column

The Humidifying Column provides a water reservoir to humidify the mixed gas. Part of the mixed gas output is guided through the column. The water inside the column is warmed up for higher humidity uptake. The different flow rates of the humidified and dry gas mixture control the humidity in the incubation chamber.

Caution!

The Humidifying Column base heats up to 50°C/122°F when in operation.

The Humidifying Column has two gas connections (input and output) as well as an electric plug for the heating of the column (connection see Section 5.1.2). On top of the column is an opening for filling it with water (filling see Section 5.2). The maximum (∼ 130 ml) and minimum (∼ 50 ml) levels are indicated by the red labels inside the column (see Figure 4).

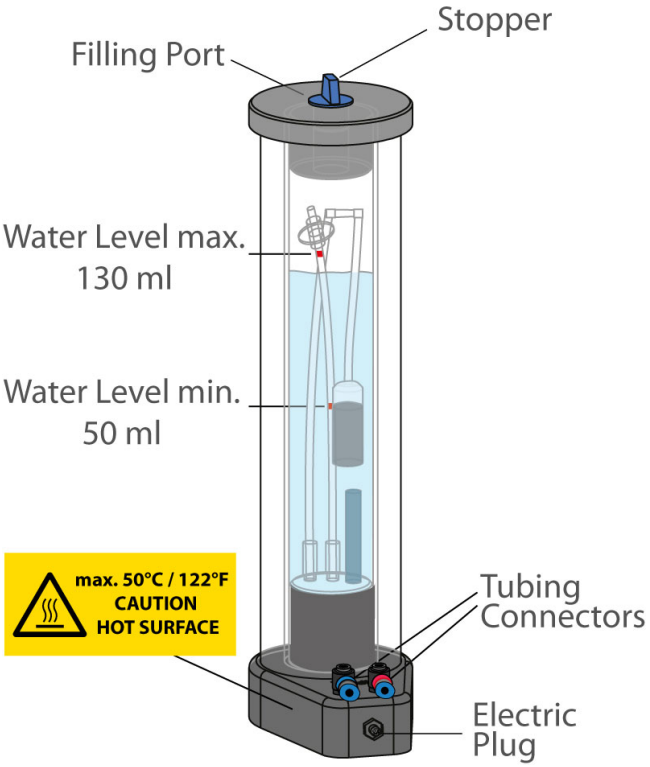


Figure 4 – Humidifying Column.

4.4 Heated Tubing Package

The Heated Tubing Package connects the gas output of the Gas Mixer to the Humidifying Column and to the Heated Chamber on the microscope stage. To actively control the humidity, two gas outputs are needed. One leads to the Humidifying Column and the other bypasses it. Both streams

are combined in the tubing leading to the stage top incubator. By varying the flow rates of the two gas streams, the humidity can be controlled.

The gas tubing carrying humidified gas is heated up to avoid condensation. For this purpose, a heating wire is inserted in the tubing. The wire is split off of the electrical cable under the insulating jacket enclosing the tubing. The exact connection of the tubing is shown in Figure 5.

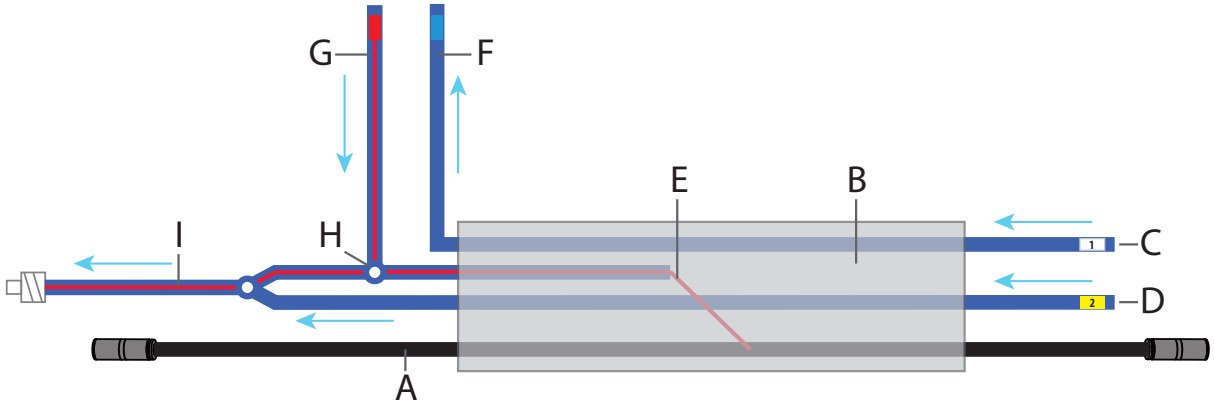


Figure 5 – Heated Tubing Package: Electrical cable for the heating of the Humidifying Column (A), Insulating jacket (B), Mixed gas output white and yellow (C and D), heated wire split off the electrical cable (E), gas input to Humidifying Column (F), gas output from Humidifying Column (G), merging point, where the heating wire is led into the tubing (H), gas tubing to the Heated Chamber (I).

Caution!

Do not bend or cut the Heated Tubing. The heating wire inside the tubing may be damaged and become non-functional.

4.5 Humidity Sensor

The humidity sensor is fixed in the side wall of the Heated Lid of the Heating System and measures the humidity inside the incubation chamber. It sits inside a small cavity in a black polymer block (Figure 6). This cavity must not be obstructed (e.g. covered with tape, pressed flat against the wall of a disposable, etc.) for the sensor to function properly. The humidity sensor is connected with a cable to the Gas Mixer and measures the humidity inside the incubation chamber, enabling fast, active humidity control.

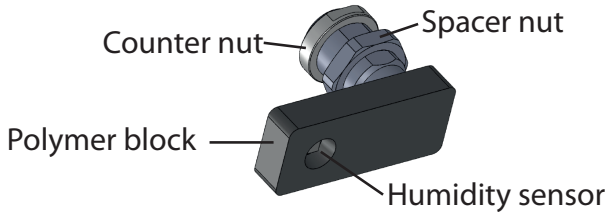


Figure 6 – Polymer block with humidity sensor and nuts for fixation in the Heated Lid side wall.

To ensure accurate humidity measurements , the humidity sensor must be mounted securely in the plastic through-hole in the side of the Heated Lid. To do this, please follow the steps below:

1. Check that the spacer nut is fixed on the humidity sensor's threaded connector.
2. Place the threaded part of the sensor through the opening in the Heated Lid's wall, so that the sensor is inside the chamber (Figure 7). The spacer nut fits into the cavity of the wall.
3. Screw the counter nut from the outside onto the thread and tighten it snugly against the outer wall of the Heated Lid using the wrench provided with the Gas Mixer. The flat cut-away on the counter nut should be facing away from the lid, allowing the wrench to fit properly over the nut. Be careful not to over tighten the nut.

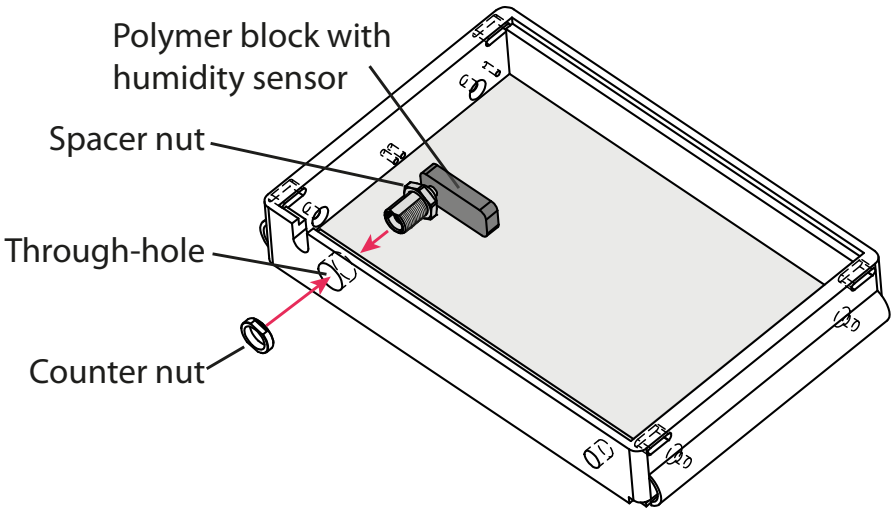


Figure 7 – Installation of the humidity sensor in the side wall of the Heated Lid.

Caution!

Make sure that the polymer block is not in contact with the Heated Lid. Heat transfer from the Heated Lid to the sensor can lead to low relative humidity readings, which in turn can lead to condensation in the chamber.

The connection of the humidity sensor with the Gas Mixer is shown in Section 5.1.3.

5 Operation

The components must be assembled in the following order:

1. Connect all tubing and cables.
2. Apply 1 bar (14.5 psi) to the connected gas tubing.
3. Switch on the Gas Mixer.

Caution!

Not maintaining this order may lead to device malfunction or damage!

The procedure is explained in detail in the following sections.

5.1 Installation and Connection

The Gas Mixer and Humidifying Column should be placed next to the microscope stand. The tubing of the Heated Tubing Package must reach the Heated Lid and Heated Plate on the microscope stage.

Caution!

Do not place the Gas Mixer and Humidifying Column on the microscope stage! The vibration might disturb the measurement.

All tubing and cables are connected to the back of the Gas Mixer (Figure 8).

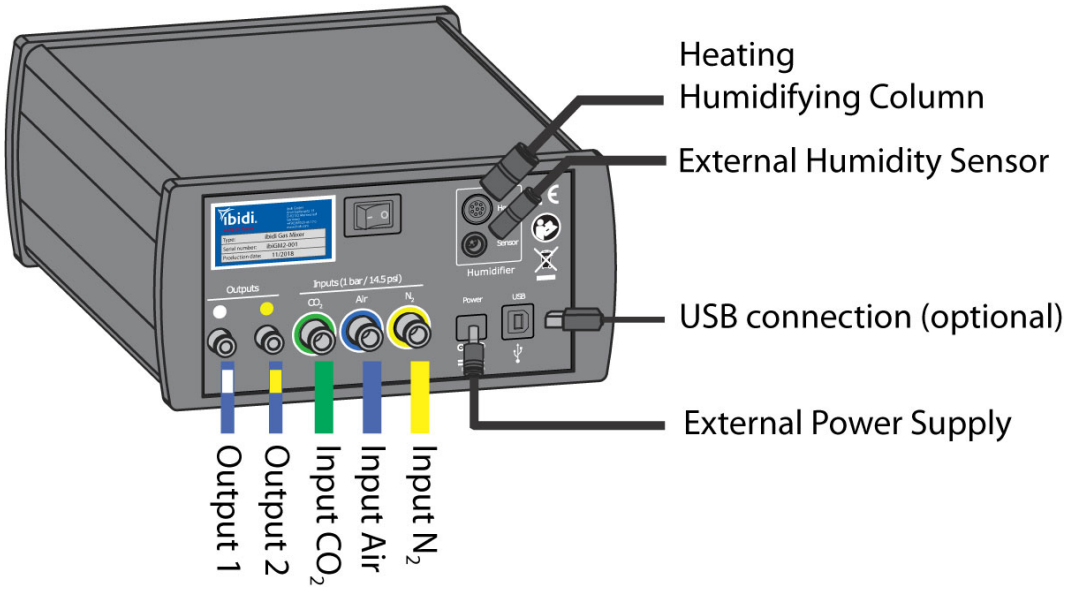


Figure 8 – Overview of the connection of all cables and tubing to the Gas Mixer. In the CO_2 version the N_2 port is not available.

5.1.1 Connection to the Gas Supply

The gases from the bottle or laboratory gas line need to be connected to the Gas Mixer with the OD 6 mm polyurethane tubing of the respective color. This tubing is delivered with the system.

Table 5 – Available connections and color code in the two different Gas Mixer versions

Gas Input	Gas Pressure	Tubing Color
Air	1 bar/14.5 psi	blue
CO_2	1 bar/14.5 psi	green
N_2*	1 bar/14.5 psi	yellow

*CO_2/O_2 version only

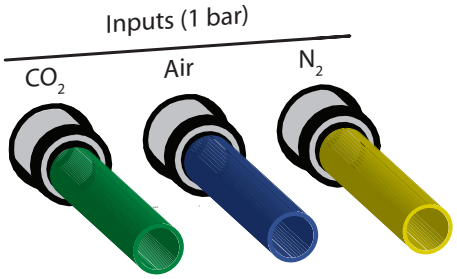


Figure 9 – Connection of the gas tubing to the Gas Mixer. The N_2 port is closed and not available in the CO_2 version.

To connect the gas tubing to the Gas Mixer, push the tubing into the push-in fittings. The functional principle is shown in Figure 10.

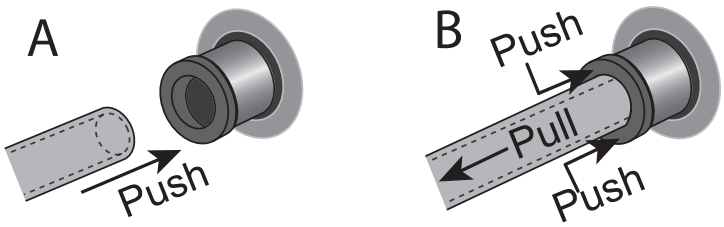


Figure 10 – How to connect and disconnect the gas tubing to the push–in fittings. Push in to connect (A). Push and pull to disconnect (B).

Caution!

Always connect all gas input ports with the appropriate gas (1 bar/14.5 psi). Do not leave any input ports open.
CO_2/O_2 version: In case the N_2 input is not needed (CO_2/O_2 Version only), close the N_2 input port using the provided dead-end tubing (Figure 11).

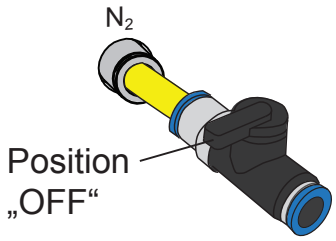


Figure 11 – Close the N_2 port of the Gas Mixer with the dead end tubing if not needed (CO_2/O_2 version only).

Warning!

Only disconnect the tubing when there is no pressure on the gas line. First, switch off the pressure from the gas line. Control the remaining pressure on the gas line's pressure reducing valve. Then, remove the tubing.

5.1.2 Connection of the Heated Tubing Package and the Humidifying Column

There are two lines coming from the Gas Mixer output. One goes to the Humidifying Column, the other directly to the Heated Chamber.

Table 6 – Connection of the gas lines with color code of the mixed gas

Color Code	
Gas Mixer output 1 (white)	Blue tubing with white label
Gas Mixer output 2 (yellow)	Blue tubing with yellow label
Humidifying Column output red	Blue tubing with red label
Humidifying Column output blue	Blue tubing with blue label

1. Connect the two gas outlets of the Gas Mixer to the white (1) and yellow (2) marked gas tubing to Output 1 and Output 2 on the back wall of the Gas Mixer.
2. Connect the plug of the electrical cable (next to the white and yellow gas tubing) to the "Heater" socket.
3. Connect the blue and red marked gas tubing to the respective inputs on the Humidifying Column.
4. To connect the electrical cable to the Humidifying Column, plug the cable into the connector on the column's base. Please make sure that the red dot on the cable and connector line up. Gently push to connect the plug.

5. To connect the mixed and humidified gas output to the Heated Chamber, screw the Luer Lock connector into the metal adapter that is fixed in the wall of the Heated Lid.

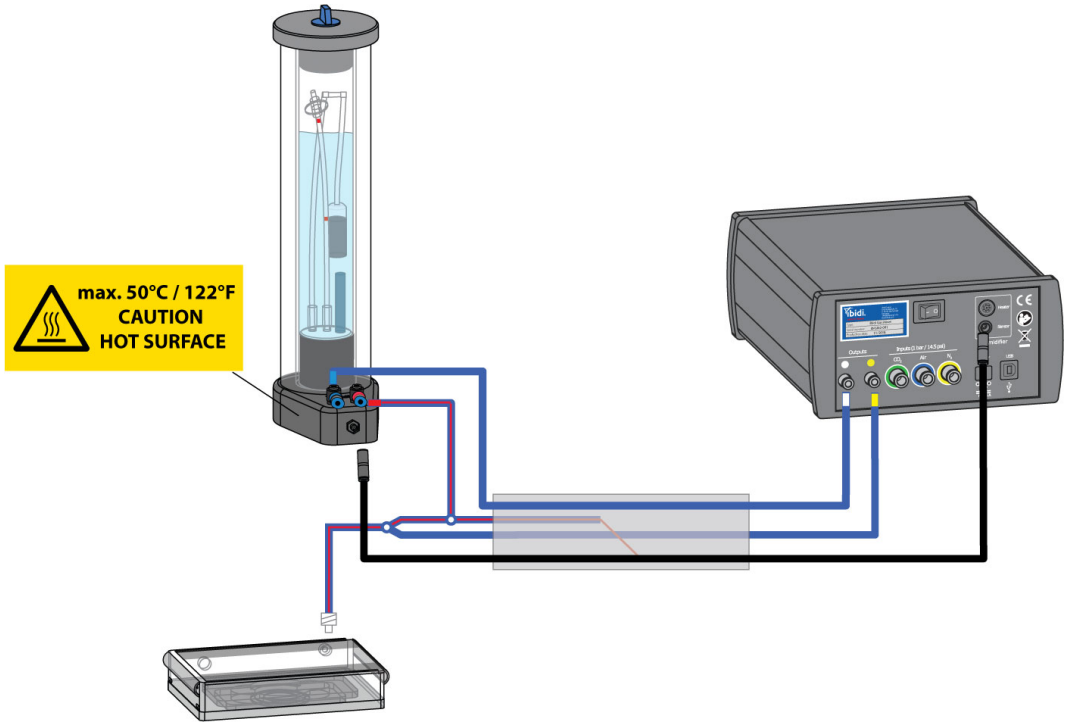


Figure 12 – Connection of the Heated Tubing Package to the Gas Incubation System.

Caution!

Do not disconnect the Heated Tubing while the Gas Mixer is running. The resulting sudden change of pressure may lead to damage to the Gas Mixer.
Be careful not to switch the red and blue inputs on the Humdifying Column, as this can also damage the gas mixer.

5.1.3 Connection of Humidity Sensor Cable

The humidity sensor should be mounted in the Heated Lid of the Heating System (see Section 4.5). To operate the Gas Incubation System with humidity control, the humidity sensor cable must be connected between the Gas Mixer and the humidity sensor.

1. Plug the humidity sensor cable into the Gas Mixer in the connector labeled ”Sensor”. Make sure that the red dots on the cable and connector line up.

2. Connect the other end to the humidity sensor mounted in the Heated Lid, as shown in Figure 13. Again make sure that the red dots on cable and connector line up.

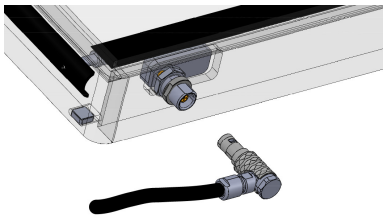


Figure 13 – Connection of the humidity sensor cable to the humidity sensor.

5.1.4 Connection of Power Supply (and USB optionally)

To finish the setup, the power supply must be connected. Optionally, the USB cable can be used to connect the Gas Mixer to an external computer. This enables the use of the IncuControl software delivered with the system (Section 6) for data logging and control of the parameters of the experiment.

1. Plug the power supply into the connector on the Gas Mixer labeled "Power".
2. Connect the other end to the wall socket.
3. Optional: Connect the computer to the Gas Mixer with the supplied USB cable.

5.2 (Re)-Filling the Humidifying Column

Before the first use, the Humidifying Column must be filled with 130 ml of ultra-pure water. During operation, the column should always be refilled before the water level falls below the minimum mark.

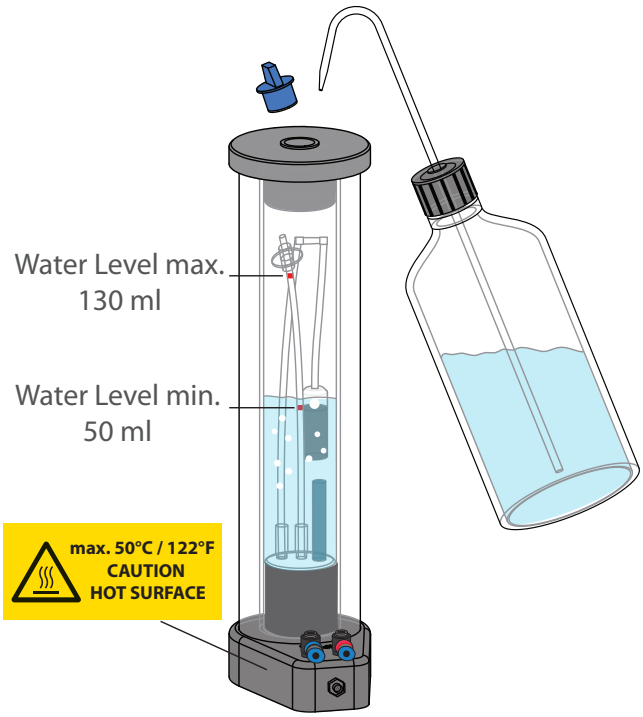


Figure 14 – (Re-)Filling the Humidifying Column.

Open the blue stopper on top of the column and use a wash bottle to fill it with ultra-pure water. The water level must be in between the minimum and maximum mark (Figure 14).

The re-filling of the Humidifying column can be done during operation, without stopping the Gas Mixer. Note that re-filling the column during operation may lead to a temporary drop in the chamber's humidity.

The length of intervals between refillings depends on set points of the humidity and flow on the Gas Mixer.

Caution!

Always close the filling port tightly to prevent gas leakage and malfunction of the system!

Warning!

Never disassemble the column while it is plugged into the Gas Mixer or when still hot after it has been in use. The heating element in the column can cause serious burns.

5.3 Switching on the System

After connecting all cables and tubing, and after filling the Humidifying Column ultra-pure water, the system is ready to start.

1. Set your gas regulation valve to the recommended pressure of 1 bar (14.5 psi).
2. Switch on the Gas Mixer and wait for "self test" (all systems) and "heat up" (only CO_2/O_2 versions).
3. Set the parameters on the display (Section 5.4).
4. Check that the display shows "Run" (see Section 5.4.1).
5. Give the system time to equilibrate all parameters for a minimum of 30 minutes.

Note

Immediately after powering up, the Gas Mixer runs a flow sensor calibration routine for several seconds. The display shows "self test".
After this, the Gas Mixer for CO_2/O_2 will automatically start a warm-up routine for 5 minutes. The display shows a blinking "heat up". After 5 minutes, the device will begin normal operation. The CO_2 version is ready for operation immediately after the "self test".

For most applications, we recommend using the values given in Table 7. The full specification range is indicated in the specifications (Section 1.4).

Caution!

Be aware that when the system is switched on, some parts of the tubing are actively heated: The Humidifying Column base (max. 55°C/131°F), the water in the Humidifying Column (max. 60°C/140°F) and the Heated Tubing Package (max. 40°C/104°F outside).

5.4 Setting Parameters in the Front Display

All control parameters can be manually set on the controller using the buttons and the display on the front panel.

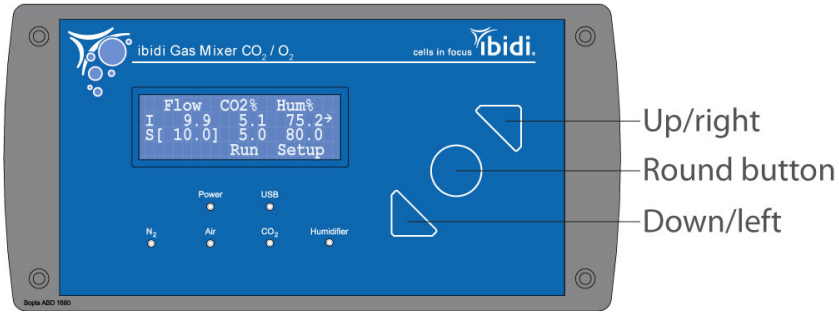


Figure 15 – Gas Mixer front display and set buttons.

The display shows the measured ('I' = instantaneous) and set ('S' = set) temperatures of all the channels.

The cursor position is indicated with square brackets ("[ ]"). You can move the cursor using the "left" and "right" buttons. If you want to select a parameter or a function, press the round button and the square brackets will change to angle brackets ("< >"). Now you are able to change the value with the "up" and "down" buttons. To confirm the changed value, you must press the round button once more.

5.4.1 Run/Stop Mode

Set the whole system to run or stop mode by manipulating the setting in the display's bottom line to "Run" (= system is running) or "Stop" (= system is not running).

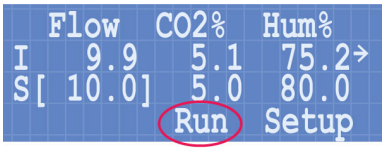


Figure 16 – Main display indicating "Run"

5.4.2 Incubation Parameters

Table 7 – Recommended parameters for the operation of the Gas Incubation System

Parameter	Recommended Values
CO_2 (%)	5–10% depending on buffer
O_2* (%)	20% (see Figure 17)
Humidity (%)	90–95%
Flow rate	10 l/h
Column Temperature (°C)	50°C

*for CO_2/O_2 version only

CO_2 regulation CO_2 is standardly set to 5% in cell culture experiments.

Humidity regulation Humidity values at the lower end of the control range can only be achieved using dried compressed air or with a compressed air pump in a very dry environment. The exact value of the lower limit will vary depending on the amount of water vapor present in the input gases used, the pressure, and the temperature of the measured environment.

Flow rate At flow rates below 7 l/h, gas mixes may not reach the highest possible humidities. If this is the case, augment the gas flow rate until the humidity is reached.

O_2 regulation/hypoxia (Gas Mixer for CO_2 and O_2 only) To generate hypoxia conditions, set the oxygen to the target value. The O_2 will be replaced by nitrogen (N_2) until the set value is reached.

Please keep in mind that in a CO_2/air mixture, the O_2 concentration will drop when the CO_2 concentration is raised. The maximum oxygen concentration thus decreases according to the CO_2 value you have chosen. Please see the graphic below for estimating the maximum O_2 concentration in your gas mix for a set CO_2 concentration. For example, when setting the CO_2 concentration to the common value of 5%, the maximum oxygen level is decreased to 20% O_2 (see Figure 17).

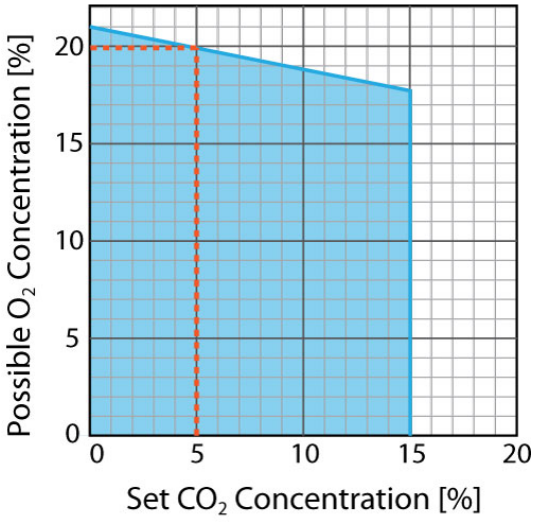


Figure 17 – Maximum O_2 concentrations at different CO_2 concentrations in gas mixtures from the Gas Mixer.

5.4.3 Setup Menu

The Setup menu offers the possibility to change the settings of the individual channels and the display. For standard operation no changes need to be made. Enter the setup menu by navigating to the "Setup" entry and pressing the round button. The mode dialog will open.

Mode Each channel of the Gas Mixer can be set to an "On" or "Off" state. For standard operation all channels must be turned on. Only if you are using the CO_2/O_2 version without nitrogen (N_2) connection, it is recommended to turn the O_2 channel off. In this case make sure that the dead-end tubing is attached to the N_2 port and closed.

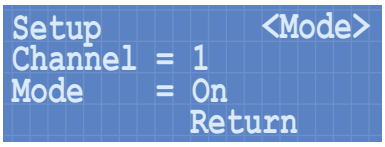


Figure 18 – Mode dialog

1. Press the round button once more to move the cursor to the channel number.
2. Select the respective channel and confirm with the round button.
3. Select "On" or "Off" and confirm with the round button.
4. To move on navigate to "Return" and press the round button.

Table 8 – Channel numbers are dedicated as follows:

Channel number	Parameter
Channel 1	Flow (outlet gas flow rate)
Channel 2	CO_2
Channel 3	Humidity
Channel 4	T-C (Humidifying Column Temperature)
Channel 5	O_2*

*CO_2/O_2 version only

Alarms In this dialog it is possible to set the alarm limits for divergent control parameters. Move from the mode menu to the alarm menu by pressing the "right" button.

The maximum and minimum limits of the alarm can be set for each individual channel. If the alarm is activated (which happens when the current value goes under/over the low/high limits), the current value (I) blinks, showing alternately the value and "low" or "high", respectively. To stop the display blinking, navigate to the blinking channel and press the round button once.

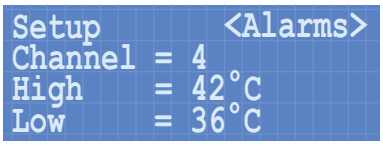


Figure 19 – Alarm settings dialog

Preferences Set the brightness and contrast of the display in the preferences menu.

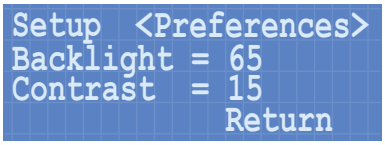


Figure 20 – Preferences dialog

Info Info about serial number and firmware version is shown in the info dialog.

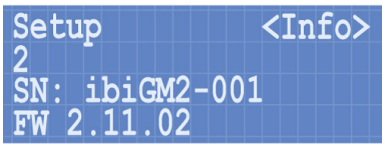


Figure 21 – Info display

6 IncuControl Software

The Gas Mixer has a USB interface for computer control and data logging. For this purpose, ibidi provides the IncuControl software that comes with the controller or can be downloaded from the ibidi website.

For more details, please refer to the IncuControl instructions.

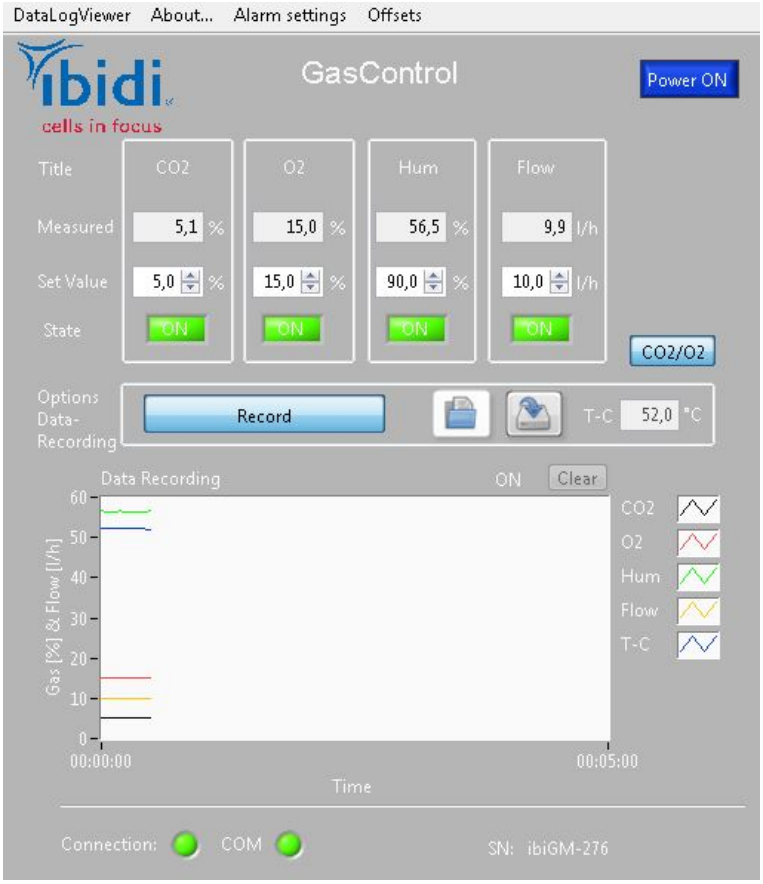


Figure 22 – Gas Mixer control window in IncuControl.

Prohibitions on the use of ibidi software:

- Copying software for other than backup purposes
- Transfering or licensing of the right to use software to a third party
- Disclosure of confidential information regarding software
- Modification of software

7 Maintenance

7.1 CO_2 Sensor Auto-Recalibration

At least once per week the Gas Mixer must be turned off for 5 minutes. This is necessary for the flow sensor's onboard automatic calibration routine, which needs an atmospheric CO_2 measurement to compensate sensor drift. When again switching on the system, the CO_2 sensor calibration is run automatically (see Section 5.3).

7.2 Disinfection and Cleaning

7.2.1 Wiping the Outer Surface

To clean the Gas Mixer and Humidifying Column from the outside, switch the Gas Mixer off and disconnect all cables and tubing. Leave the instrument to cool down for approximately 5 minutes. Use only dry cloth or a cloth dampened with water (ultrapure) or common lab disinfection solutions that are based on quaternary ammonium compounds (e.g., Barrycidal 36 or Pharmacidal). Fingerprints can be removed using ethanol or isopropanol (70%) or lens/eyeglass cleaning wipes.

Caution!

70% ethanol or isopropanol can be used to wipe the Humidifying Column from the outside. Do not use any other organic solvents. Do not expose to ethanol or isopropanol for an extended period of time. It can lead to damage of the Humidifying Column.

7.2.2 Cleaning the Column's Inner Face

Microbial contamination in the Humidifying Column is usually not a problem since distilled water and light-starved environments (microscopy rooms) do not support microbe growth. If, however, contamination has occurred, disinfect the Humidifying Column by running the system with 3% hydrogen peroxide for 1 hour. Subsequently wash with water. The use of anti-microbial agents is not recommended since they can be transported as aerosol and end up in the specimen.

7.3 Influence of Surrounding Temperature and Airflow

The surrounding, ambient temperature affects the temperature inside the microscopy chambers. Devices, such as computers and camera controllers, can significantly heat up small rooms. In this case, we recommend equilibrating the room temperature to the typical experimental conditions at least 2–3 hours before starting the experiment.

Airflow can enhance the effect of temperature and humidity changes in the vicinity of the incubation chamber. In cases where airflow (e.g., air conditioning) cannot be stopped, we recommend protecting the microscope as much as possible from it.

8 Troubleshooting

8.1 Focus not Stable

Focus drift is detrimental for most microscopy experiments, especially long duration time-lapse experiments. Focus stability is mainly influenced by mechanical changes and temperature variations. Follow these recommendations to keep your cells in focus:

- Switch on all components (e.g., heating, gas incubation, computer, or other equipment) at least 60 minutes before starting the experiment.
- After you put the sample onto the microscope, wait 30 minutes before starting a time-lapse experiment to achieve temperature and immersion oil equilibration[2].
- Keep the room temperature as stable as possible. Air conditioning should either be working continuously or switched off.
- Do not change the temperature during the experiments. Avoid door/window openings, as this could rapidly change the temperature.
- Eliminate all sources of mechanical vibrations. Use a damped table for your microscope.

8.2 Evaporation Is too High

Depending on the incubating conditions, small volumes might evaporate quickly, especially during long-term experiments. If you have an actively controlled humidifying device (e.g. ibidi Gas Incubation System), increase the set value for relative humidity. Additionally, we suggest using silicone oil (e.g. Anti-Evaporation Oil, ibidi, 50051) to decrease evaporation.

Covering the medium with sterile silicone oil prevents all evaporation effects and is compatible with cell culture. Please do not use mineral oil, as this can be harmful to your cultureware.

Equilibrate oil and medium inside the incubator overnight. This step helps to avoid the formation of air bubbles, and pre-warms the solutions to 37°C. Afterwards, fill your slide with cells and medium. Cover the medium's surface with an appropriate amount of silicone oil. Don't drip the oil directly onto the surface, but let it run down the edges of the cell culture vessel. Details about avoiding evaporation are given on the ibidi web site in Application Note 12 "Avoiding Evaporation".

8.3 Gas Flow too Low

8.3.1 Gas Input Open Without Connected Gas

Always connect all gas input ports with the appropriate gas and pressure (1 bar/14.5 psi). Do not leave any input ports open. In case N_2 is not needed or available, close the input port by using the provided dead-end tubing.

[2] If the experiment needs to be started immediately, either after placing the sample on the microscope or after closing the lid, we recommend checking the focus for 20 minutes. In the first few minutes after starting the experiment, temperature equilibration might influence the focus/z-position of the cells.

8.3.2 Gas Pressure too Low

Always use the recommended pressure of 1 bar/14.5 psi.

8.3.3 Set Values Are too High or too Low

Please make sure the set values are set to ambient air values (0% CO2, 21% O2) in case the corresponding gas is not connected. Additionally, keep in mind that the maximum O_2 concentration depends on the set CO_2 concentration. See page 28 for further details.

8.4 Condensation Inside the Stage Top Incubator

Check the temperature of the chamber (Heated Lid and Heated Plate). Make sure the humidity sensor is not in contact with the Heated Lid. In case of condensation, decrease the humidity and air-dry the incubator if necessary.

Please contact ibidi at techsupport@ibidi.com for further troubleshooting help.

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