

Instruction Manual

ibidi Heating System Slide/Dish – Silver Line



12110 ibidi Heating System Slide/Dish – Silver Line



Contact

ibidi GmbH

Lochhamer Schlag 11
82166 Gräfelfing
Germany

Phone: +49 89 / 520 46 17 - 0

Fax: +49 89 / 520 46 17 - 59

E-mail: info@ibidi.de

Internet: ibidi.com

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1 Preamble

1.1 Introduction

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

Before using the ibidi Heating System Slide/Dish, 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, see the online version on ibidi.com or request a replacement via techsupport@ibidi.com.

To ensure safe operation, the ibidi Heating System Slide/Dish must only be operated with the supplied components and according to the instruction manual.

For Research Use Only! Not for use in diagnostic procedures.

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 messages in the text are displayed in a gray shaded box. Please see section 1.6 for general safety considerations.



WARNING – A potentially hazardous situation which, if not avoided, could result in serious injury or even death.



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.

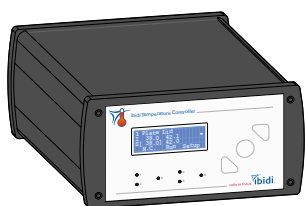


This label is positioned on the back of the device and prompts you to read the manual before using the device.

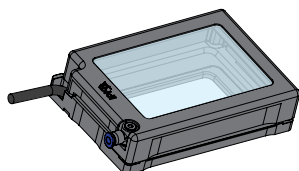


Product disposal: The symbol indicates that this product must be recycled/disposed of separately from other household waste. See page 12 for details.

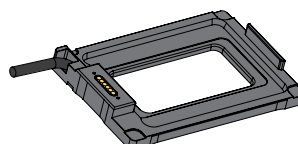
1.3 Nomenclature



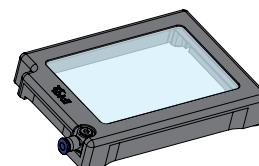
Temperature Controller



Incubation Chamber



Heated Plate



Heated Lid

1.4 Specifications

Only operate the Heating System in the range of the specifications given below:

Table 1: Specifications of the ibidi Heating System Slide/Dish

Electrical Specifications Power Supply	
Protection class	I
International protection marking (IEC 60529)	IP 20
Overvoltage category (IEC 664)	II
External power supply	AC 100–240 V, 50/60 Hz, 2 A
Input line voltage Temperature Controller	DC 24 V, 6.67 A, 160 W
Output voltage per heating channel	DC max. 24 V, max. 2 A
Operating and Storage Conditions	
Operating site	Indoor use only
Operating temperature	18–30°C/64–86°F (at least 5°C/9°F less than set temperature)
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	
Temperature Controller	90 mm × 170 mm × 230 mm Weight: 1.75 kg/3.86 lbs
Heated Lid	20 mm × 85.5 mm × 127.5 mm Connection to the gas tubing: Push-in fitting, OD 4 mm
Heated Plate	15 mm × 85.5 mm × 127.5 mm Observation area: 40 mm × 82 mm Length of cable: 1.5 m

Table 1: (continued)

Heated Plate assembled with Heated Lid	Height: 28 mm Weight: 230 g/0.51 lbs (without cable) Weight: 320 g/0.71 lbs (cable included)
Holders all Dish Holder	47 mm × 97 mm Height without Holder: 8 mm (12 mm at the handles) Height with top: 18–22 mm Observation area: \varnothing 28 mm Weight Holder (top): 20 g/0.044 lbs Weight Holder (bottom): 30 g/0.066 lbs
Slide Holder	Height: 8 mm (12 mm at the handles) Observation area: 54.3 mm × 23 mm Weight Holder: 20 g/0.044 lbs
USB cable	Length: 1.8 m
Power supply cable	Length: 2.0 m (power supply to wall) Length: 1.5 m (power supply to device)

Temperature Control Range

Heated Lid	5 degree above ambient temperature (min. 18°C) to 50°C Accuracy: $\pm 0.2^\circ\text{C}$ (at sensor location) Uniformity: $\pm 5^\circ\text{C}$ (entire heated glass)
Heated Plate	5 degree above ambient temperature (min. 18°C) to 46°C Accuracy: $\pm 0.2^\circ\text{C}$ (at sensor location) Uniformity: $\pm 0.5^\circ\text{C}$ (entire heated plate)

Sample Temperature

Temperature stability	$\pm 0.2^\circ\text{C}$
Recommended Temperatures before Adjustment	See Section 5.4

USB Interface

Connector type at controller	USB 2.0 Connector Type B
Recommended USB cable	Tripp Lite UR022-006 (shielded)
USB driver for PC	FTDI VCP driver

Microscope Requirements

Working distance condenser	≥ 28.5 mm
Stage holder	Holder for standard multiwell plates

Optical Properties Glass Lid

Glass	Selected HQ Floatglass
Thickness of the glass plate	1.1 mm

Table 1: (continued)

Refractive index glass	$n_D = 1.520$ (588 nm)
ITO coating	Thickness: 100 nm
Passivation layer	Thickness: 20–25 nm
Refractive index ITO with passivation layer	1.95

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 ibidi Heating System Slide/Dish 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 ibidi Heating System Slide/Dish under conditions that pose a risk of explosion, implosion, or the release of gases.
- Do not place flammable solids, liquids, gases, or gas outlets near the system (e.g., matches, ethanol, propane gas, solvents). Do not bring these products in contact with any other component of the system either.
- Do not operate a damaged ibidi Heating System Slide/Dish. If the housing seems damaged or something is rattling inside the controller, contact the [ibidi technical support](#) for repair.
- Only operate the Heated Lid and Heated Plate properly installed on a microscope stage.

- Some accessible parts of the Heated Plate and Heated Lid can reach temperatures up to 55°C. Avoid touching the temperature-controlled parts of the system when you have set the Temperature Controller to high temperatures.

**CAUTION**

- Ensure that the external power supply is easily accessible. The ibidi Heating System Slide/Dish 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 ibidi Heating System Slide/Dish.
- 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 ibidi Heating System Slide/Dish 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 ibidi Heating System Slide/Dish where it could be exposed to high levels of dust or to outside air or ventilation outlets.
- Install the ibidi Heating System Slide/Dish in a location that enables easy access for maintenance.
- Do not place heavy objects on the instrument.
- Install the Temperature Controller in a horizontal and stable position, which includes a table, bench or desk upon which the instrument is installed.
- The heated glass plates of the incubation chamber can break on mechanical impact. If so, the glass shards can lead to injuries if handled.
- Be aware that when switched on, a 10 V DC voltage is applied to the underside of the glass on the Heated Lid. Do not touch the underside or put it in contact with anything conductive. This could cause a short circuit that may damage the Temperature Controller and/or the heating devices.

1.7 Limited Warranty

Products manufactured by ibidi, unless otherwise specified, are warranted 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.

1.8 Transporting the ibidi Heating System Slide/Dish

The weight of the Temperature Controller is approx. 1.75 kg/3.86 lbs. Moving the Temperature Controller during operation can pose a risk of personal injury or damage to the instrument.

For transport, switch off the Temperature Controller and then disconnect the heated components from the controller. Carry the devices carefully and avoid mechanical shocks.



WARNING – Hot surface (max. 55°C)! Do not touch Heated Lid and Heated Plate when hot. Always disconnect the instrument from the power supply before transport and leave the instrument to cool down for approx. 5 minutes.

1.9 Repairing the ibidi Heating System Slide/Dish

For inquiries concerning repair service contact ibidi GmbH technical support (techsupport@ibidi.com) and provide the model name and serial number of your system.



CAUTION – Do not try to repair the ibidi Heating System Slide/Dish by yourself. Disassembly of the ibidi Heating System Slide/Dish is not allowed. Disassembly poses a risk of personal injury or damage to the devices. Contact ibidi technical support if there is a need to disassemble a device.

1.10 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).

1.10.1 EU Directive WEEE

The ibidi Heating System Slide/Dish 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.

1.10.2 EU Directive RoHS

RoHS conformity is declared in the EU-conformity in Section 1.11.

1.11 Regulatory Statement

EG-Konformitätserklärung EC Declaration of Conformity

Wir / We

ibidi GmbH
Lochhamer Schlag 11
D-82166 Gräfelfing

erklären hiermit die Übereinstimmung des genannten Produktes mit der Richtlinie 2014/35/EU - Niederspannungsrichtlinie und mit der Richtlinie 2014/30/EU über die Elektromagnetische Verträglichkeit.

Bei Änderungen am Produkt, die nicht von uns autorisiert wurden, verliert diese Erklärung ihre Gültigkeit.

We declare the compliance of the product with the requirements of the Directive 2014/35/EU - Low Voltage Directive and with the Directive 2014/30/EU about the Electromagnetic Compatibility.

Any modification to the product, not authorized by us, will invalidate this declaration.

Laborgerät / laboratory equipment:

ibidi Temperature Controller ibiTC3-XXX with ibidi accessories

Der oben beschriebene Gegenstand erfüllt die Vorschriften der Richtlinie 2011/65/EU vom 08. Juni 2011 zur Beschränkung der Verwendung bestimmter gefährlicher Stoffe in Elektro- und Elektronikgeräten.

The object of the declaration described above is in conformity with Directive 2011/65/EU of 8 June 2011 on the restriction of the use of certain hazardous substances in electrical and electronic equipment.

Das Produkt entspricht den unten aufgeführten Normen:
The product meets the requirements of the following standards:

DIN EN 61010-1:2020

Sicherheitsbestimmungen für elektrische Mess-, Steuer-, Regel- und Laborgeräte - Teil 1: Allgemeine Anforderungen Safety requirements for electrical equipment for measurement, control and laboratory use - Part 1: General requirements

DIN EN 61326-1:2013

Elektrische Mess-, Steuer-, Regel und Laborgeräte. EMV-Anforderungen. Allgemeine Anforderungen
Electrical equipment for measurement, control and laboratory use. EMC requirements. General requirements

Das Produkt ist gekennzeichnet mit/ The product is marked with



Gräfelfing, den 29.07.2021
Gräfelfing, 2021-07-29

Ort/Datum
Place/date

Dr. Valentin Kahl
Geschäftsführer

Name, Funktion
Name, Function

Valentin Kahl

Unterschrift
Signature

Diese Erklärung bescheinigt die Übereinstimmung mit den genannten Richtlinien, ist jedoch keine Zusicherung von Eigenschaften. Die Sicherheitshinweise der Produktdokumentation sind zu beachten.

This declaration certifies the conformity to the specified directives but not includes any warranted quality of the instrument. The safety documentation of the product shall be considered in detail



2 Intended Use

The ibidi Heating System Slide/Dish is a stage top incubator for live cell imaging that fits onto inverted microscope stages with a mounting frame for multiwell plates. The ibidi Heating System Slide/Dish, consisting of an Incubation Chamber with Heated Plate and Heated Lid and a Temperature Controller, provides a platform for keeping microscopy slides and dishes at a constant temperature during an experiment on a microscope.

The modular concept of the Heated Plate and a choice between two different Holders for labware (Slide Holder, Dish Holder) enables the use of a variety of microscopy labware, including standard 35 mm dishes and regular slides.

For full control of the incubation environment, the ibidi Heating System Slide/Dish can be combined with the ibidi Gas Incubation System to regulate the CO₂ and O₂ concentrations as well as the humidity.

The ibidi Heating System Slide/Dish is for research use only! It is not intended for use in diagnostic procedures.

3 Principle

Physiological Conditions in Live Cell Imaging

Cells react sensitively to changes in their environment. Factors such as temperature, humidity, and CO₂/O₂ levels significantly influence the outcome of cell culture assays. In order to achieve biologically relevant and reproducible results, it is crucial to maintain optimal conditions on the microscope stage during live cell imaging experiments.

To enable high-quality live cell imaging under physiological conditions, the following parameters need to be considered:

Environment:

- Temperature
- Carbon Dioxide (CO₂) Levels and pH of the Medium
- Humidity and Evaporation
- Oxygen (O₂) Levels

Optical Aspects:

- Condensation
- Imaging Method and Photon Dose

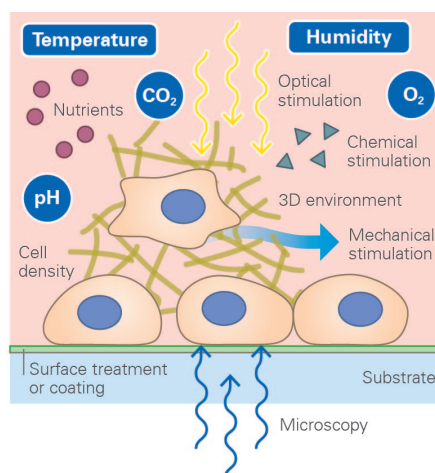


Figure 1: Live cell imaging conditions.

The Heating System combined with the Gas Incubation System supplies complete incubator conditions for your live cell imaging directly on the microscope stage.

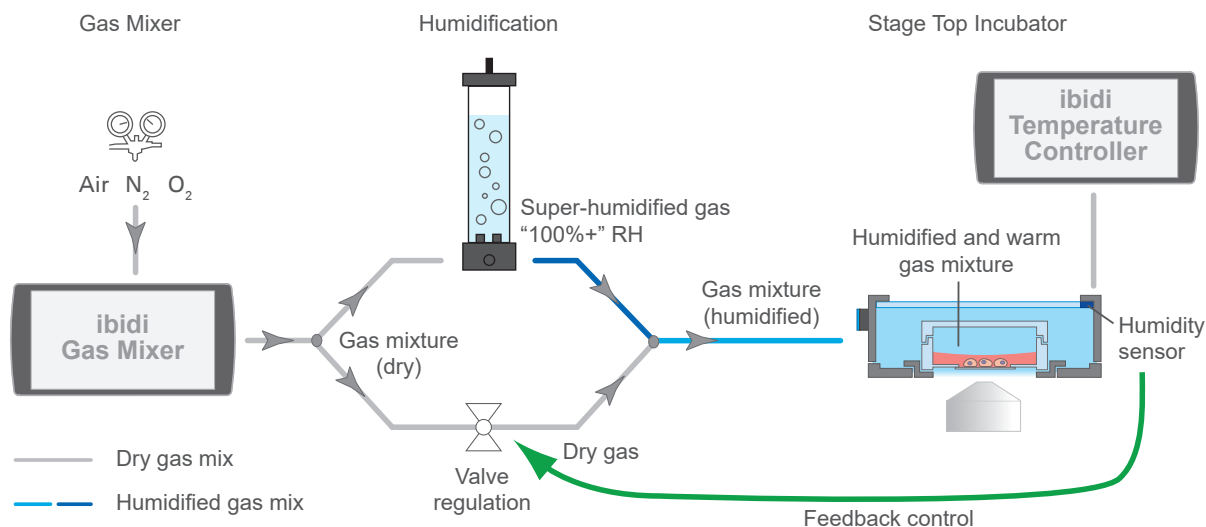


Figure 2: Schematic view of the ibidi Stage Top Incubation System, combining the Heating System with the Gas Incubation System.

The Gas Incubation System provides a controlled atmosphere of gas (CO₂ or CO₂ and O₂) as well as a defined humidity in the emitted gas stream that is flushed continuously through the incubation chamber. Oxygen can be reduced during the experiment, enabling the user to perform hypoxia experiments (CO₂/O₂ version only).

Humidity in ambient air can lead to condensation on all surfaces, especially on the lids of cell culture vessels. If these surfaces are within the optical pathway, small water droplets will cause light scattering. This diminishes the optical quality of transmitted light microscopy (i.e., phase contrast). To ensure the highest image quality, condensation on any surface should be prevented during live cell imaging experiments.

The independently controlled Heated Lid of the ibidi Heating System prevents from condensation during live cell imaging. By heating the lid to a temperature higher than the plate, a vertical temperature gradient is created. This gradient, and active humidity control prevent the formation of condensation on the lid of the cell culture vessel.

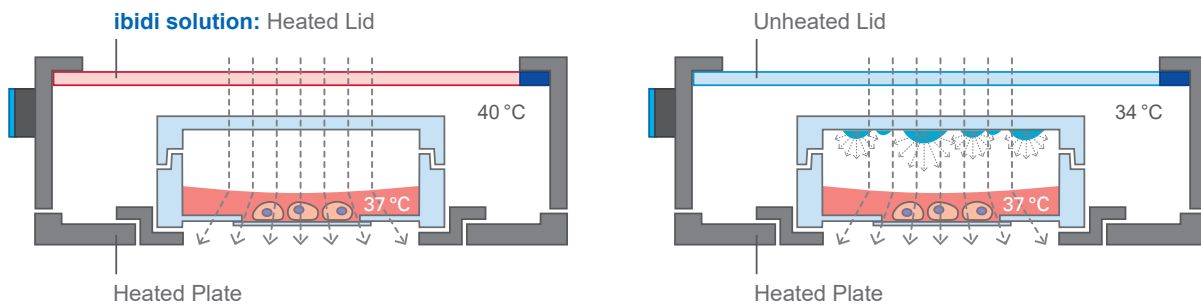


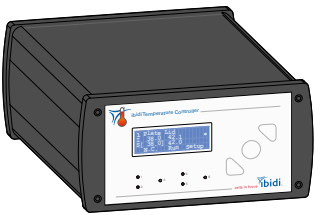
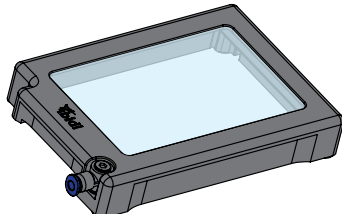
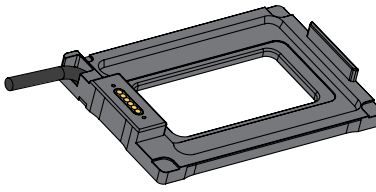
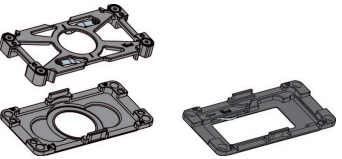

Figure 3: The Heating System prevents condensation on the cell culture vessel lid, as well as on the Heated Lid itself.

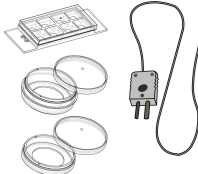




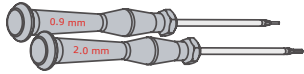


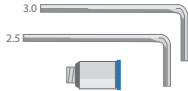
4 Equipment

This section provides a brief overview of all parts of the ibidi Heating System Slide/Dish including a description of the characteristics.

4.1 Components of the ibidi Heating System Slide/Dish

The components of the ibidi Heating System Slide/Dish are listed below.

Description	Drawing
Temperature Controller	
Heated Lid	
Heated Plate with electrical cable to connect to the Temperature Controller	
Slide/Dish Holder	
Bridge cable to connect to the Gas Mixer	

Description	Drawing
Temperature Adjustment Set: 1 temperature sensor 1 perforated ibidi μ -Slide 8 Well 1 perforated ibidi μ -Dish ^{35 mm, high} 1 perforated ibidi μ -Dish ^{35 mm, low}	
USB cable to connect the Temperature Controller with a computer	
Country-specific power cord to connect the external power supply to the wall socket	
External power supply for the Temperature Controller	
USB flash drive with IncuControl software	
Hex key 0.9 and 2.0 mm	
Crosshead (Phillips) screwdriver PH0	
Cable clip for optional use	
Push-in fitting exchange kit	

4.2 Stage Top Incubator Overview

The components of the ibidi Heating System Slide/Dish are set up as a stage top incubator as shown in Figure 4. The ibidi Heating System Slide/Dish can be combined with the ibidi Gas Incubation System – Silver Line in order to provide CO₂ and O₂ (optional) control and a defined humidified atmosphere in addition to temperature control. Detailed information on the ibidi Gas Incubation System – Silver Line is given in the [Gas Incubation System instructions](#).

The Incubation Chamber Slide/Dish fits on an inverted microscope stage equipped with a mounting frame for multiwell plates. The Heated Plate of the Incubation Chamber is connected to the Temperature Controller. The electrical connection to the Heated Lid is established via connection pins to the Heated Plate (Sections 4.4 and 4.5).

The Heated Plate can be equipped with different Holders (Slide Holder, Dish Holder, Section 4.6) to hold several geometries of slides and dishes (Section 5.5).

Optionally, the Objective Heater Universal – Silver Line can be connected to the Temperature Controller to keep the objective warm, thereby avoiding cooling of the sample by heat transfer from the sample to the objective while imaging. Detailed information on the Objective Heater Universal is given in the [Objective Heater Universal – Silver Line instructions](#).

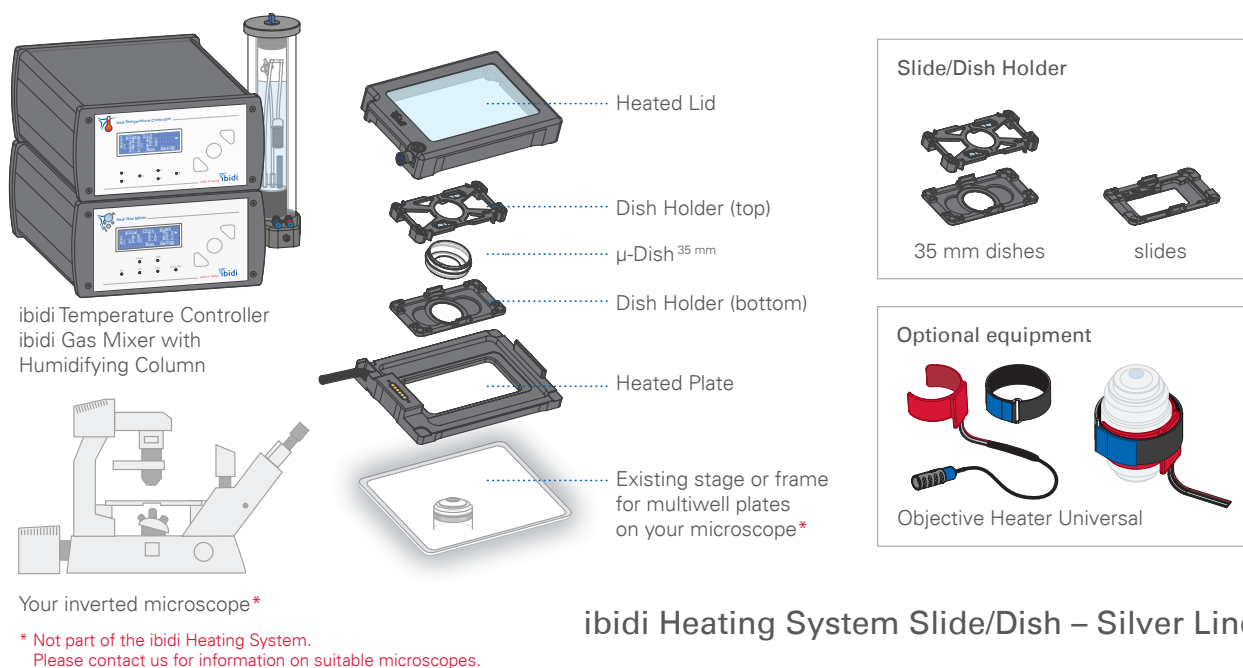


Figure 4: Overview of the components of the ibidi Heating System Slide/Dish, with optional equipment.

4.3 Temperature Controller

The Temperature Controller is designed to precisely operate the different heating units (e.g., Heated Lid and Heated Plate of the Incubation Chamber Slide/Dish – Silver Line), and optionally the Objective Heater Universal – Silver Line (six control channels available). Additionally, an external Temperature Sensor can be connected to read out the sample temperature, for example.

The front display shows the set ('S' = set) and actual ('I' = is) values. The settings can be adjusted via the control buttons (Section 5.3).

If you wish to control the Temperature Controller via PC, use the IncuControl software (Section 6).

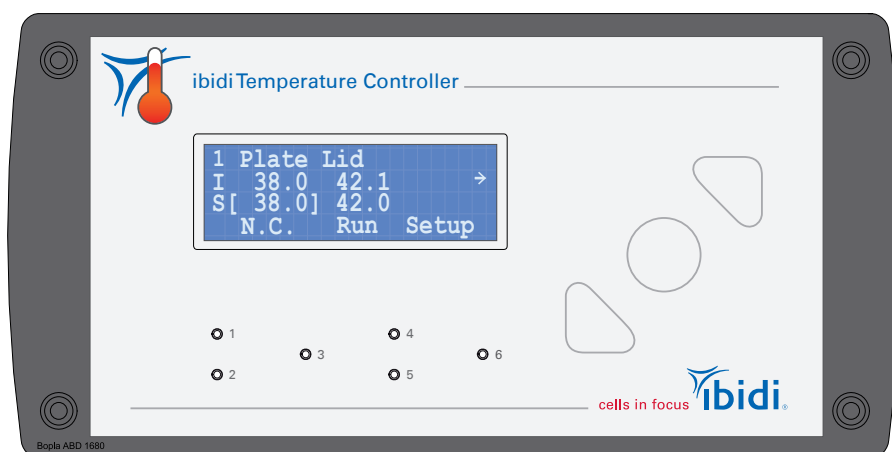


Figure 5: Front view of the Temperature Controller.

The LEDs on the front indicate the status of the channels that are connected (Figure 5).

Control LEDs Channel 1–6	
LED off	Channel inactive
LED on	Channel active
LED fast blinking	Channel error
Please contact ibidi at techsupport@ibidi.com .	

All electrical connectors are located on the back of the Temperature Controller (Figure 6). For connecting the heating devices, two sizes of ports are available. The larger ports 1/2 and 4/5, which each accommodate two output channels, and the smaller ports for the output channels 3 and 6. Please find which heating units (e.g., Incubation Chamber, Objective Heater) can be connected to which port in Table 6 (Section 5). Refer to Section 5.1 for setting up the ibidi Heating System Slide/Dish.

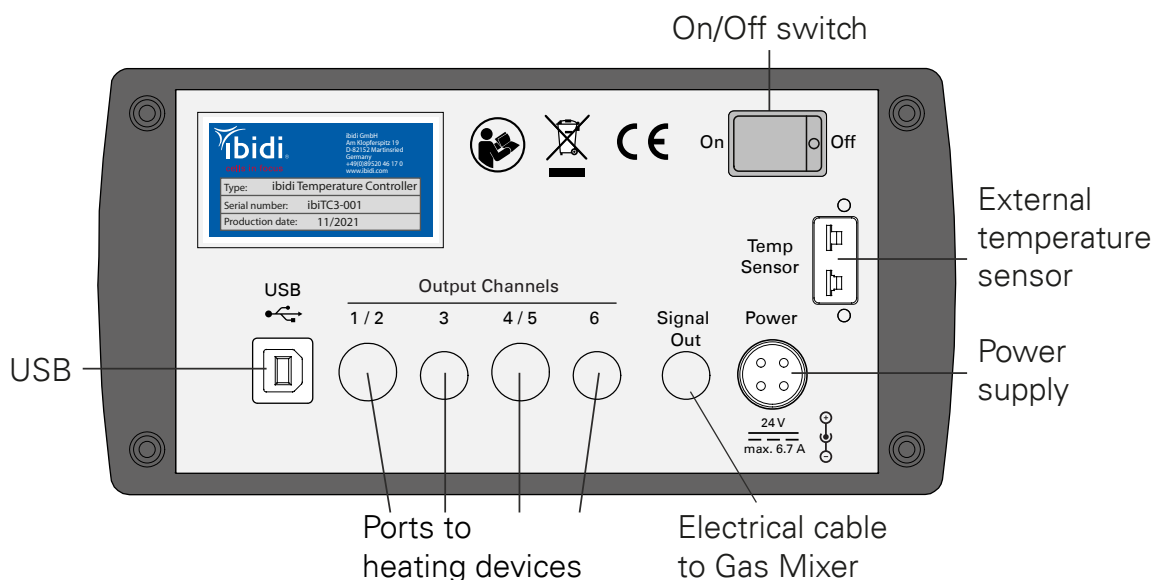


Figure 6: Rear view of the Temperature Controller.

4.4 Heated Plate

The Heated Plate provides the base for the ibidi Heating System Slide/Dish. It fits into any microscope stage with a mounting frame for multiwell plates.

To minimize xy-movement of the Heated Plate inside the opening of the mounting frame for multiwell plates on the microscope stage, the Heated Plate's clamping mechanism can be used for fixation (Figure 8):

- Place the Heated Plate into the opening of the mounting frame for multiwell plates.
- Loosen the screw with the crosshead screwdriver PH0 to unlock the clamping mechanism (Figure 8, left side).

- A small spring inside the clamping mechanism pushes out the clamping bolt. (Figure 8, A and B).
- Tighten the screw again. The Heated Plate now fits perfectly into the opening of the mounting frame.

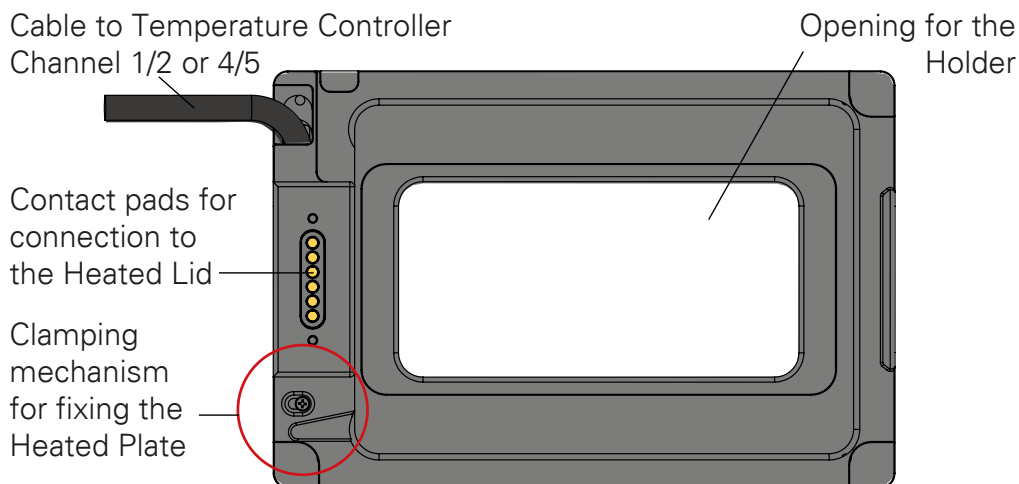


Figure 7: Heated Plate, top view.

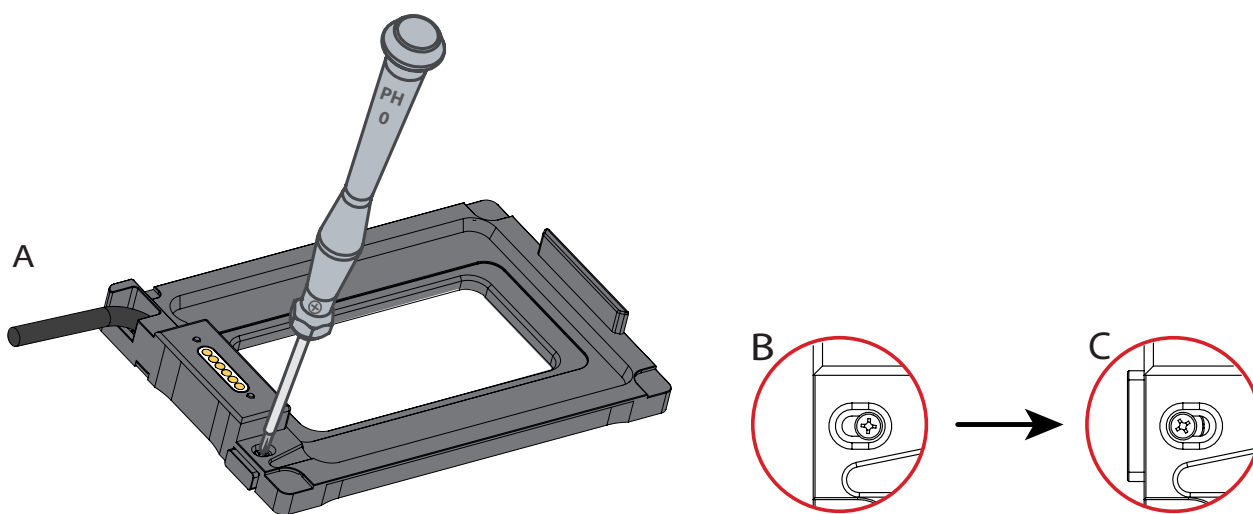


Figure 8: Clamping mechanism to fix the plate firmly inside the microscope stage. (A) Use a crosshead screwdriver PH0 to unlock the mechanism. Clamping mechanism (B) locked, (C) unlocked.

The Slide Holder or Dish Holder (Section 4.6) is placed into the opening of the Heated Plate, where it is held in place by magnets.

The electrical cable (1.5 m) is connected to the Temperature Controller.

The electrical pins provide electricity for the heating of the Heated Lid (Section 4.5). The pins also connect to the temperature and humidity sensors that are integrated into the Heated Lid and send back the information to the Temperature Controller.

4.5 Heated Lid

The Heated Lid (Figure 9) provides excellent optical quality, and also allows for the use of all standard microscopy techniques, including differential interference contrast (DIC). Due to the height of the lid, condensers with a working distance of ≥ 28.5 mm need to be used.

The frame and the glass plate of the Heated Lid are heated. The Heated Lid fits exactly and securely onto the Heated Plate. The electrical pins connect to the Heated Plate to supply the Heated Lid with electricity for heating and to lead the temperature and humidity sensor's feedback to the Temperature Controller.

For gas incubation, the Heated Lid is equipped with a push-in fitting for the gas tubing (Figures 9 and 10). The Humidity sensor is integrated into the Heated Lid (Figure 9 B). Detailed information on the Gas Incubation System is given in its [instruction manual](#).

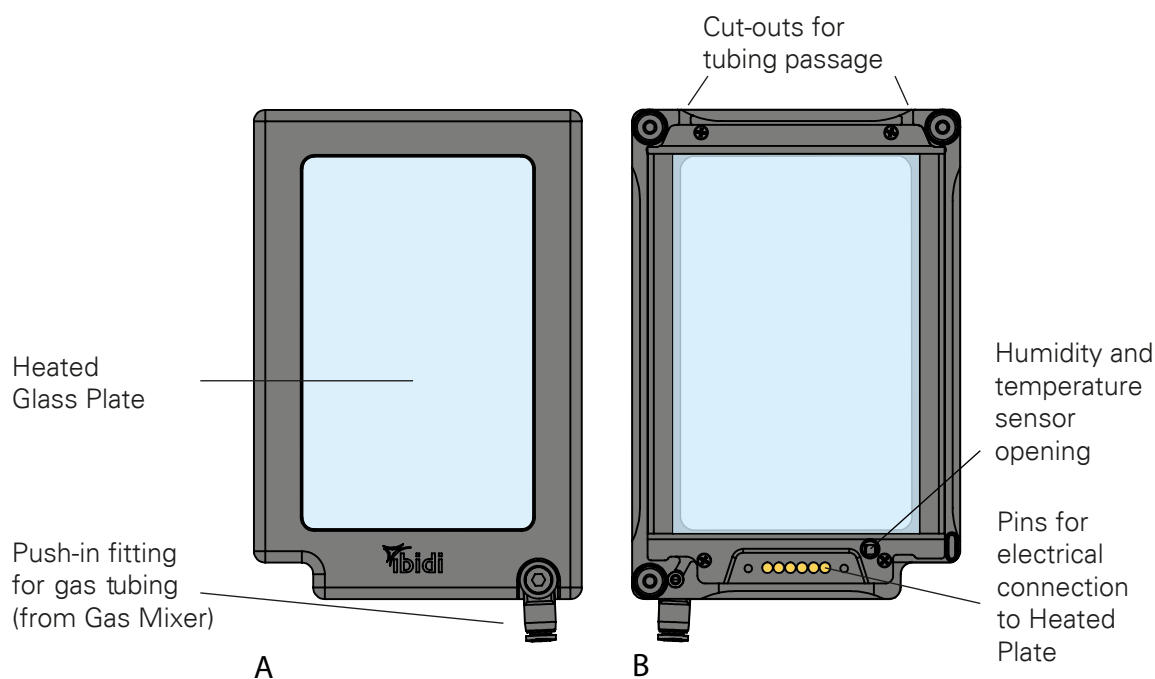


Figure 9: Heated Lid top (A) and bottom (B) view.

The Heated Lid of the Incubation Chamber Slide/Dish has a push-in fitting for optional connection to the ibidi Gas Incubation System. Either a push-in fitting directed sideways (Figure 10 A) or a push-in fitting directed upwards (Figure 10 B) is installed to the Heated Lid. In order to exchange the push-in fitting from sideways to upwards, or vice versa, use the hex keys as shown in Figure 10. Use the hex key 3.0 to remove or insert the push-in fitting, sideways. Use the hex key 2.5 to remove or insert the push-in fitting, upwards.

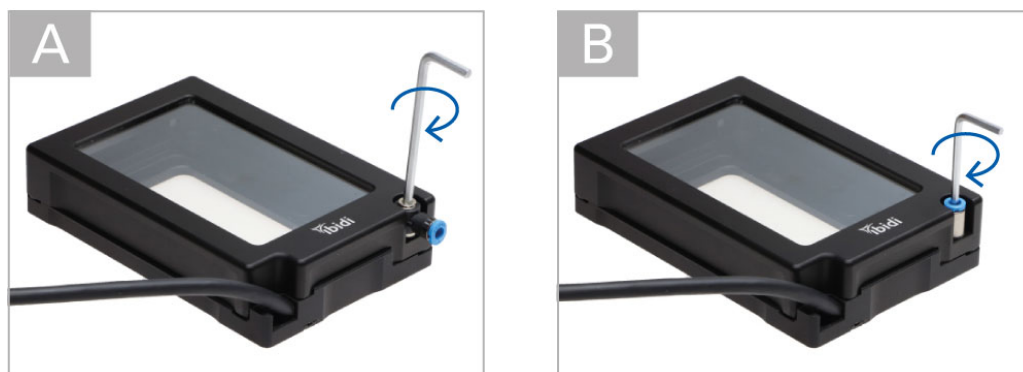


Figure 10: Exchange the push-in fitting for gas supply from sideways (A) to upwards (B).

The Heated Lid is equipped with two cut-outs that allow for passage of tubing in and out of the Incubation Chamber (e.g., for perfusion experiments). If the cut-outs are not used, please close them with the small magnetic plate (Figure 11).

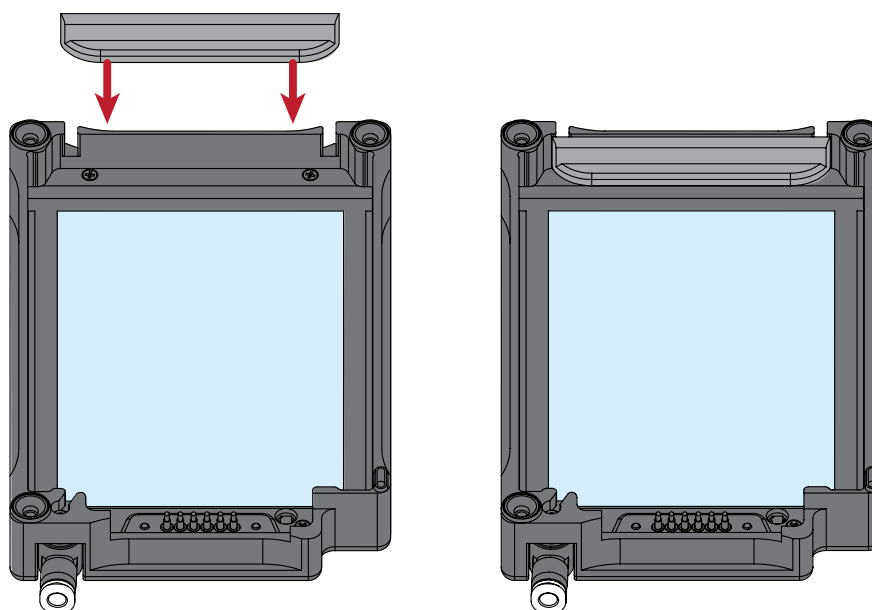


Figure 11: Put the magnetic plate onto the lid to close the cut-outs for tubing insertion.

4.6 Slide/Dish Holder

The ibidi Heating System Slide/Dish comes with a Slide Holder and a Dish Holder. The Holder holds the sample and fits into the Heated Plate. The Holder is held in place by strong neodymium magnets, which helps holding the slides and dishes firmly in position during microscope stage movements. Further, contact between the metallic Holder and the Heated Plate maximizes the heat transfer and ensures stable heating of the sample. The Heated Plate passively heats the Holders.

Use the handles to place/remove the Holder into/from the Plate of the ibidi Heating System Slide/Dish.

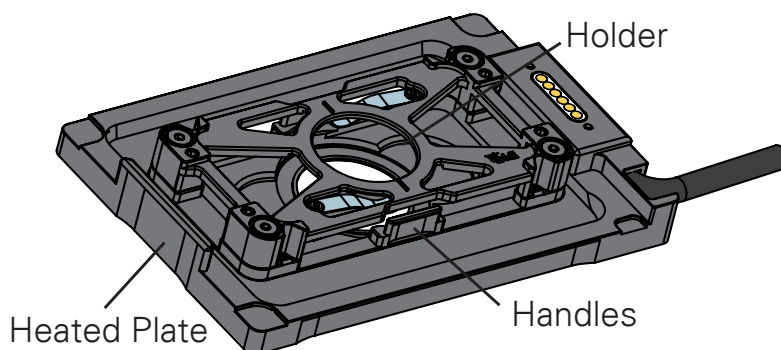


Figure 12: Heated Plate with inserted Dish Holder.



WARNING – Heated Plate, Heated Lid, and Holders all contain strong neodymium magnets! Please contact us for a non-magnetic system if permanent magnetic fields are detrimental to your experiment.

4.6.1 Slide Holder

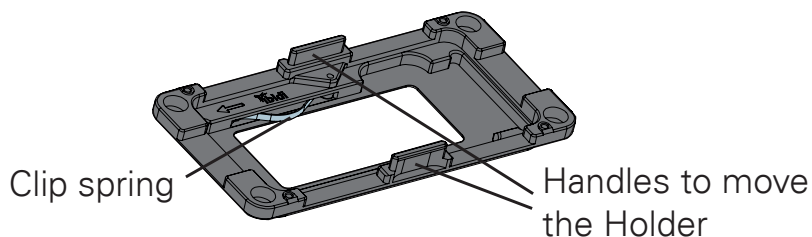


Figure 13: Slide Holder.

The Slide Holder comes as one piece, without top (Figure 13). The slides are held in position by a clip spring. A stopper at the side wall of the Holder facilitates the positioning of shorter (60 mm) slides (Figure 14 B).

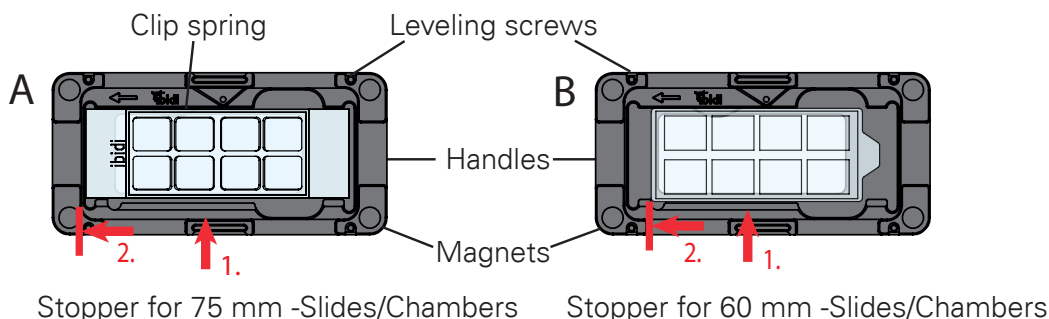


Figure 14: Top view of the Slide Holder equipped with an ibidi μ -Slide 8 Well (A) and equipped with a Nunc™ Lab-Tek™ Chambered Coverglass (B). Red arrows indicate the steps for insertion of a slide. 1. Carefully slide the slide or coverglass via the slanted edge into the cavity and press it gently against the clip spring. 2. Push the slide towards one of the two stoppers for 75 or 60 mm slides, respectively, to center the observation area.

4.6.2 Dish Holder

The Dish Holder has a lower part (bottom) that fits into the Heated Plate, and an upper part (top) to press the sample down.

The Dish Holder fits most of the commercially available 35 mm dishes (compatible dishes are listed in Section 5.5). The clip springs on the Holder compensate for slightly different heights. To minimize the tilting of the upper part, the gap can be adjusted with four screws (Figure 16). For this purpose, a screw driver (hex key 2.0 mm) is supplied.

For very low dishes (e.g., ibidi μ -Dish ^{35 mm, low}) flip the Holder upside down (Figure 17).

For optimal flat positioning on the microscope stage, the Dish Holder can be leveled with four leveling screws on the edges (Section 4.6.3).

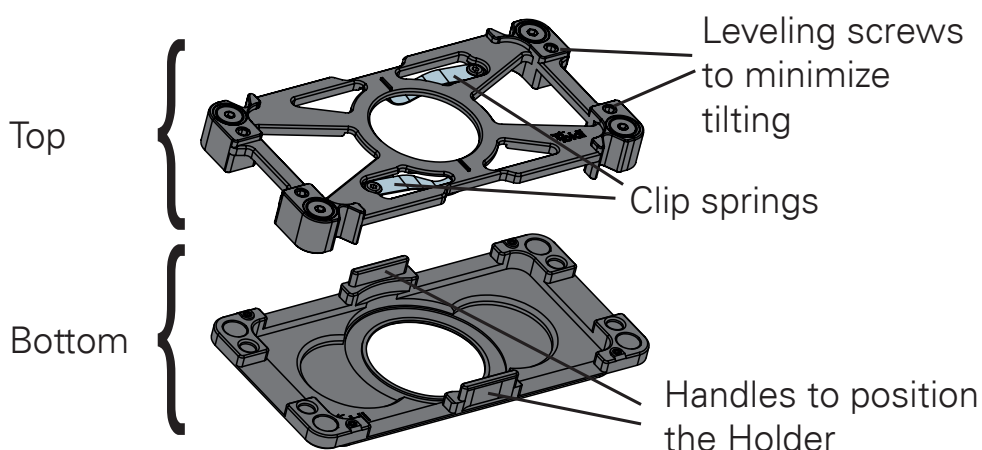


Figure 15: Dish Holder.

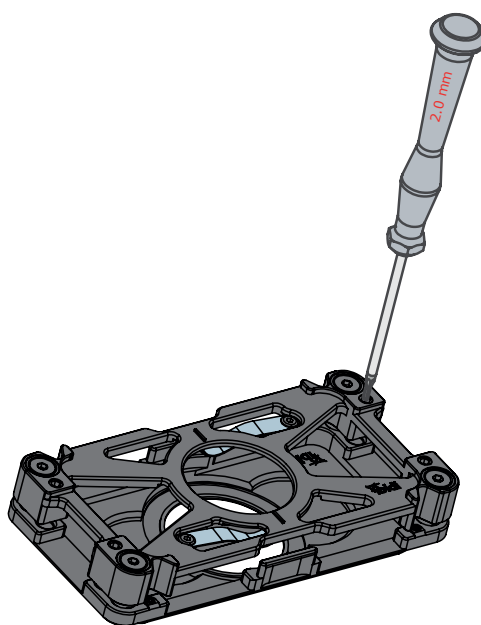


Figure 16: Use the 2.0 mm hex key to adjust the four screws to minimize the tilting of the upper part of the Holder.

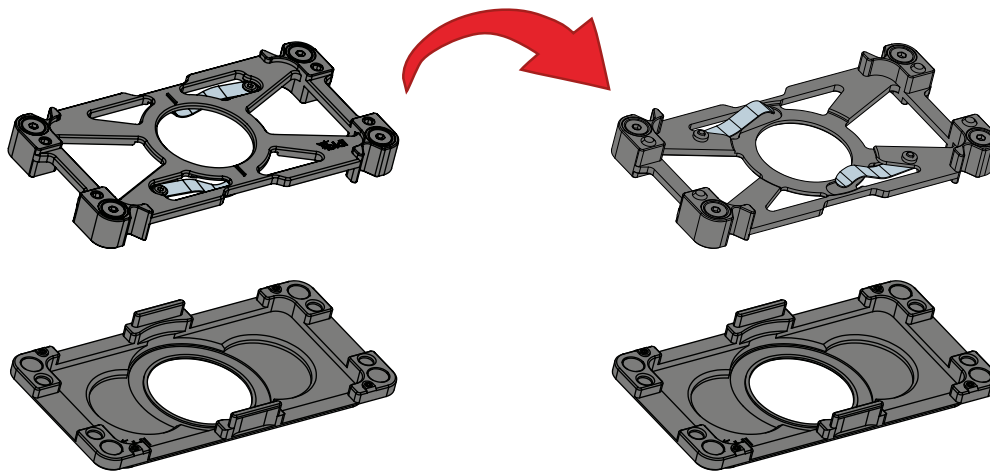


Figure 17: Flip around the top of the Dish Holder to obtain the best fit for very low 35 mm dishes.

4.6.3 Holder Leveling

The Dish Holder and Slide Holder can be leveled (e.g., to adjust the focus position for a tilted sample). Use the 0.9 mm hex key to adjust the leveling screws at each of the Holder's corners. The leveling screws on the Holders can be accessed even if there is a sample in the Holder and the Holder (top) is in place (Figure 18).

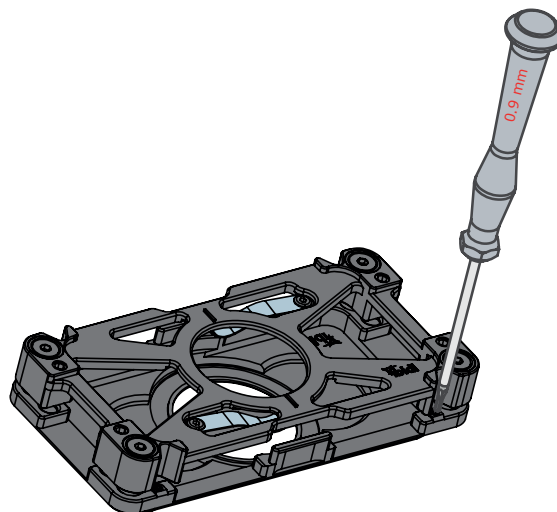


Figure 18: Leveling the Dish Holder with a 0.9 mm hex key. The principle also applies to the Slide Holder.

4.7 Compatible Slides and Dishes

All tested compatible slides and dishes are listed in the table below. Slides and Dishes that are not listed may also be compatible.

Table 4: Overview of Compatible Slides

Company	Product	Cat. No.
ibidi	all μ -Slides	see ibidi.com
Thermo Fisher	Nunc™ Lab-Tek™ Chamber Slide System, 8 wells	177402
Thermo Fisher	Nunc™ Lab-Tek™ II Chamber Slide™ System	154534
Eppendorf	Cell Imaging Coverglass with 8 chambers	0030 742.036

Table 5: Overview of Compatible 35 mm Dishes

Company	Product	Cat. No.
ibidi	μ -Dish ^{35 mm} , high	see ibidi.com
ibidi	μ -Dish ^{35 mm} Grid-500	see ibidi.com
ibidi	μ -Dish ^{35 mm} Glas Bottom	see ibidi.com
Greiner	Cell Culture Dish, PS, 35/10 mm, four inner rings, vents, CELLSTAR® TC	627170
Greiner	Cell Culture Dish, PS, 35/10 mm, vents, CELLSTAR®, TC	627160
Greiner	CELLVIEW Cell Culture Dish, PS, 35/10 mm, Glass Bottom, 4 Compartments, TC	627870
Greiner	CELLVIEW Cell Culture Dish, PS, 35/10 mm, Glass Bottom, 1 Compartment, TC	627860
Corning	Corning® 35 mm TC-Treated Culture Dish	430165
Thermo Fisher	Nunc™ Cell Culture/Petri Dishes, 35 mm, TC	153066
Eppendorf	Cell Culture Dish, 35 mm, TC	30700112
Fisher Scientific	Corning™ Falcon™ EASY GRIP 35×10 mm Dish	351008
WPI	Fluorodish Cell Culture Dish - 35mm	FD35-100
WillCo	WillCo–dish® Glass Bottom Dishes 35×10 mm	GWST-3522

4.8 Cable Clip for Optional Use



CAUTION – ibidi GmbH does not accept any liability for damages resulting from the use of the cable clip on your microscope. Please check with your microscope provider and/or your institution’s technical support if using the cable clip on the microscope stage is allowed.

The cable clip can optionally be used on your microscope stage to reduce the strain from any cables and gas tubing on the Incubation Chamber to a minimum. Please check with your microscope

provider and/or your institution’s technical support if modifications to the microscope stage are allowed.

The cable clip comes with a self-adhesive tape on the underside. To use the clip, define the optimal position of the clip on your stage, before removing the protective foil from the self-adhesive tape and mounting the clip to the stage by slight pressure. Open the clamp and introduce all cable and tubing leading from the Incubation Chamber to the controller(s). When using the ibidi Heating System Slide/Dish, these are the electrical cable from the Heated Plate and the gas tubing from the Gas Incubation System.

If there is no more need for the clamp, it can easily be removed by soaking the adhesive tape with isopropanol (70%) for about 20–30 s, followed by careful removal of the clip via circular movements. Dry away remaining isopropanol with a lint-free tissue.

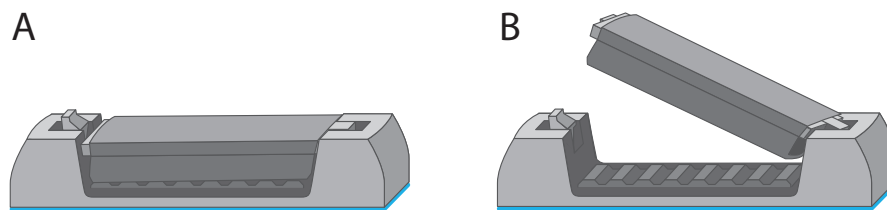


Figure 19: Cable clip for optional use on microscope stage. (A) closed, (B) open.



CAUTION – Please consult the manufacturer of the microscope stage regarding compatibility with isopropanol beforehand. The use of isopropanol or other types of organic solvents may remove the instrument’s paint.

5 Operation

Before starting an experiment, check that the ibidi Heating System Slide/Dish fits on your microscope stage, and that your cell culture vessels are compatible with the Holder. Connect all parts (Section 5.1) and perform a temperature adjustment as explained in Section 5.4.



NOTE The Temperature Controller only measures the temperature from the sensors in the Heated Lid and Heated Plate. The temperature in the sample must be adjusted for your specific setup. Follow the instructions in Section 5.4.

5.1 Installation and Connection of the Components

The components of the ibidi Heating System Slide/Dish are connected as shown in Figure 20.

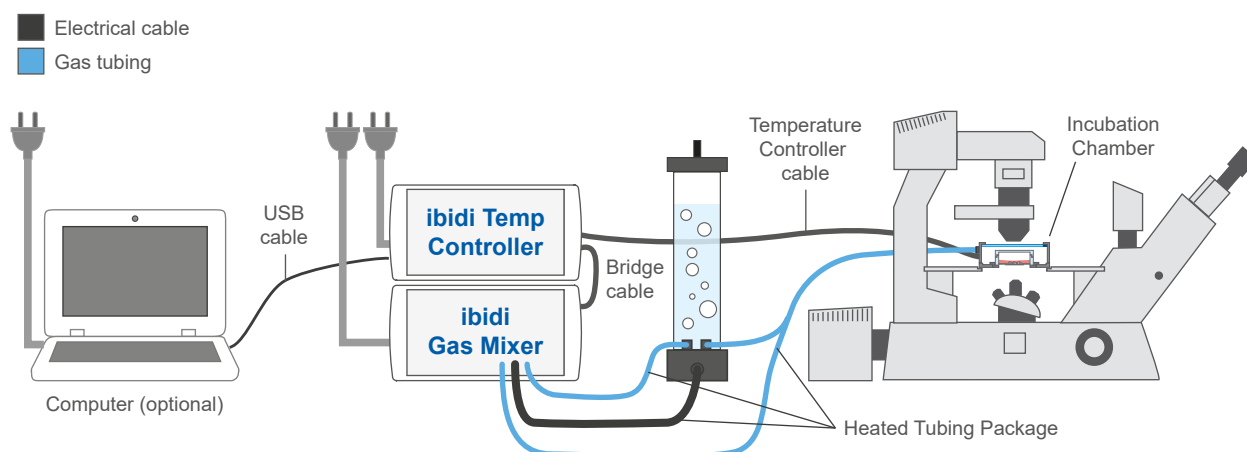


Figure 20: Installation of the components of the ibidi Heating System Slide/Dish.

1. Place the Temperature Controller next to the microscope and connect the power supply.
2. Confirm that the power switch of the Temperature Controller is off.
3. Insert the Heated Plate into the opening of the mounting frame on the microscope stage.
4. Insert an empty, unsterile slide or dish into the respective Holder and put the assembly into the opening of the Heated Plate.
5. Place the Heated Lid onto the Heated Plate.
6. Plug the electrical cable of the Incubation Chamber into the connector port 1/2 or 4/5 at the back of the Temperature Controller.
7. Switch on the controller by the dip switch. All heating components that are recognized are shown in the Temperature Controller's display.

8. Let the system equilibrate for at least 30 minutes.

Optional: To setup communication with the IncuControl Software, the USB cable must be connected to the Temperature Controller and the computer (Section 6).

To adjust the sample temperature, use the temperature sensor in the Temperature Adjustment Set and plug it into the corresponding connector at the back of the Temperature Controller. The actual temperature is shown in the lower left corner of the display (Figure 23). The sensor is then dipped into the sample (Section 5.4).

5.2 Start Operation of the ibidi Heating System Slide/Dish

Switch on the Temperature Controller by the dip switch at the back. Make sure the display shows “RUN” (see Section 5.3.1). The system immediately starts heating up the heating devices (e.g., Heated Plate, Heated Lid).



WARNING – Be aware that when the system is switched on, 10V DC voltage is applied to the underside of the glass plate. Do not touch the underside or contact it with anything conductive! This could cause a short circuit that may destroy the controller and/or the lid.

If you are not sure which temperature is set (e.g., when operating the system the first time), it is recommended to check the settings without your sample.

5.3 Setting Parameters in the Front Display

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

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, press the round button once more.

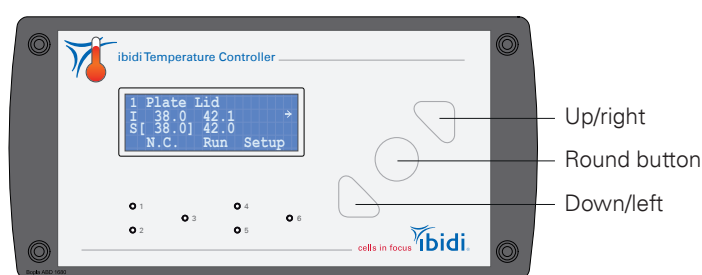


Figure 21: Temperature Controller front display and set buttons.

Each heating unit that is connected (e.g., Incubation Chamber or Objective Heater) is shown on an individual display. The display number (1–4) is indicated in the upper left corner of each display (Figure 21 and Table 6). Navigate between the displays using the “left” and “right” buttons.

Depending on the heated components used, different connector ports (output channels) at the back of the Temperature Controller can be used (Table 6). Accordingly, the information of one, two or three heated components with the designations Plate, Lid, Glass or Obj is shown per display.

Table 6: Output channel assignment and display of the Temperature Controller

Display No.	Channel No.	Column title(s)	Components to connect
1	Channel 1/2	“Plate Lid”	Incubation Chamber, Heating System Dish/Slide
	Channel 1/2 and 3	“Plate Lid Glass”	or Incubation Chamber, Heating System Multi-Well Plate
2	Channel 3	“Obj”	Objective Heater Universal
3	Channel 4/5	“Plate Lid”	Incubation Chamber, Heating System Dish/Slide
	Channel 4/5 and 6	“Plate Lid Glass”	or Incubation Chamber, Heating System Multi-Well Plate
4	Channel 6	“Obj”	Objective Heater Universal

The first three lines of the display show the respective set (“S” = set) and actual (“I” = is) temperature values of the corresponding components (Figure 23). If a component of a heating unit is not connected properly, “N.C.” will be shown in the “I”-value line.

The bottom line shows the temperature of the temperature sensor (if connected), as well as the state of the device (“Run” or “Stop”). On the bottom right, you can switch to the settings menu via “Setup”.

5.3.1 Run/Stop Mode

Set the whole system to run or stop mode by changing the setting in the bottom line of the display to “Run” (= system is running) or “Stop” (= system is not running), respectively.

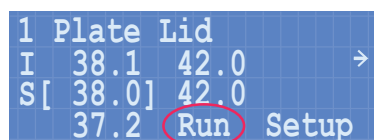


Figure 22: Main display indicating “Run”.

5.3.2 Incubation Parameters

Set the parameters via the front display and wait for the temperature to equilibrate. It is recommended to start the system at least 30 minutes before inserting cells. Recommended values are given in Table 7.

Before starting an experiment, an initial temperature adjustment must be performed (see Section 5.4).

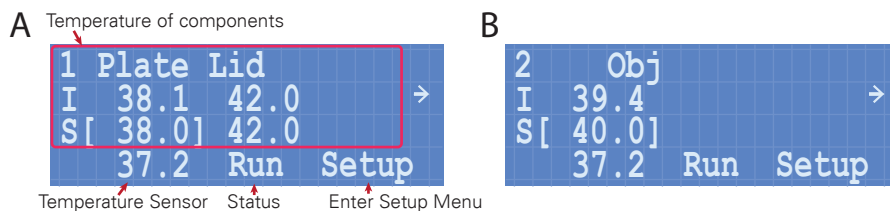


Figure 23: Display of the incubation parameters (set and actual values) (A) Display No. 1, (B) Display No. 2.

5.3.3 Select Temperature Sensor

At first use, the external temperature sensor may not yet be selected. In this case, the display shows “NoSel”. To select the thermocouple type K sensor that is provided with the Temperature Adjustment Set, please select “NoSel” by using the set buttons and choose “TC-K” from the list (Figure 24).

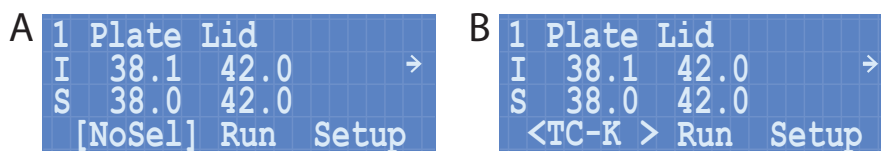


Figure 24: Select temperature sensor. (A) No temperature sensor is selected, (B) Select the thermocouple type K (TC-K) as the temperature sensor by using the set buttons.

5.3.4 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 Temperature Controller can be set to an “On” or “Off” state. For standard operation all channels must be turned on.

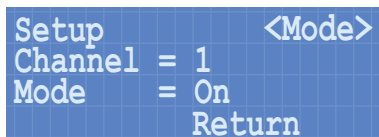


Figure 25: Mode dialog.

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.

Alarms In this dialog, it is possible to set the alarm limits for deviating control parameters. Move from the mode menu to the alarm menu by pressing the “up/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 below/above 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.

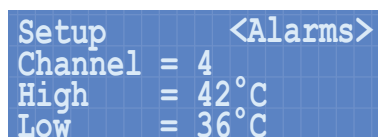


Figure 26: Alarm settings dialog.

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

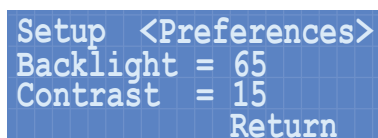


Figure 27: Preferences dialog.

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

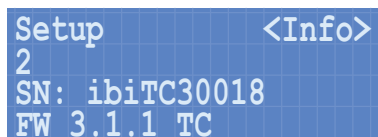


Figure 28: Info display.

5.4 Temperature Adjustment in the Sample

The ibidi Heating System Slide/Dish controls the temperature of the actively heated components (Heated Lid and Heated Plate). The temperature in the sample results from the interaction of all heated components with the supporting microscopy stage and the sample. The temperature at the position of the cells must be measured and adjusted for each specific setup to ensure the optimal temperature at the sample (e.g., 37°C).

When starting the temperature adjustment in the sample in your specific setup, use the recommended temperature settings (for samples that should be 37°C):

Table 7: Recommended temperature settings before optimization

Device	Temperature
Heated Lid	42°C
Heated Plate	38°C

To measure and adjust the sample temperature, use the provided Temperature Adjustment Set (temperature sensor and culture vessel with perforated lid). The temperature of the heated components has to be adjusted in small steps. Follow this procedure to adjust the sample temperature:

1. Set all your peripheral experimental parameters, such as room temperature, air conditioning, airflow, illumination, microscope settings, etc.
2. Connect the external temperature sensor (thermocouple type K) to the plug at the Temperature Controller's back. The connected external temperature sensor will appear in the display.
3. Fill the provided culture vessel (depending on what you want to use for your experiments) with water or medium. Volumes should be the same amount you need for your experiments.
4. Put the loose end of the thermocouple through the hole in the lid of the culture vessel and make sure it dips into the liquid. In case of a channel slide (e.g., μ -Slide Luer) introduce the sensor wire from the Luer ports into the channel.
5. Place the culture vessel into the Holder of the Heated Plate and put the magnetic holder on top.
6. Close the Incubation Chamber with the Heated Lid.
7. After 30 minutes, check the temperature of the external sensor on the display or in the Incu-Control software. If the sample temperature is still too low, raise the set values for the Heated Plate and Heated Lid for 0.5–1°C each, and wait until the sample temperature is stable again (at least 10–15 min).
8. When the sample temperature has reached the desired value, write down the set values for the Heated Plate and Heated Lid and use these settings for all upcoming experiments with the same sample holder.

NOTE

The temperature of the Heated Lid must be set to at least 1–2 degrees higher than the temperature of the Heated Plate!

The temperature adjustment must be repeated from time to time (at least once a year), especially if one of the following conditions has changed:

- Room temperature
- Air conditioning
- Chamber type or objective lens
- Humidification and gas flow
- Use of an XL-Incubator
- Use of an Objective Heater

5.5 Sample Preparation

Check that the cell cultureware you intend to use fits into the Slide Holder or Dish Holder of the ibidi Heating System Slide/Dish (Section).

Prepare the cells according to your protocol and place the culture vessel into the Holder. When using the Dish Holder, fixate the Dish with the Top (Section 4.6.2). Place the assembly into the opening of the Heated Plate and close the Heated Lid.

NOTE

Let the temperature of the system equilibrate for a minimum of 30 min before you start your experiments.

6 IncuControl Software

The Temperature Controller 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](#).

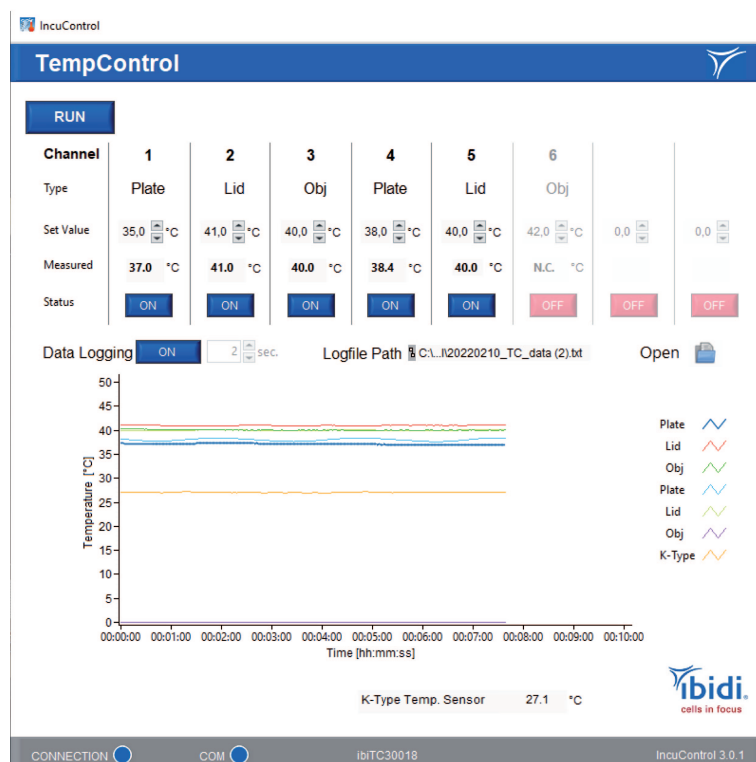


Figure 29: Temperature control window in IncuControl.

Prohibitions on the use of ibidi software:

The following actions are prohibited:

- Copying software for other than backup purposes
- Transferring 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 Disinfection and Cleaning

The heated components and the Temperature Controller do not have to be sterile, because the incubation chamber has no direct contact with the cells and the cell culture medium. If disinfection is necessary for some reason, we recommend using isopropanol (70%) or common lab disinfection solutions based on quaternary ammonium compounds (e.g., Barrycidal 36 or Pharmacidal).

All parts of the ibidi Heating System Slide/Dish can be cleaned from the outside. We recommend using ultrapure water for cleaning. Fingerprints on the Heated Lid can be removed using isopropanol (70%) or lens/eyeglass cleaning wipes.



CAUTION – When cleaning the heated glass plate(s), be careful when wiping the inner surface of the lid not to damage the electro-conductive coating. Also take care not to damage the glass plate.

The use of ethanol or other types of organic solvents may remove the instrument's paint.

To clean the heated components and/or the Temperature Controller switch off the Temperature Controller and disconnect all electrical cables. Leave the instrument to cool down for approx. 5 minutes.



WARNING – Hot surface (max. 55°C)! Do not touch Heated Lid, Heated Plate, and Heated Glass Bottom when hot.

7.2 Influence of Ambient Temperature and Ventilation

The ambient temperature affects the conditions inside the incubation chamber. 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.

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

The use of an XL-Incubator and/or an objective heater minimizes those effects and helps significantly to stabilize surrounding conditions.

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 equilibration¹.
- 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 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 Condensation Inside the Stage Top Incubator

Check the temperature of the chamber (Heated Lid and Heated Plate). 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.

¹If 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.



ibidi GmbH

Lochhamer Schlag 11
82166 Gräfelfing
Germany

Toll free within Germany:

Phone: 0800/00 11 11 28
Fax: 0800/00 11 11 29

International calls:

Phone: +49 89/520 46 17 - 0
Fax: +49 89/520 46 17 - 59

E-Mail: info@ibidi.com
ibidi.com

