



# Instruction Manual

## ibidi Gas Incubation System for CO<sub>2</sub> and O<sub>2</sub>

---

Version 1.3



## Table of Content

Safety Considerations .....	3
Nomenclature.....	4
Regulatory Statement .....	6
Specifications .....	7
Preface .....	9
Notices .....	9
Limited Warranty.....	10
Installation Requirements.....	11
Installation and Connecting Cables .....	12
Maintenance and Operation .....	16
Waste Disposal .....	17
1 Working Principle .....	18
2 Equipment.....	20
2.1 Product Description .....	20
2.2 Gas Mixer and Humidifying Column .....	20
2.3 Sterilization and Cleaning.....	20
2.4 Menu Navigation .....	21
2.5 Starting Operation and Recommended Settings .....	22
2.6 CO <sub>2</sub> Calibration .....	24
3 Control Software .....	24
4 Troubleshooting .....	24
4.1 Focus Not Stable .....	24
4.2 Evaporation Too High .....	24
4.2.1 Parafilm Procedure .....	25
4.2.2 Silicone Oil Procedure (ibidi's Anti-Evaporation Oil).....	25
4.3 Value of Relative Humidity (RH) not Stable .....	25
4.4 Gas Flow too low.....	25
4.4.1 Gas Input open without connected gas.....	25
4.4.2 Gas Pressure too low .....	25
4.4.3 Set Values are too high or too low.....	25
4.5 Condensation Inside the Incubator.....	25
5 WEEE/RoHS Compliance Statement .....	26
6 Appendix .....	27
6.1 Gas Consumption During Operation.....	27
6.2 Gas Humidification and Refilling Time.....	28

## Safety Considerations

To ensure operation safety, the ibidi heating system must be operated correctly and maintained according to a regular schedule. Carefully read this manual to fully understand all safety precautions listed in this manual before operating the instrument. Also, please take a moment to understand what the signal words **WARNING!**, **CAUTION**, and **NOTE** mean in this manual.

### Safety Symbols

#### **WARNING!**

A **WARNING!** indicates a potentially hazardous situation that, if not avoided, could result in serious injury or even death.

#### **CAUTION**

A **CAUTION** indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against damaging the equipment or the instrument.

Do not proceed beyond a **WARNING!** or **CAUTION** notice until you understand the hazardous conditions and have taken the appropriate steps.

#### **NOTE**

A **NOTE** provides additional information to help the operator achieve optimal instrument and assay performance.

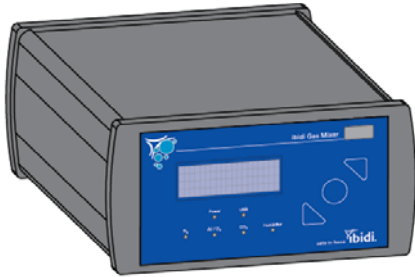


Read the Manual label. This label indicates that you have to read the manual before using the instrument (RTFM).

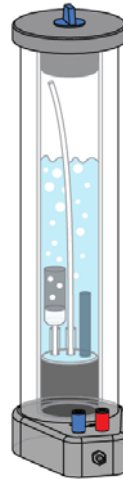


This symbol indicates important notes.

## Nomenclature



**GAS MIXER**



**HUMIDIFYING COLUMN**

**WARNING!** Only operate the ibidi Gas Incubation System with the supplied cables and plugs. If you use other attachments, you are at risk of electric shock and fire.

**WARNING!** Do not operate the ibidi Gas Incubation System with substances, or under conditions, which can cause a risk of explosion, implosion, or the release of gases. Only operate the ibidi Gas Incubation System with aqueous solutions.

**CAUTION:** Ensure that the power plug is easily accessible. The ibidi Gas Incubation System must be installed in a way that does not hinder access to the power plug.

**CAUTION:** Operate the Gas Incubation System only on a power socket with protective conductor to ground the housing of the Gas Mixer.

**CAUTION:** The weight of the ibidi Gas Mixer is approx. 2.1 kg. The weight of the Humidifying Column is approx. 0.63 kg. Provide a secure stand for the Gas Mixer and the Humidifying Column, where they cannot be accidentally dropped. Moving the instruments poses a risk of personal injury or damage to them.

**CAUTION:** Manually opening the ibidi Gas Incubation System is not allowed. Opening the equipment yourself poses a risk of personal injury or damage to the Gas Mixer and the Humidifying Column. Contact ibidi service personnel, if you need to open the instruments.

**CAUTION:** The ibidi Gas Incubation System includes heated tubing that controls the gas flow's temperature. Some accessible parts of the tubing and the Humidifying Column can reach temperatures of up to 60°C. Avoid touching the temperature-controlled parts of the system.

**CAUTION:** Only use the supplied, external power supply. The correct voltage/wattage is indicated on the external power supply. Using the wrong power supply can lead to malfunction, such as the overheating of the Gas Mixer, which can cause fires.

**CAUTION:** Only the ibidi staff is allowed to service and open the Gas Mixer and Humidifying Column. Unplug the power cord before having the system serviced, unless otherwise noted. Connect the Gas Mixer only to the supplied, external power supply. Do not use extension cords. Have a certified electrician immediately replace any damaged external cords, plugs, or cables. Not doing so poses a risk of personal injury or damage to the system.

**CAUTION:** Only use the ibidi Gas Incubation System in dry rooms. Do not use the ibidi Gas Incubation System in a cold room.

**CAUTION:** When the ibidi Gas Incubation System is not in use, remove the external power supply from the main power source.

**CAUTION:** Do not use ethanol or other types of organic solvents to clean the Gas Mixer, as they may remove the paint.

**CAUTION:** If contamination has occurred, sterilize the Humidifier Column by rinsing with a 70% Ethanol-in-Water mixture. Wash with sterile, ultra-pure water afterwards.

**CAUTION:** Use the instrument only for cell experiments with aqueous solutions.

**CAUTION:** Do not use the instrument with hazardous substances or substances/materials that pose a risk of infections.

# Regulatory Statement

in preparation

## Specifications

<b>Gas Incubation System for CO<sub>2</sub> and O<sub>2</sub></b>
---

<b>Power Consumption</b>	
Type	External Power supply
Supply Voltage	230 V / 50 Hz - 115 V / 60 Hz
Input Voltage / Wattage	24 V – 120 W (DC)

<b>Gas Supply (Input)</b>	
Pressurized Air (Blue color coding)	1 bar optimum / 14.5 psi (0.8-1.2 bar / 11.6 – 17.4 psi)
CO <sub>2</sub> (Green color coding)	1 bar optimum / 14.5 psi (0.8-1.2 bar / 11.6 – 17.4 psi)
N <sub>2</sub> (Yellow color coding)	1 bar optimum / 14.5 psi (0.8-1.2 bar / 11.6 – 17.4 psi)
O <sub>2</sub> (Black color coding)	1 bar optimum / 14.5 psi (0.8-1.2 bar / 11.6 – 17.4 psi)
Tubing for all gasses (Supplied with the system)	PUR/PUN tubing 4 mm inner diameter 6 mm outer diameter 1 mm wall thickness

<b>Surrounding Conditions</b>	
Storage Temperature	-5 – 50°C
Operating Temperature	15 – 40°C (Indoor use only)
Humidity	80% relative humidity up to 31°C, 30% relative humidity up to 40°C
Operating Altitude	max. 2000 m (atmospheric pressure 800 – 1060 hPa)

<b>CO<sub>2</sub> Control</b>	
Control Range	0.1% – 20%
Accuracy	0.1% – 0.5% (absolute)

<b>O<sub>2</sub> Control*</b>	
Control Range	0% - 21%
Accuracy	0.5% (absolute)

<b>Humidity Control</b>	
Control Range	20% - 99% (relative humidity)
Accuracy	1% (absolute)

<b>Gas Flow</b>	
Control Range	5 – 20 l/h

<b>Humidifying Column</b>	
Humidifying Liquid	Ultra-pure Water
Volume	1/3 minimum = 50 ml 2/3 maximum = 130 ml
Refilling	80 ml ultra-pure water (approx. after 7 days*)

<b>Net Dimensions (h×w×d)</b>	
Gas Mixer	90 x 170 x 230 mm <sup>3</sup>
Humidifying Column	290 x 70 x 110 mm <sup>3</sup>

<b>Weight</b>	
Gas Mixer	2.1 kg
Humidifying Column, Empty/Full	630 g / 710 g

\*See Appendix for calculation.



## Preface

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

Before using the ibidi 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. When not using the instrument, keep this manual in a safe place. If this manual becomes lost, you can order a replacement from ibidi GmbH or download the pdf document on [www.ibidi.com](http://www.ibidi.com).

## Notices

1. ibidi shall not be held liable, either directly or indirectly, for any consequential damage incurred as a result of product use.
2. Prohibitions regarding the use of the ibidi software:
  - copying software for any reason other than making a backup
  - transferring or licensing the right to use the software to a third party
  - disclosure of confidential information regarding the software
  - any modification of the software
  - using the software on multiple workstations, network terminals, or by other methods
3. The contents of this manual are subject to change, without notice, for product improvement.
4. This manual is considered complete and accurate at the time of publication.
5. This manual does not guarantee the validity of any patent rights or other rights.
6. If an ibidi software program has failed, causing an error or improper operation, this may be caused by a conflict with another program operating on your notebook (PC). In this case, take corrective action by uninstalling the conflicting product(s).
7. ibidi is a registered trademark of ibidi GmbH in Germany and in other countries.

## Limited Warranty

Products sold by ibidi, unless otherwise specified, have a warranty for a period of one year from the date of shipment that covers 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 CONSUMABLES, DISPOSABLES AND PARTS NOT SUPPLIED BY AN AUTHORIZED IBIDI DISTRIBUTOR.
5. CORROSION DUE TO THE USE OF IMPROPER SOLVENTS, SAMPLES, OR DUE TO SURROUNDING GASES.
6. ACCIDENTS BEYOND IBIDI'S CONTROL, INCLUDING NATURAL DISASTERS.

This warranty does not cover consumables like cell culture dishes, reagents and the like.

The warranty for all parts supplied and any repair provided under this warranty expires on the warranty expiration date of the original product. For inquiries concerning repair service, contact ibidi after confirming the model name and serial number of your ibidi system.

## Installation Requirements

To ensure operational safety, observe the following conditions:

1. Only operate the Gas Incubation System with the supplied external power supply.
2. Only connect the power cable of the Gas Incubation System to an electrical socket containing a protective conductor terminal.
3. Ensure that the power plug of the power supply is easily accessible. The ibidi Gas Incubation System must be installed in a way that it does not hinder the access to the external power supply and its power plug.
4. Only operate the Humidifying Column with the original ibidi Gas Mixer.
5. Operate the Gas Incubation System in a room temperature range of 15 – 40°C.
6. Operate the Gas Incubation System in a humidity range of 0 – 80% RH up to 31°C and 30% RH up to 40 °C.
7. Operate the Gas Incubation System in an atmospheric pressure range of 800 – 1060 hPa.
8. Do not operate the Gas Incubation System under conditions that pose a risk of explosion, implosion, or the release of gases.
9. Avoid strong magnetic fields and sources of high frequency. The Gas Incubation System may not function properly when near a strong magnetic field or high frequency source.
10. Avoid vibrations from vacuum pumps, centrifuges, electric motors, processing equipment, and machine tools.
11. Avoid dust and corrosive gas. Do not install the Gas Incubation System where it may be exposed to dust, especially in locations next to outside air or ventilation outlets.
12. Only use ultrapure water when cleaning the Gas Incubation System.
13. Do not install the Gas Incubation System in a location where it may be exposed to direct sunlight.
14. Install the Gas Incubation System in a horizontal and stable position. This may include a table, bench, or desk on which the instrument has been installed.
15. Ensure that no air conditioner blows air directly onto the Gas Incubation System. This may prevent stable conditions.
16. Install the Gas Incubation System in a location that allows easy access for all maintenance.
17. Operate the Gas Incubation System with the recommended pressure only.

**NOTE:** The above conditions do not guarantee optimal performance of this Gas Incubation System.

## Installation and Connecting Cables



### Order of Installation

1. Connect all tubing and cables.
2. Apply 1 bar to the connected tubing.
3. Switch on the Gas Mixer.



**WARNING!** Always connect tubing and cables first. Then apply the corresponding pressure from your gas regulation. Switch on the Gas Mixer after you are 100% sure that the recommended pressure of 1 bar is present. Not maintaining this hierarchy may lead to functional disorder or damage to the instrument.

The ibidi Gas Mixer needs CO<sub>2</sub> and pressurized air. Additionally, depending on model and configuration, O<sub>2</sub> and N<sub>2</sub> are necessary.

Pressurized air can be supplied by the lab gas line or a small air pump or compressor.

With the O<sub>2</sub> version, Hypoxia conditions can be created by using pure Nitrogen (N<sub>2</sub>).

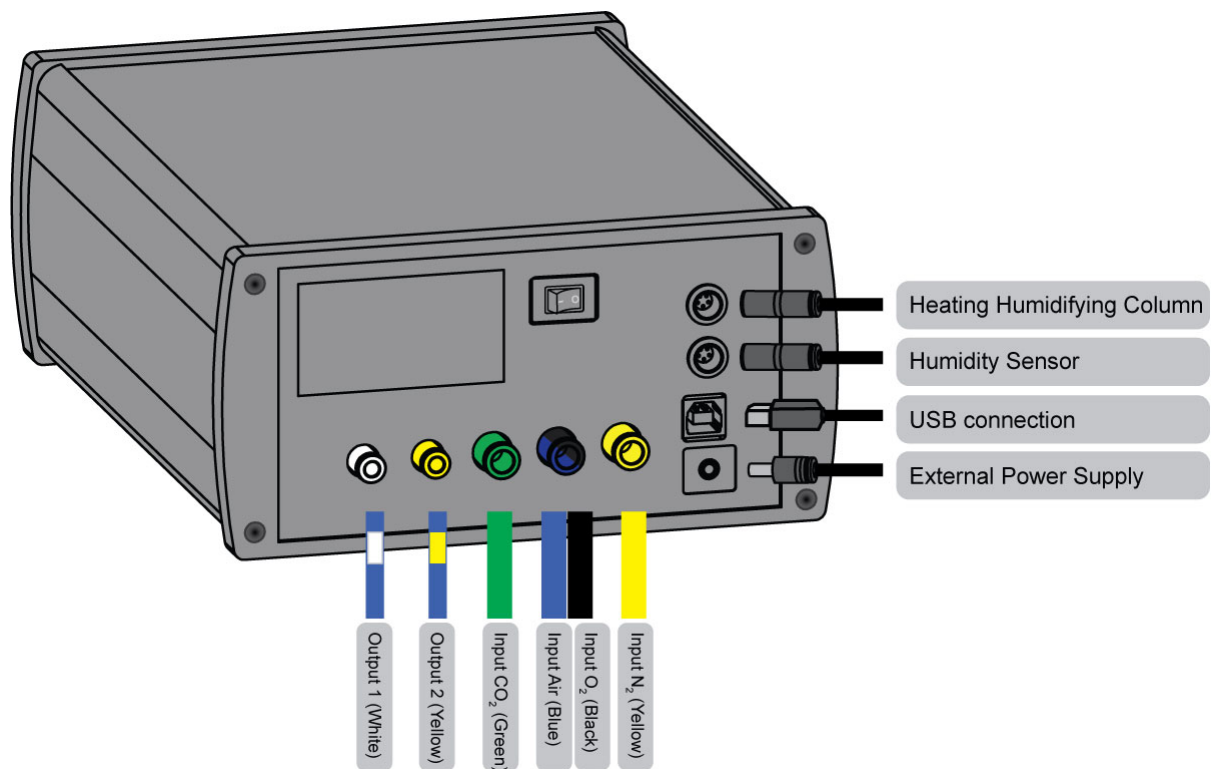
Always use the recommended pressure. Using different pressures may lead to major malfunction.

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

<b>Model</b> <b>Gas Input</b>	<b>CO<sub>2</sub></b> 0-20% CO <sub>2</sub>	<b>CO<sub>2</sub> + O<sub>2</sub></b> <b>Hypoxia</b> 0-20% CO <sub>2</sub> 0-21% O <sub>2</sub>	<b>CO<sub>2</sub> + O<sub>2</sub></b> <b>Hyperoxia*</b> 0-20% CO <sub>2</sub> 21-50% O <sub>2</sub>
CO <sub>2</sub>	1 bar CO <sub>2</sub>	1 bar CO <sub>2</sub>	1 bar CO <sub>2</sub>
AIR / (O <sub>2</sub> )*	1 bar AIR	1 bar AIR	1 bar O <sub>2</sub>
N <sub>2</sub>	-	1 bar N <sub>2</sub>	1 bar N <sub>2</sub> or AIR

\* Please contact [info@ibidi.de](mailto:info@ibidi.de) for availability.

## Connecting the Gas Mixer



Name	Function
Heating Humidifying Column	Electrical connection to heat the water and the tubing for the humidifying column
Humidity Sensor	Connects the humidity sensor to the Gas Mixer. The sensor is located inside the stage top incubator.
USB Connection	Socket for connecting the Gas Mixer to a PC/Notebook in order to computer-control the device. This feature will be available soon.
External Power Supply	The connector to the electrical external power supply (DC, 24 V, 5 A, 120 W)
Input N <sub>2</sub> (Yellow)	Input Tubing (Inner Ø 4 mm, Outer Ø 6 mm), 1 bar
Input Air/O <sub>2</sub> (Blue/Black)	Input Tubing (Inner Ø 4 mm, Outer Ø 6 mm), 1 bar
Input CO <sub>2</sub> (Green)	Input Tubing (Inner Ø 4 mm, Outer Ø 6 mm), 1 bar
Output 2 (Yellow)	Output Tubing (Inner Ø 2.5 mm, Outer Ø 4 mm)
Output 1 (White)	Output Tubing (Inner Ø 2.5 mm, Outer Ø 4 mm)

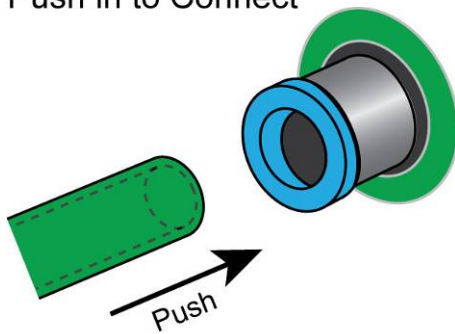
**WARNING!** Only operate the ibidi Gas Incubation System with the supplied cables and plugs. The use of other, non-supplied parts poses a risk of electric shock and fire.

**CAUTION:** Ensure that the power plug of the external power supply is easily accessible. The Gas Incubation must be installed in a way that it does not hinder access to the power plug. All ingoing and outgoing connections can be found at the rear panel of the instrument.

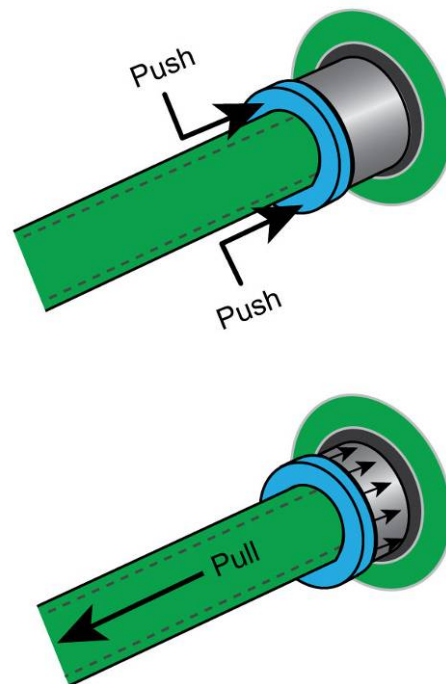
### Connecting the Gas Tubing

The gasses from your bottle or gas line need to connect the PUR/PUN tubing with 4 mm inner diameter, 6 mm outer diameter, and 1 mm wall thickness. This tubing is delivered with the system. After connecting the tubing as shown, set your gas regulation valve to the recommended pressure of 1 bar (14.5 psi).

Push in to Connect

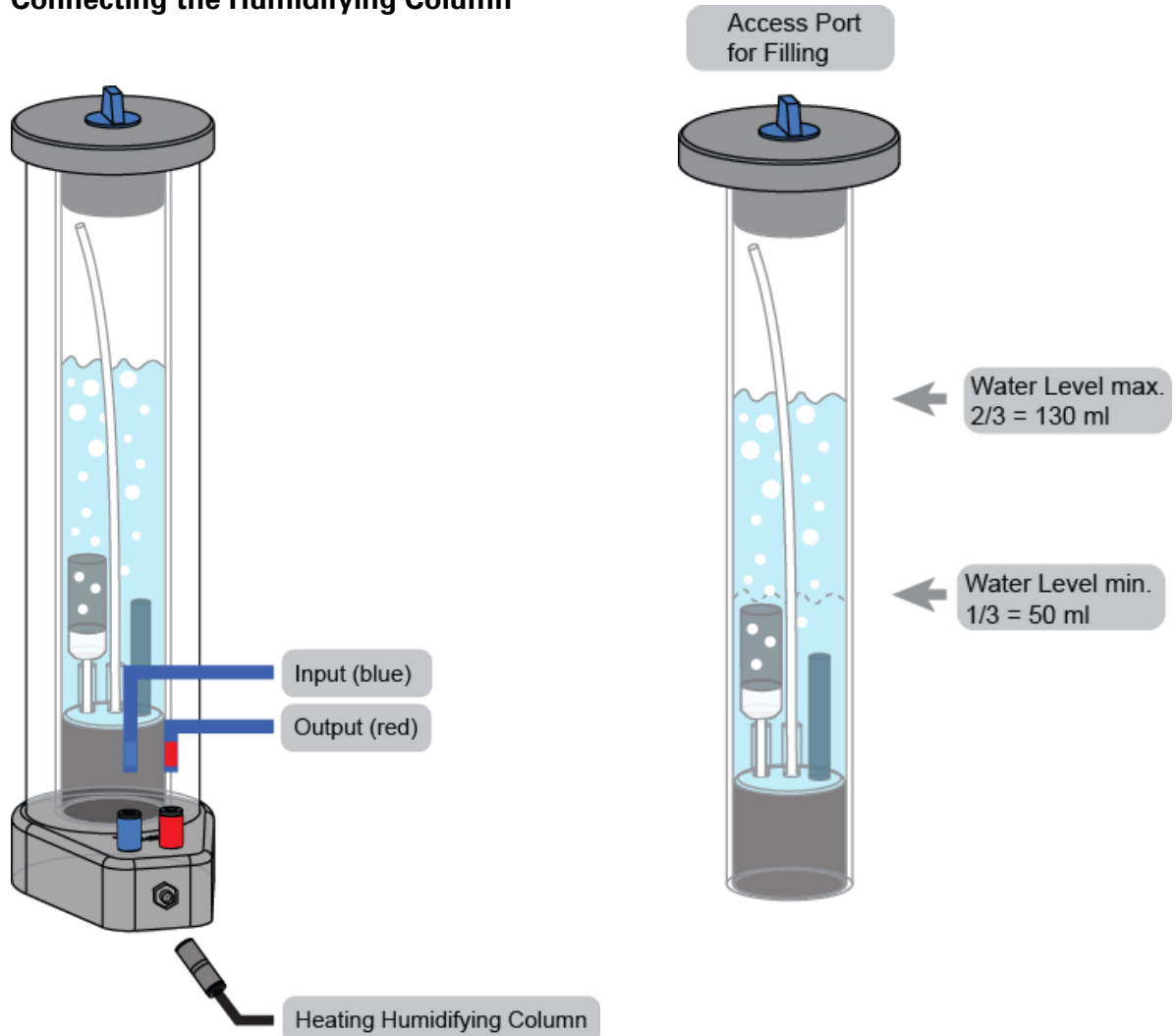


Push & Pull to Disconnect



**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.

## Connecting the Humidifying Column



For connecting the cable to the Humidifying Column plug in the cable. Please make sure the small notch is in the correct position. Gently push to connect the plug. After that, tighten the housing by using the metal screw.

After making all of the connections, fill the inside of the Humidifying Column with 130 ml of ultra-pure water. Refill with another 80 ml of ultra-pure water, after the water level minimum is reached.

**WARNING!** Do not push hard while turning the plug to fit the notch. Applying too much force may lead to damage of the pins and the electrical connection.

**WARNING!** Only operate the Humidifying Column with the supplied electric cable and tubing. The use of other, non-supplied parts poses a risk of electric shock and fire.

**WARNING!** Only operate the Humidifying Column using ultra-pure water.

**CAUTION:** Do not operate the Humidifying Column when empty. The minimum filling level is 1/3 of the total volume (50 ml). The maximum level is 2/3 of the total volume (130 ml). Operating the system without any water may lead to functional disorder or damage to the instrument.

## Maintenance and Operation

Pay close attention to the operating environment around the Gas Incubation System and always keep it clean, so that the instrument can be used in a stabilized condition over a long period. Do not place anything heavy on the Gas Mixer.

### Cleaning the Gas Mixer

To clean, remove the power plug from the electrical socket. Only use a dry cloth or cloth soaked with water (ultrapure) to clean the instrument.

**CAUTION:** Do not use ethanol or other types of organic solvents to clean the instrument, as they may remove the instrument's paint.

### Cleaning the Humidifying Column

To clean, remove the power plug from the electrical socket. Only use a dry cloth or cloth soaked with water (ultrapure) to clean the instrument.

**CAUTION:** Do not use ethanol or other types of organic solvents for prolonged periods of time. The use of anti-microbial agents is not recommended, since they can be transported as aerosol and end up in the specimen.

**CAUTION:** Ethanol and water are the only compatible solvents to clean the Humidifying Column. Do not use any other organic solvents.

### Transporting the Gas Incubation System

To transport, remove the power plug from the electrical socket. Carry the instrument carefully to avoid any mechanical shocks.

**CAUTION:** The weight of the Gas Mixer is approx. 2.1 kg. Dropping the instrument, while moving it, may cause personal injury or damage to the unit.

### Functional Disorder

In the case of a functional disorder, unplug the power cable of the Gas Mixer and wait for five minutes. Then reconnect the power cable and switch on the instrument again. If there is still a functional disorder, switch off the device, unplug the power cable, and contact the ibidi service.

### Repairing the ibidi Gas Incubation System

Do not try to repair the instrument yourself. Contact the ibidi service department to schedule a repair.

**CAUTION:** The manual opening of the instrument is not allowed. Opening the instrument manually poses a risk of personal injury or damage to the instrument. Contact ibidi service personnel, if you need to open the instrument.



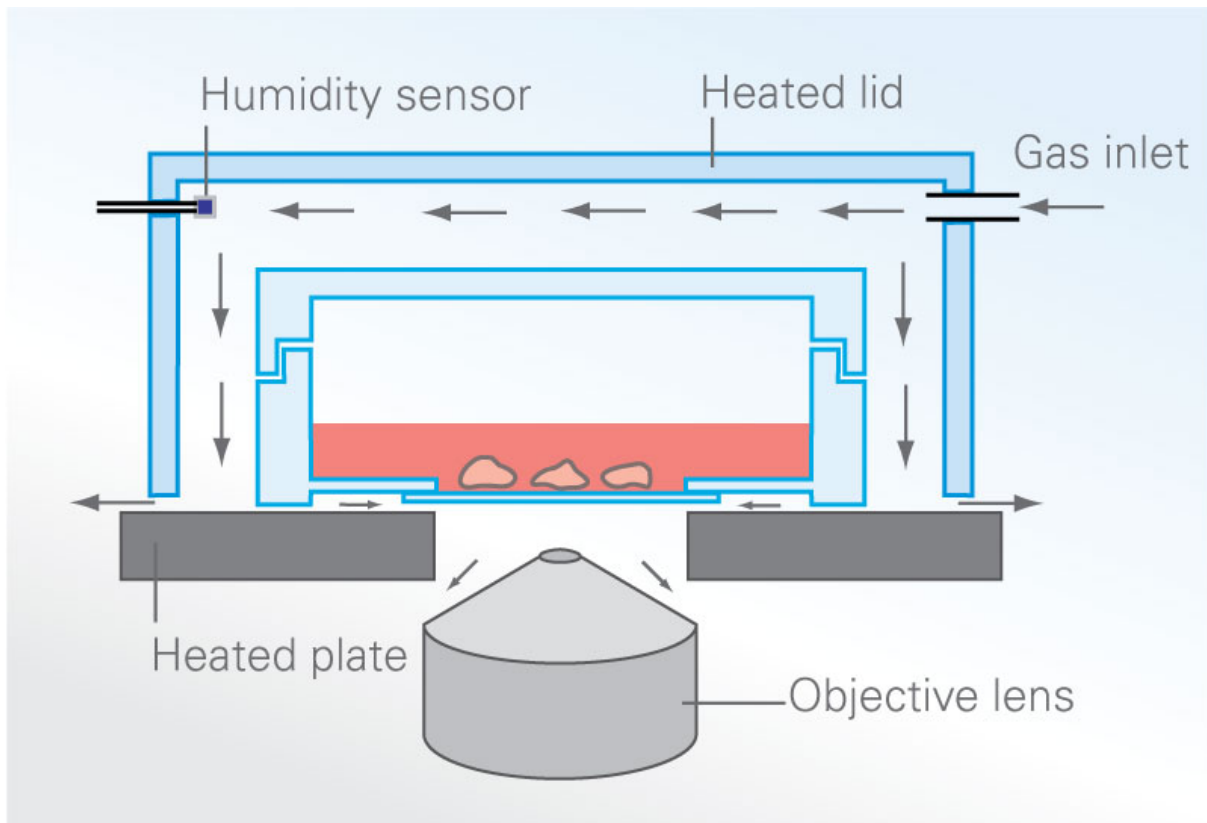
## Waste Disposal

Waste disposal is your own responsibility. Any by-product or waste must be handed to a company that specializes in waste recovery. Do not dispose the ibidi Gas Incubation System in a trash bin or at a public waste disposable site. For detailed disposal information, please contact the ibidi service department.

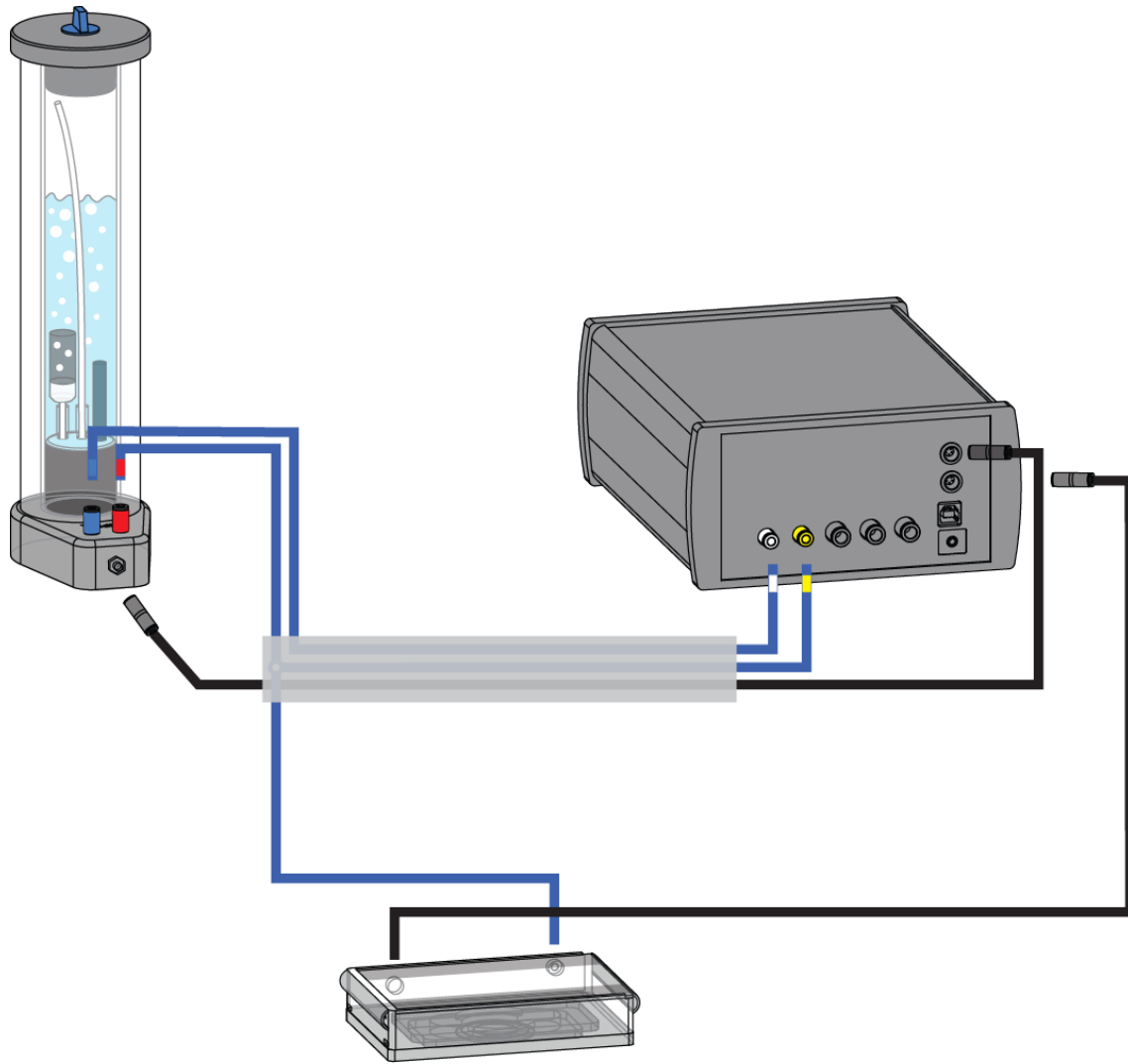
## 1 Working Principle

The ibidi Gas Incubation System provides both humid and CO<sub>2</sub>-rich air for stage-top incubators like the ibidi Heating System. The gas mixture is continuously flushed through the stage top incubator, ensuring a maximum humidity and an optimal pH for CO<sub>2</sub>-buffered liquids. With the O<sub>2</sub> hypoxia version, oxygen can be reduced during the experiment.

We recommend an air flow that replaces the air inside the stage top incubator every minute by continuous flow. For the ibidi Heating System a gas flow of 10 l per hour and 90% relative humidity (RH) is recommended.



**Figure 1: Schematic Setup of a Stage Top Incubator (37°C, 5% CO<sub>2</sub>)**



**Figure 2: Schematic setup of the ibidi Gas Incubation System with an ibidi Heating System**

## 2 Equipment

### 2.1 Product Description

A brief product overview is provided in this section, including the nomenclature for descriptions mentioned later in this document.

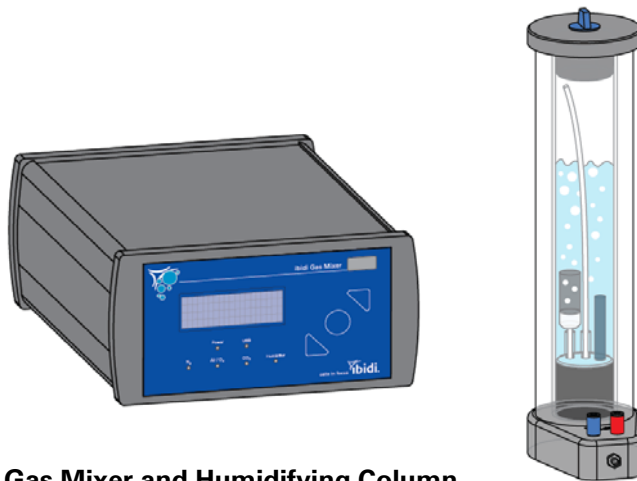
The ibidi Gas Incubation System for CO<sub>2</sub> and O<sub>2</sub> contains:

Gas Mixer with External Power Supply	3.5 m Cable
Humidifying Column	1.2 m Tubing to incubator 0.5 m Cable/Tubing to Gas Mixer
Humidity Sensor	1.5 m Cable
Tubing for CO <sub>2</sub>	5 m Tubing
Tubing for Air	5 m Tubing
Tubing for N <sub>2</sub> *	5 m Tubing
Tubing for O <sub>2</sub> **	5 m Tubing
USB Cable	Coming Soon

\*For O<sub>2</sub> versions only.

\*\* For hyperoxia only. Please contact [info@ibidi.de](mailto:info@ibidi.de) for availability.

### 2.2 Gas Mixer and Humidifying Column



**Figure 3: Gas Mixer and Humidifying Column**

### 2.3 Sterilization and Cleaning

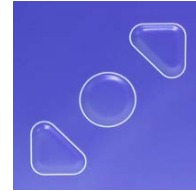
Microbial contamination is usually not a problem since distilled water and light-starved environments (microscopy room) do not support growth. If, however, contamination has occurred, sterilize the Humidifier Column by rinsing it with 70% of EtOH. Subsequently wash with water (do not expose to EtOH for prolonged periods of time). The use of anti-microbial agents is not recommended since they can be transported as aerosol and end up in the specimen.

Water is an efficient CO<sub>2</sub> buffer. Therefore, equilibration of the Humidifier Column by bubbling it with the desired CO<sub>2</sub> concentration for several minutes before the experiment may be required.

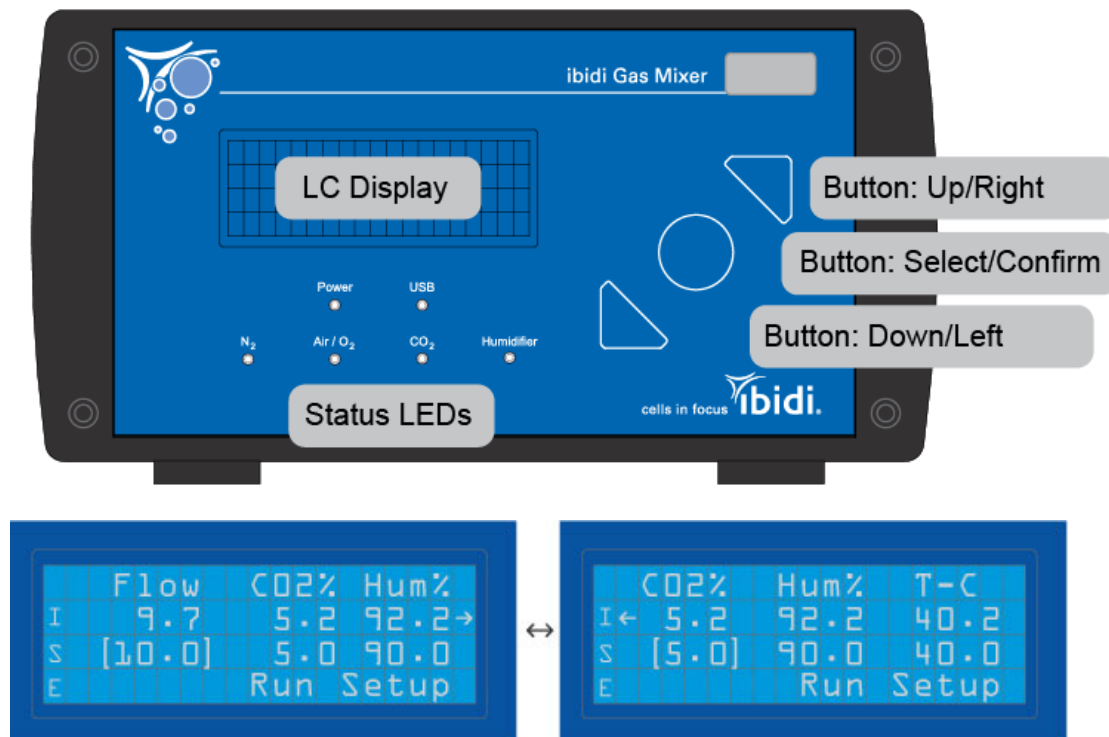
## 2.4 Menu Navigation

All control parameters can be manually set on the Gas Mixer using the buttons and the LC display on the front panel. The button functions are:

- Up / Right
- Down / Left
- Select / Confirm



The cursor position is indicated with square brackets ('[xx.x]'). You can move the cursor using the 'Left' and 'Right' buttons. If you want to select a parameter or a function, you need to press the 'Select' button. When you select a parameter, the square brackets will change to angle brackets ('<xx.x>'). Now you are able to change the value up or down. To confirm the changed value, you must press the 'Confirm' button once more. When selecting a function, you will see the individual function view, which is where you can change the parameters or access further functions.



A small arrow on the right side indicates that there are more parameters to select. Move the cursor to the arrow and 'Confirm' to enter.

The 'Run/Stop' button can be used for longer manipulations, inside the open heating system. Under opened conditions, CO<sub>2</sub> and humidity cannot reach normal values. If you want to open the system for longer than 1 min we recommend to 'Stop' the system. After closing the system set the Gas Mixer to 'Run' again. This way humidity and CO<sub>2</sub> will be quickly controlled immediately after closing the system.

## 2.5 Starting Operation and Recommended Settings



### Order of Installation

1. Connect all tubing and cables.
2. Apply 1 bar to the connected tubing.
3. Switch on the Gas Mixer.



**WARNING!** Always connect tubing and cables first. Then apply the corresponding pressure from your gas regulation. Switch on the Gas Mixer after you are 100% sure that the recommended pressure of 1 bar (14.5 psi) is present. Not maintaining this hierarchy may lead to functional disorder or damage to the instrument.

After switching on, the Gas Mixer will automatically start a warm-up routine and a self-test for 5 minutes. You can skip this routine after 2 min in case the instrument is already warmed up, e.g. after a restart. After this, the measured values will be shown.

For an optimal start, we recommend using the following values:

	Recommended Values	Full specifications range
<b>Flow</b> = Gas flow	10 l/h	5-20 l/h
<b>CO<sub>2</sub>%</b> = CO <sub>2</sub> in gas mix	5-10% depending on buffer	0.1-20%
<b>Hum%</b> = Rel. Humidity	90-95%	20-99%
<b>T-C</b> = Temperature of water in humidifying column	50°C	Max 50°C
<b>O<sub>2</sub>%*</b>	0-5%	0-21%

\*For O<sub>2</sub> versions only.



After switching on, please give the system time to equilibrate all parameters for a minimum of 30 minutes.



Please let the temperature of the entire imaging system equilibrate for a minimum of 30 minutes before you start.



Be aware that when the system is switched on, some parts of the tubing are actively heated to up to 65°C.

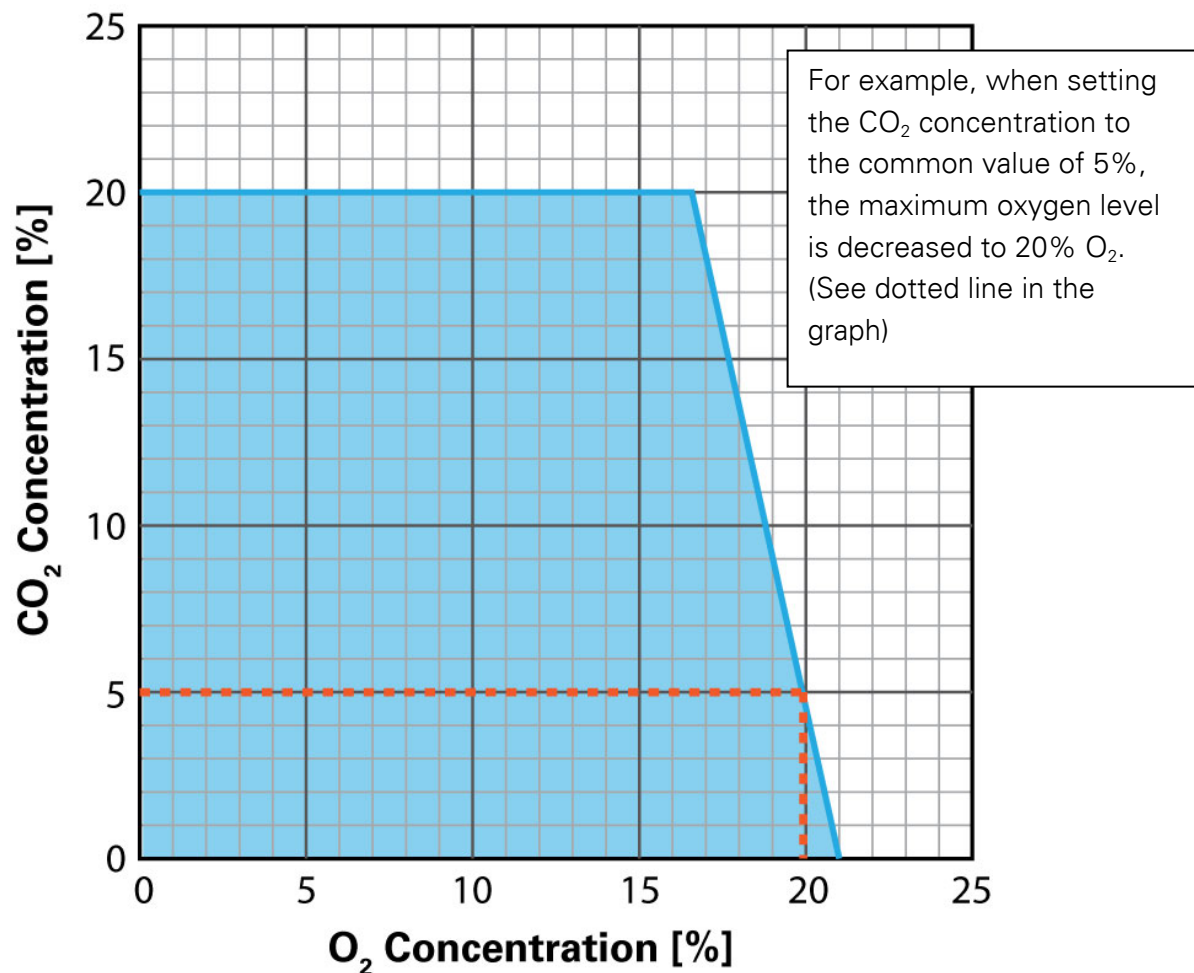


Please make sure the humidity sensor is exactly at 37°C. Otherwise the humidity control cannot reach high values over 90% relative humidity.

In case the measured humidity scatters to much, reduce the temperature of the water inside the humidifying column (T-C) to 45°C.

In case there are condensation effects after some hours, decrease the humidity accordingly.

Please keep in mind that CO<sub>2</sub> replaces some of the natural 21% O<sub>2</sub> level from your pressurized air. The maximum oxygen concentration is decreasing according to the CO<sub>2</sub> value you have chosen. Please see the graphic below for estimating the maximum O<sub>2</sub> concentration in your gas mix for a set CO<sub>2</sub> concentration.



## 2.6 CO<sub>2</sub> Calibration

After switching on, the Gas Mixer auto-calibrates the CO<sub>2</sub> value using external air as a reference. This process takes 2 minutes.

After 7 days of running continuously the Gas Mixer will auto-calibrate again. This short routine runs automatically.

## 3 Control Software

The ibidi Gas Incubation System can be computer-controlled. This feature will be available soon.

## 4 Troubleshooting

### 4.1 Focus Not Stable

Focus drift is a disturbing effect, especially during 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 (heating, gas incubation, computer, other equipment) at least 60 minutes before starting the experiment.
- After you put the  $\mu$ -Slide onto the microscope, wait 20 minutes before starting a time-lapse experiment to achieve temperature and immersion oil equilibration. (\*)
- 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.

(\*) In the case that the experiment needs to be started immediately, either after placing the slide on the microscope, or after closing the lid, we recommend controlling the focus for 20 minutes. In the first minutes after starting the experiment, temperature equilibration might influence the focus/z-position of the cells.

### 4.2 Evaporation Too High

Depending on the incubating conditions, small volumes might evaporate quickly, especially during long-term experiments. We suggest increasing the set value of the relative humidity. For special experiments, we recommend using Parafilm (Pechiney Plastic Packaging Company) or a silicone oil (ibidi Anti-Evaporation Oil, Cat. No. 50051) to decrease evaporation.



#### **4.2.1 Parafilm Procedure**

Small stripes of Parafilm fitted onto the reservoirs of the slides are very effective at preventing evaporation effects. Fill the slide, as recommended, then adapt the Parafilm until it fits tightly.

#### **4.2.2 Silicone Oil Procedure (ibidi's Anti-Evaporation Oil)**

Covering the medium with sterile silicone oil prevents all evaporation effects and is compatible with cell culture. Please don't use mineral oil, as this would be harmful to the ibidi  $\mu$ -Slides.

Equilibrate oil and medium inside the incubator overnight. This step helps 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 by pressing the pipette tip directly on the upper side of the reservoir. For example, when using  $\mu$ -Slide VI<sup>0.4</sup>, fill each reservoir with 30  $\mu$ l cell-free medium and 30  $\mu$ l silicone oil.

### **4.3 Value of Relative Humidity (RH) not Stable**

In case the measured humidity scatters to much, reduce the temperature of the water inside the humidifying column (T-C) to 45°C.

### **4.4 Gas Flow too low**

#### **4.4.1 Gas Input open without connected gas**

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

#### **4.4.2 Gas Pressure too low**

Always use the recommended pressure of 1 bar (14.5 psi).

#### **4.4.3 Set Values are too high or too low**

Please make sure the set values are set to ambient air values (0% CO<sub>2</sub>, 21% O<sub>2</sub>) in case the corresponding gas is not connected. Additionally, keep in mind that the maximum O<sub>2</sub> concentration depends on the set CO<sub>2</sub> concentration. Please see page 24 for further details.

### **4.5 Condensation Inside the Incubator**

Check the temperature of the incubator (Heated Lid, Heated Plate). In case there are condensation effects after some hours, decrease the humidity accordingly and air-dry the incubator if necessary.

Please contact us at [info@ibidi.com](mailto:info@ibidi.com) for further troubleshooting help.

## 5 WEEE/RoHS Compliance Statement

EU Directives WEEE and RoHS

To Our Valued Customers:

ibidi GmbH is committed to being a good corporate citizen. As part of that commitment, we strive to maintain an environmentally conscious manufacturing operation. 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).

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. We will, however, continue to monitor the application of the RoHS Directive in relation to our products and will comply with any changes, as they apply.

Recycling is offered for our products that fall within the scope of the WEEE Directive. These specific products, available for sale after August 13, 2005, will be identified with a “wheelie bin” symbol.



When you see this symbol, do not dispose of this product with Municipal Waste. Special Collection/Disposal is required.

Please contact us for feedback or further information.

ibidi GmbH  
Am Klopferspitz 19  
82152 Planegg-Martinsried  
Germany  
[www.ibidi.com](http://www.ibidi.com)  
[info@ibidi.de](mailto:info@ibidi.de)  
Tel.: +49 89 520 4617 0  
Fax: +49 89 520 4617 59

## 6 Appendix

### 6.1 Gas Consumption During Operation

A normal 25 kg bottle of CO<sub>2</sub> gas can last for years, if used only for the ibidi Gas Incubation System. See the calculation example below.

Calculation example for CO<sub>2</sub> consumption:

Usual CO<sub>2</sub> bottle: 25 kg gas CO<sub>2</sub>  
Density of gaseous CO<sub>2</sub>: 2 kg/m<sup>3</sup> = 2 kg/1000 liters  
Example Settings: 5 % CO<sub>2</sub> in gas mixture. Gas flow rate of 10 l/h.

How long does the 25 kg bottle last?

$$\text{Duration} = \frac{\text{Mass of gas}}{\text{Density (CO}_2\text{)} \cdot \text{Flow rate} \cdot \text{Percentage}}$$

$$\text{Duration} = \frac{25 \text{ kg} \cdot 1000 \text{ l} \cdot \text{h}}{2 \text{ kg} \cdot 10 \text{ l} \cdot 0.05} = 25,000 \text{ h} = 1040 \text{ days}$$

Answer: Ca. 1040 days if used non-stop.

## 6.2 Gas Humidification and Refilling Time

For a minimum of evaporation, the gas mix needs to be humidified. This allows for long-term experiments. To see how long, check the calculation example below.

Calculation example for water consumption:

Mass of water available  $m_{\text{Water}} = 80 \text{ g}$  (=80 ml)

Density  $D_{\text{Air (37°C)}} = 1.1248 \text{ kg/m}^3$

Gas flow rate of 10 l/h = 0.01 m<sup>3</sup>/h =  $F_{\text{air}}$

$x_{\text{Mollier}} = 42 \text{ g/kg}$  (Mass of water at 80% relative humidity at 37°C)

Mass flow of air ( $M_{\text{air}}$ ):

$$M_{\text{air}} = F_{\text{air}} \cdot D_{\text{air}} = 11.25 \text{ g/h}$$

Mass flow of water ( $M_{\text{water}}$ ):

$$M_{\text{water}} = M_{\text{air}} \cdot x = 0.4725 \text{ g/h}$$

Time for consumption (t):

$$t = \frac{m_{\text{water}}}{M_{\text{water}}} = 169.3 \text{ h} = 7 \text{ d}$$

Answer: Ca. 7 days if non-stop used.