my-Control
for
MiniBio Reactors 250, 500 and 1000 ml

OPERATOR MANUAL

Software Version mE.2.9.X; Document Version 2.91

applikon®
BIOTECHNOLOGY

TABLE OF CONTENTS

1 SAFETY

1.1 SAFETY SYMBOLS

The following symbols are used on the equipment and in this manual.

WARNING
Important issue concerning personnel health and device safety. Refer to this manual.

WARNING
Risk of electrical shock hazard.

WARNING
Hot surface.

INFORMATION
Additional information.

Disposal instructions

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1.2 SAFETY WARNINGS

GENERAL

- This set of equipment has been designed in accordance with EN61010-1 "Safety Requirements for Electrical Equipment for Measurement, Control and Laboratory Use", and has been supplied in a safe condition.
- The Hardware manual contains information and warnings, which have to be followed by the user to ensure safe installation, operation and to retain the equipment in safe condition. Carefully read this manual before putting the ***my***-Control into operation.
- Before switching-on the equipment, make sure that it is set to the line voltage. Refer to the Hardware Manual, section 3.2.
- This equipment has been designed for bioprocess control; it must not be used for other purposes!

WARNING
Risk of electrical shock hazard.

- The colored metal front panel (with any mounted pumps or micro valves) of the ***my***-Control cabinet can be replaced by a panel with a different color. However, removing the metal panel will make hazardous parts accessible and must therefore be performed by qualified personnel. The ***my***-Control must not be switched on or used when the colored metal front panel is not properly in place.
- Any interruption of the protective conductor inside or outside the ***my***-Control or disconnection of the protective conductor terminal will make the device hazardous. Intentional interruption is prohibited.
- Capacitors inside the ***my***-Control may still be charged, even if the apparatus has been disconnected from all voltage sources.
- It is not allowed to perform maintenance and/or repair on the opened device under voltage. Before removing the metal front panel or the rear panel, the power cord must be removed from the power entry socket of the ***my***-Control.
- Make sure that only fuses with the required rated current and of the specified type (International Standard IEC 127) are used for replacement. The use of makeshift fuses and the short-circuiting of fuse holders is prohibited.
- It is not permitted to connect equipment to the ***my***-Control or the bioreactor without the double isolation qualification or without the SELV (Safety Extra Low Voltage) qualification.

WARNING
The rear of the ***my***-Control cabinet must be accessible.

- The power switch of the ***my***-Control is located at the rear of the cabinet. Make sure that the ***my***-Control is installed in such a way that, in case of emergency, the power switch can be easily reached!

WARNING
Risk of overpressure in the glass bioreactor.

- The glass reactor may be damaged easily (scratches on the surface)! As a result, its overall strength is reduced. Therefore, do not apply a process pressure that exceeds 0.5 barg (7 psig). Do not obstruct the off-gas line! Make sure that the off-gas filter is not clogged.

WARNING
Risk of damaging the equipment.

- In most cases, one or more pumps are installed in the ***my*-Control**.
- Application of damaged tubes may result in fluid leaking into the pump drive.
- Verify the tube quality inside the pump head before every fermentation run.
- Do not use the tubing pump drives for other purposes than displacement of fluids (or gas).
- Advised pump tubing (pharmed) has its limitation in physical and chemical resistance. Make sure that the pump tubing and the selected reagent type are compatible.

WARNING
Hot surface.

- A Heating Blanket may be used around the bioreactor as an actuator for temperature control. During heating up the bioreactor, the outer surface of this Heating Blanket will be hot.

WARNING
Make sure to use a proper power cord.

- The ***my*-Control** comes with a detachable power cord with 3 x 1.0mm^2 wires (for the USA, the power cord complies with 18AWG which is equal to 3 x 0.82mm^2 wires).
- When the original power cord needs to be replaced by another one, make sure that the replacement cable has the same (or better) specifications than the original.

WARNING
Do not use flammable substances in the affinity of the ***my*-Control** while in operation.

- The ***my*-Control** is an electr(on)ic control device that contains relays for switching power and actuator signals. These relays may generate sparks during operation. It is therefore not allowed to use flammable substances in the affinity of the ***my*-Control** while this device is in operation.

Additional information

- Although the ***my*-Control** as a whole is not UL-certified, all used components have been selected based on conformance with the standard UL 60950 (Underwriters Laboratories Inc. Standard for Safety of Information Technology Equipment).

Disposal instructions

- This product must not be disposed of together with domestic waste.
- All users are obliged to hand in all electrical or electronic devices, regardless of whether or not they contain toxic substances, at a municipal or commercial collection point so that they can be disposed of in an environmentally acceptable manner.
- Consult your local authority or your supplier for information about disposal.

WARNING
Sensor is fragile.

- The Lumisens sensor tube body is made of glass and can break easily. **Handle with care**.

2 GENERAL

2.1 GENERAL INTRODUCTION

The Applikon autoclavable MiniBio Reactor Systems basically consist of the following parts:

- an autoclavable MiniBio Reactor with the appropriate auxiliaries like a stirrer assembly, sensors, an aeration assembly, etc.
- a ***my*-Control** bio controller for measurement and control of process variables (pH, temperature, dO_2, level and stirrer speed) with corresponding controller outputs in order to keep process conditions on set point.
- a host (PC, Tablet or Smartphone) that is used as a User Interface.

The ***my*-Control** combines and supports actuators like pumps, mass flow controllers and valves in order to optimize the use of limited bench space.
Typical characteristics of the MiniBio Reactor Systems:

- Easy setup and operation
- Cultivate using a small amount of medium
- Generation of scalable results
- Easy data handling

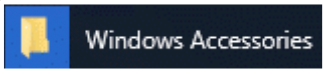
2.2 THE USER INTERFACE

Through a network that is connected to the ***my*-Control** (TCP/IP communication), different kind of devices can be used as a User Interface (UI). Since the ***my*-Control** is addressed by using its IP address, the User Interface is also called the Web UI.
Examples:

Web UI Device Type	**Connecting Network**
PC	LAN / WAN or Peer-to-Peer
Tablet	Wireless connection with WIFI-Router
Smartphone	Wireless connection with WIFI-Router

The ***my*-Control** is addressed by using its IP-address.
Start the Internet Browser at the WebUI-device and surf to the following address: http://IP-address*/. The ***my*-Control** WebUI will be displayed.

***The IP-address of the *my*-Control. The *my*-Control comes with a preset IP-address. This address can easily be customized. Refer to section 1.3 and Appendix A of the Software Reference Manual.**

Internet Explorer IE11 and Google Chrome are supported as Internet browsers.
When working on a PC with Windows 10, IE11 can be started through the Accessories.
Press  > 

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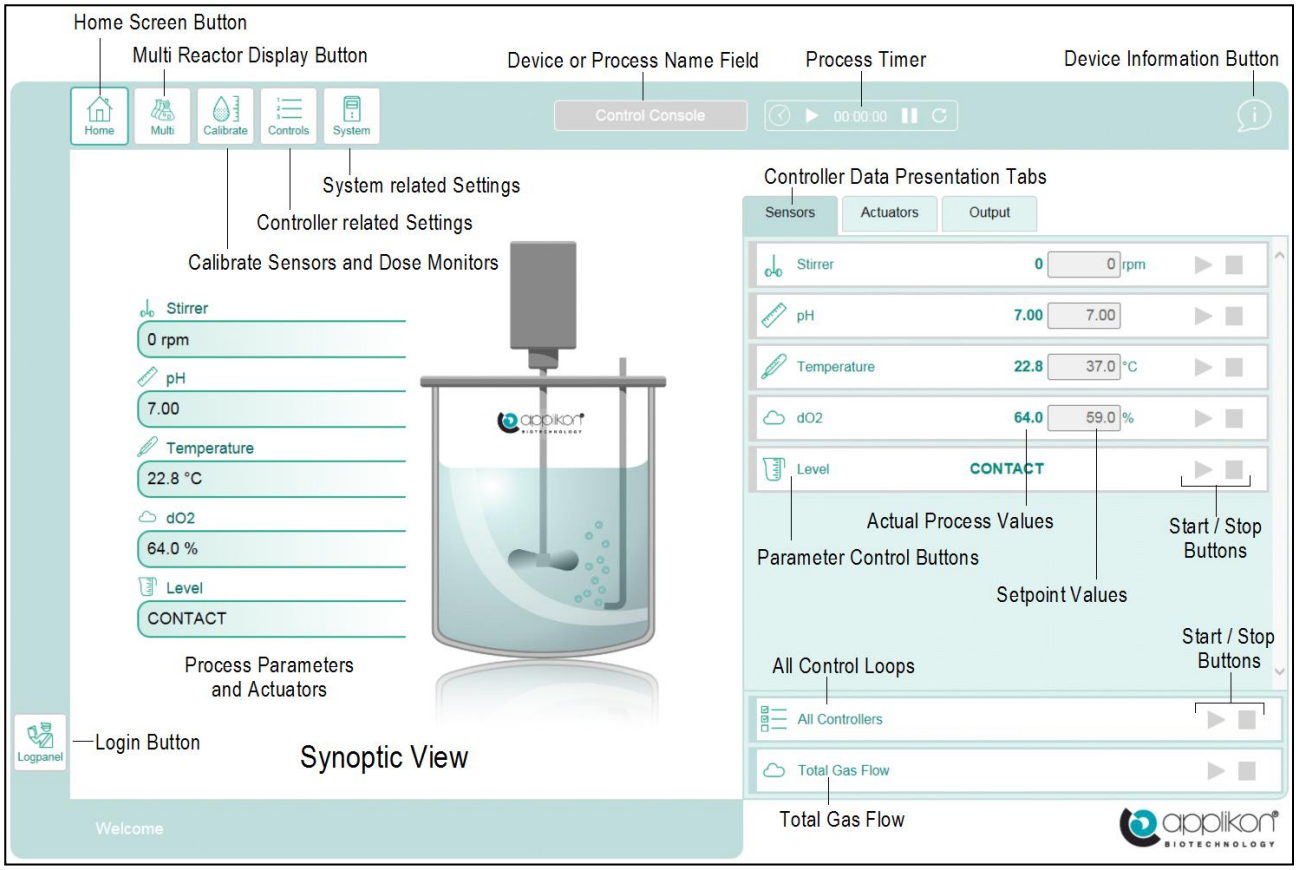
After switching on the power of the ***my*-Control** and invoking the Web Interface through the Internet browser on the PC, the ***my*-Control** displays its Home Screen (see next page).
In this Operator Manual, it is assumed that a PC is used as Web UI (***my*-Control** is operated with mouse-clicks).

2.3 HOME SCREEN

After switching on the power of the ***my*-Control** and invoking the Web UI with the Internet browser, the ***my*-Control** displays its **Home Screen**.

Example of the **Home Screen** in **View** mode (no user is logged in, control loops are "Idle"):



The presented Synoptic View shows a glass stirred tank reactor. The reactor image depends on the Bioreactor Selection. Refer to the Software Reference Manual.

The Login button (Logpanel) can be used to login as Operator or System Engineer.

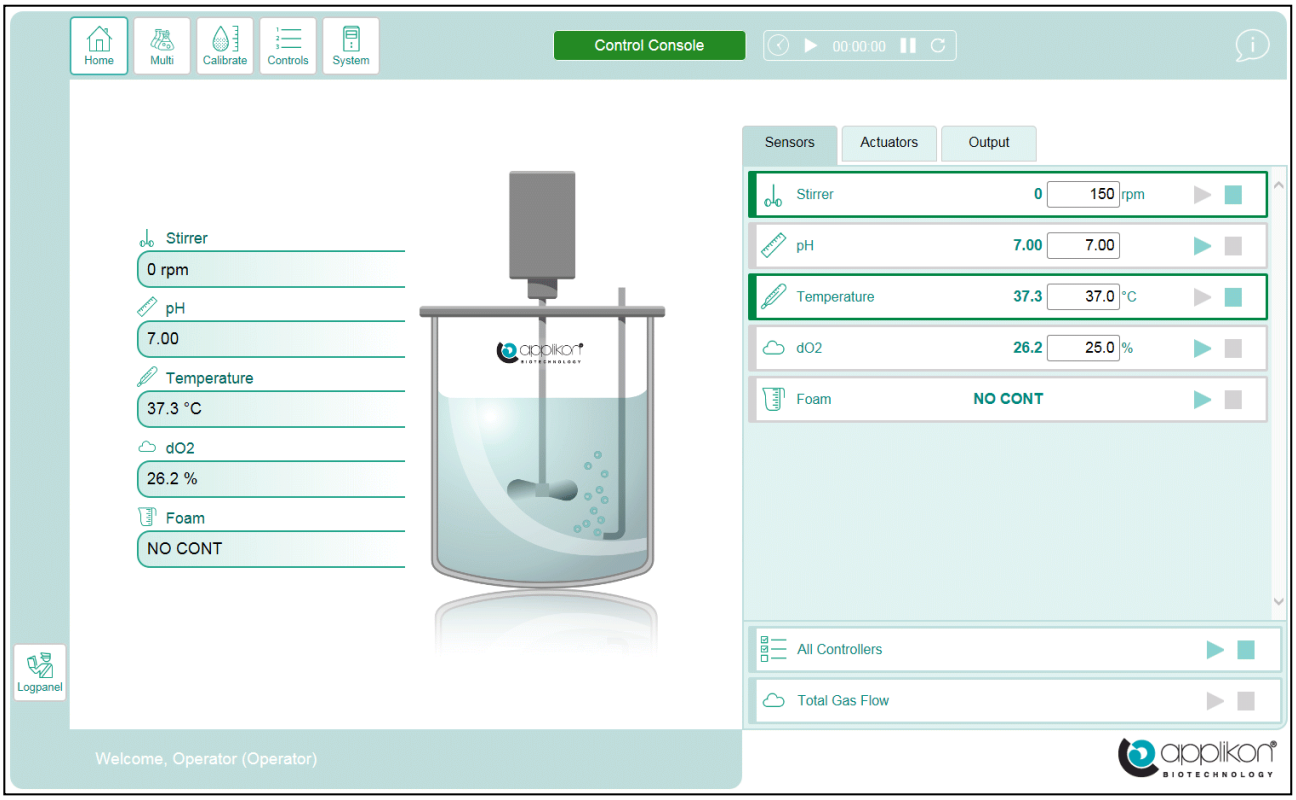
Default password for Operator and System Engineer: 0000.

For additional information regarding the Login procedure, refer to chapter 2 of the Software Reference Manual.

This section is continued on the next page.

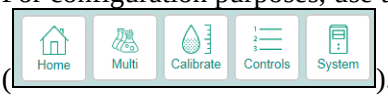
When the user is logged in as Operator or System Engineer, additional Access Rights are granted.

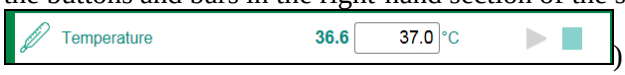
When the user is logged in as Operator and some control loops are running, the User Interface could be presented like:



When the zoom-factor of the selected Web browser is adjusted to < 100%, some details of the screen may not be presented correctly (pixels may be lost). Make sure to adjust the zoom factor of your browser to ≥ 100%.

The User Interface shows buttons, bar and process information fields. Instruction for use:

- For configuration purposes, use the buttons that are presented in the screen header (

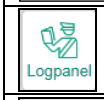
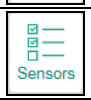
).
- For daily operation of the controller (starting and stopping control loops and editing control parameters, the buttons and bars in the right-hand section of the screen (such as  and 

) can be used.

Comprehensive information concerning the use of the ***my***-Control in combination with bioprocesses can be found in the corresponding Software Reference Manual!

2.4 GRAPHICAL PRESENTATION OF BUTTONS

For the buttons in the Web User Interface, the following icons are used:

Button	Description	Button	Description
	Home Screen button		System menu button
	Login button		Communication Settings button (I/P address, subnet mask and gateway)
	Multiple Reactor Display Mode button		Bioreactor Type selection and Control Loop Configuration button
	Calibration Menu button		System Settings button (e.g. Synoptic definition and System Preferences)
	Sensor Calibration button		
	Dose Monitor Calibration button		Control Loop Tab, based on a pH sensor
	Control Parameter menu button		Control Loop Tab, based on a dO_2 sensor
	Alarm Limit definition button		Control Loop Tab, based on a Temperature sensor
	Controller Setup button (control type and parameters)		Control Loop Tab, based on a Level sensor
	Manual Actuator Control button		Control Loop Tab, based on an Analog Input
	Controller Configuration button (assign actuators per control loop)		Control Loop Tab, based on weight (balance input)
	Actuator Property button (edit actuator name, units, type and limits)		Control Loop Tab, based on a Biomass sensor (or Conductivity)
	Sensor Property button (edit sensor name, units and priority)		Control Loop Tab, based on a Redox sensor
	Totalized Gas Flow Control button (edit control settings and warnings)		
	Trend button (presents the parameter values in a trend)		

2.5 SYSTEM TAG / TAB PRESENTATION

In the table below, the sensor and actuator symbols (as they are presented in the right side of the Home screen) are listed:

Sensor Tag	Description	Actuator Tag	Description
	pH Sensor (potentiometric or optical)		Actuator, assigned to pH Control
	dO_2 Sensor (polarographic or optical)		Actuator, assigned to dO_2 Control
	Temperature Sensor		Actuator, assigned to Temperature Control
	Level Sensor		Actuator, assigned to Level Control
	Stirrer Speed Input		Stirrer Motor
	Analog Input		Actuator, assigned to a Control Loop that is based on an Analog Input
	Balance Input		Actuator, assigned to a Weight or Flow Control Loop
	Redox Sensor		Actuator, assigned to Redox Control
	Biomass (or Conductivity) Input		Actuator, assigned to Biomass Control
			Free Actuator, not assigned to a Control Loop

2.6 ENTERING TEXT AND NUMERIC VALUES

Text (such as parameter names) and numeric values can be entered using the external or internal keyboard. If a normal PC monitor is used as User Interface, the external keyboard is used for entering alpha-numeric data.
If a PC monitor with Touch Screen (or tablet / smart phone) is used as User Interface, the internal keyboard will pop up as soon as data entry is required.

As soon as new data is typed, the  buttons will appear at the bottom of the active window.

Instead of using the <Enter> key, the  button can be used to save the entry. Pressing the  button cancels the entry without saving it.

3 ASSEMBLING THE REACTOR

3.1 GENERAL

Unpacking

- When unpacking the equipment, verify if there is any transport damage and if the reactor is complete (the way you ordered it).

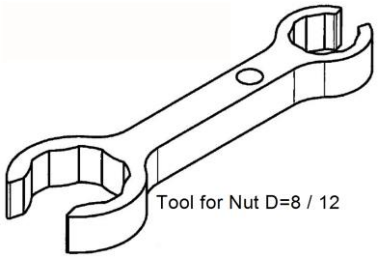
Cleaning

- Clean all parts with 70% ethanol to remove dust or dirt from shipping.

Assembling

- When assembling the reactor, make sure not to damage the threaded ports: always screw in the auxiliaries straight.
- Make sure that an O-ring is present between the auxiliary and the head plate so as to keep the interior sterile.

CAUTION
The MiniBio reactors come with a tool to fix the head plate auxiliaries. However, do not apply too much force to fasten the auxiliaries!



3.2 ASSEMBLING THE REACTOR AND HEAD PLATE

The head plate of the reactor contains a number of ports that can accommodate auxiliaries for agitation, aeration, fluid and gas addition, sampling, sensing, etc.
The exact head plate layout depends on the reactor type (volume).

For details of the available head plates and their auxiliaries, see the ***my*-**Control Hardware Manual.

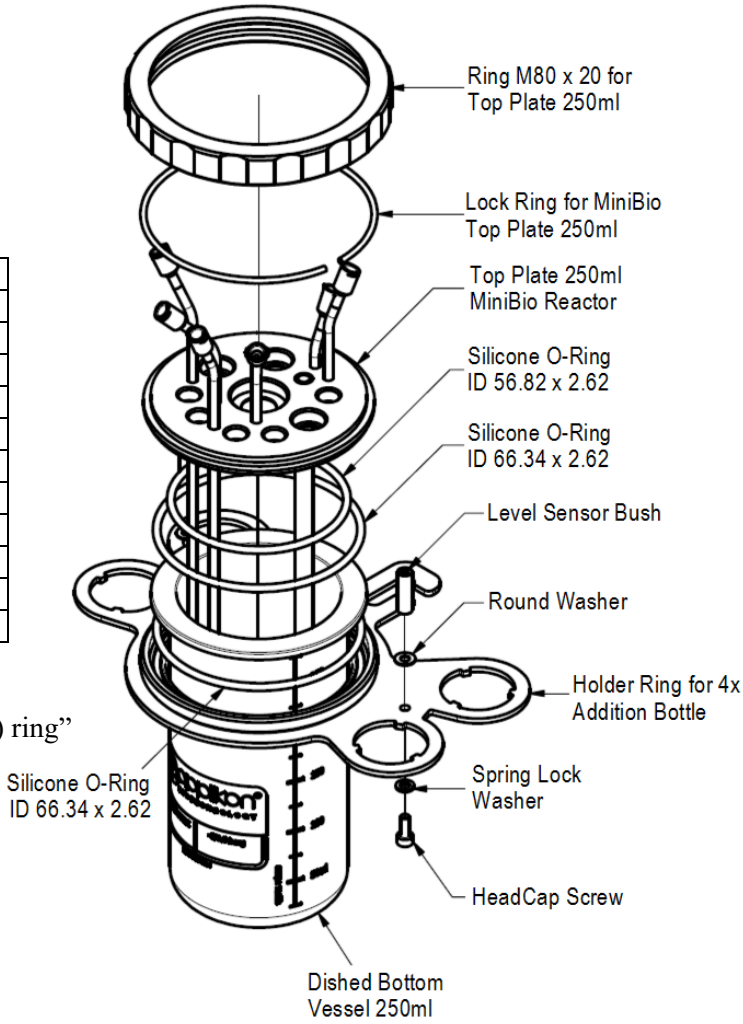
3.2.1 250 ML REACTOR

Assembling the MiniBio reactor:

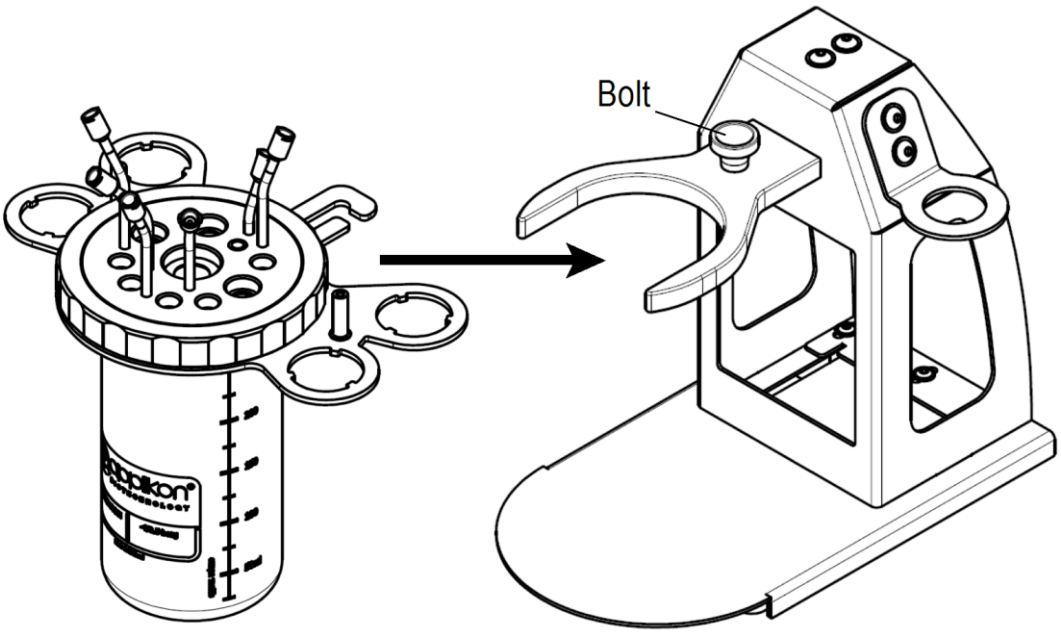
Step 1: Put the parts of the MiniBio reactor together according to the exploded view:

Part #	Description
V3MP072501	Vessel Dished Bottom 250ml
V3MP071851	Lock Ring for Top Plate 250ml
V3MP071811	Ring M80x2 for Top Plate 250ml
V3MP071031	Level Sensor Bush
V3KP070141	Holder Ring 4x Addition Bottle
V3KP070121	Top Plate 250ml with Luer Locks
V1S4ARP146	Silicone O-Ring ID 66.34x2.62
V1S4ARP140	Silicone O-Ring ID 82.34x2.62
V0N0310051	Spring Lock Washer
V0N0310001	Round Washer
V0N0305008	HeadCap Screw

The “top plate” is locked in the “(rotating) ring” by the “lock ring”.



Step 2: Place the reactor in the fork of the stand and fix the bolt that secures the reactor.



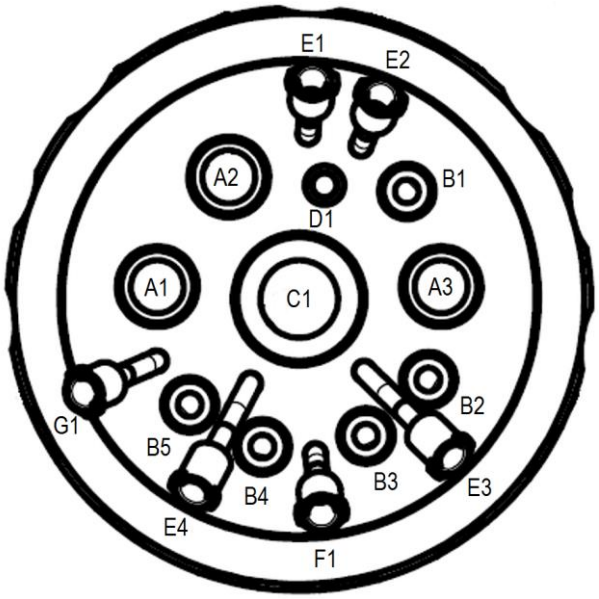
Step 3: Mount the auxiliaries in the head plate.
The head plate of the 250 ml MiniBio reactor contains welded inserts for:

- Aeration (sparging)
- Sampling
- Additions (4x)

Also the temperature pocket is welded in the head plate.

The ports and welded inserts of the head plate are listed in the table below:

Port #	Description
A1	Sensor / Condenser Port M12 x 1
A2	Sensor / Condenser Port M12 x 1
A3	Sensor / Condenser Port M12 x 1
B1	Universal Port M8x1
B2	Universal Port M8x1
B3	Universal Port M8x1
B4	Universal Port M8x1
B5	Universal Port M8x1
C1	Stirrer Port
D1	Temperature Pocket
E1 (Luer Lock)	Addition or Overlay Pipe
E2 (Luer Lock)	Addition or Overlay Pipe
E3 (Luer Lock)	Addition or Overlay Pipe
E4 (Luer Lock)	Addition or Overlay Pipe
F1 (Luer Lock)	Fixed Sample Pipe
G1 (Luer Lock)	Sparger Pipe



One of the four addition ports may be used for gas overlay.
For Microbial applications, the lower end of the sparger pipe is left open or a jet sparger tip is used:
Z813180252 Jet Sparger Tip
For Cell Culture applications, the lower end of the sparger pipe may be equipped with a porous sparger tip:
Z813180251 Porous Sparger Tip

Assembling instructions

Make sure that an O-ring is present between the auxiliary and the head plate so as to ensure sterility.
Use the tools that come with the MiniBio reactor. Do not apply excessive force.

Mount the auxiliaries in the following order:

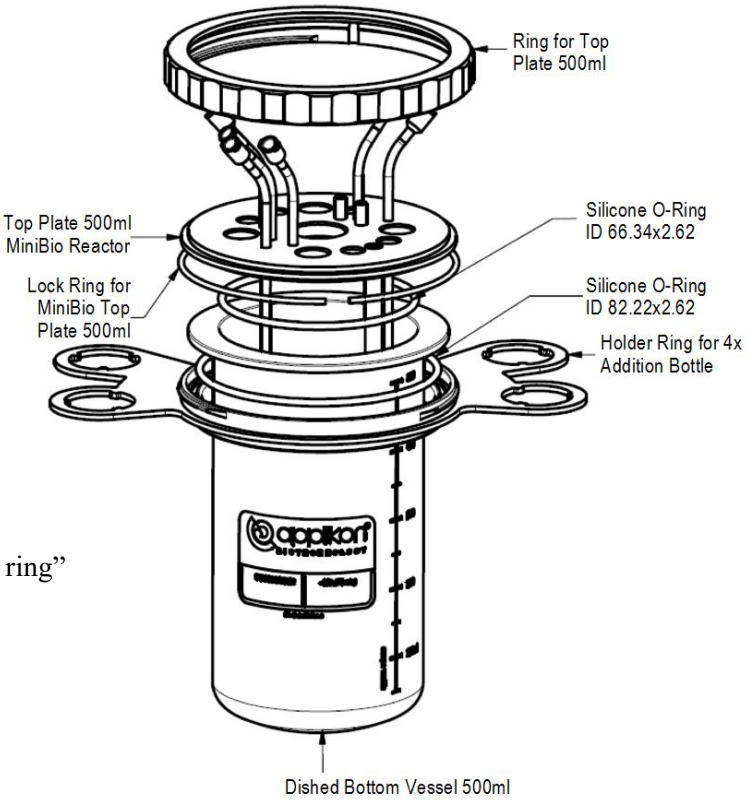
- Stirrer assembly
- Auxiliaries in the Universal M8 x 1 ports
- Sensors or sensor parts in the M12 x 1 sensor ports:
  - the pH sensor must be calibrated before it is mounted in the head plate (refer to section 4.2),
  - the optical LumiSens sensor for dO_2 measurements consists of two parts that stick together through a magnetic coupling. The Glass Tube with Sensor Tip and Threaded Magnetic Coupling must be mounted in the M12 x 1 port Head Plate port and be covered with the End Cap to prevent moisture entering the Glass Tube during autoclaving. The Sensor Head with electronics and Optical Fiber Tube is mounted later (after autoclaving). Refer to section 7.3.
- The M12 x 1 hose barb insert in the condenser port
- The temperature sensor in the thermometer pocket

3.2.2 500 ML REACTOR

Assembling the MiniBio reactor:

Step 1: Assemble the MiniBio reactor according to the exploded view:

Part #	Description
V3MP075001	Vessel Dished Bottom 500 ml
V3KP070161	Holder Ring 4x Addition Bottle
V1S4ARP152	Silicone O-Ring ID 82.22x2.62
V1S4ARP146	Silicone O-Ring ID 66.34x2.62
V3MP071951	Ring for Top Plate 500ml
V3MP071981	Lock Ring for Top Plate 500ml
V3KP070131	Top Plate 500ml with Luer Locks

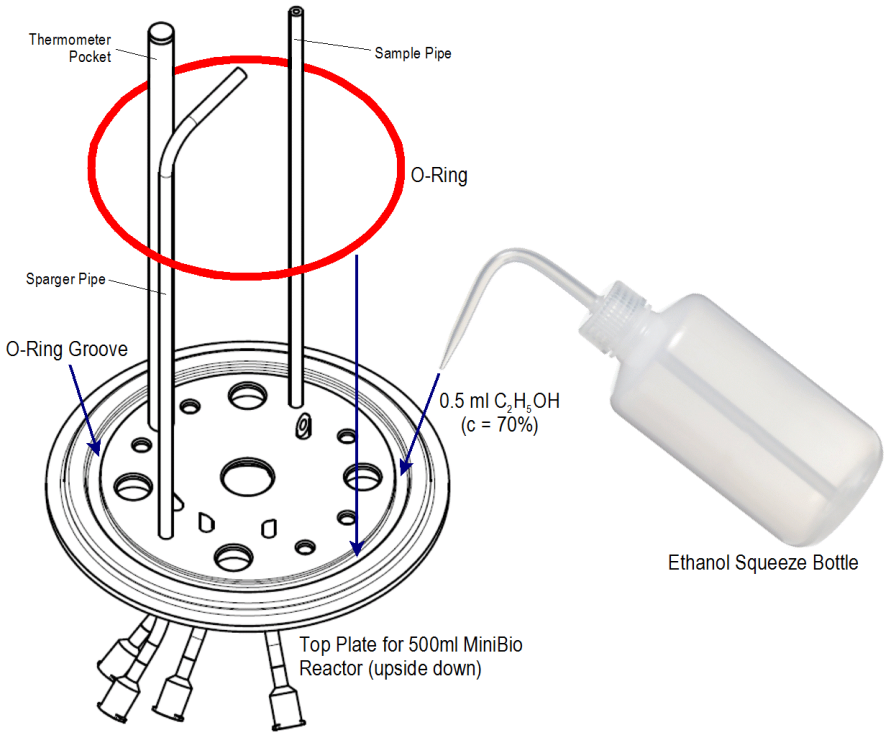


The "top plate" is locked in the "(rotating) ring" by the "lock ring".

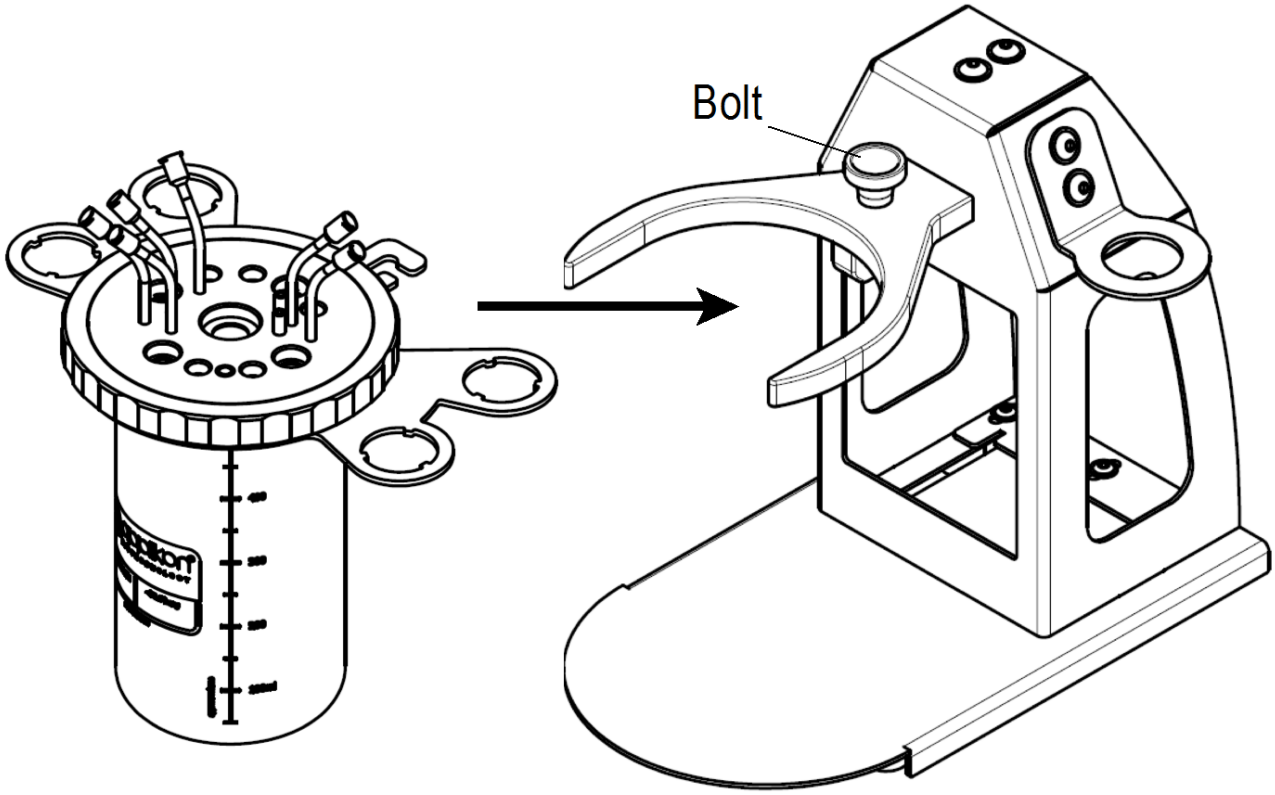
Mounting the Silicone O-ring in the bottom of the top plate:

If the O-ring in the bottom of the top plate has to be replaced, it requires a special trick to mount it without twisting.

Use an ethanol squeeze bottle to add approx. 0.5 ml ethanol (c = 70%) to the O-ring groove.
Insert the O-ring in the groove and use your thumbs to force the ring into the groove.
Since the O-ring is wetted by the ethanol, it will not pop out any longer.



Step 2: Place the reactor in the fork of the stand and fix the bolt that secures the reactor.



Step 3: Mount the auxiliaries onto the head plate.
The head plate of the 500 ml MiniBio reactor contains welded inserts for:

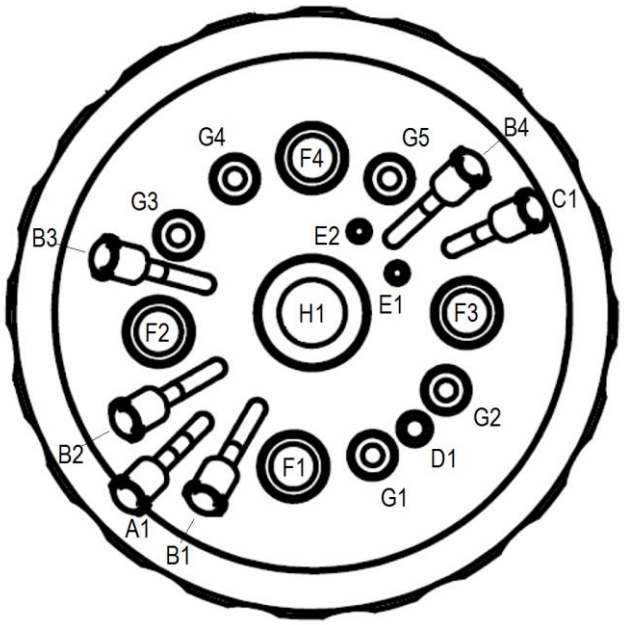
- Aeration (sparging)
- Sampling
- Additions (4x)

Also the temperature pocket is welded in the head plate.

This step is continued on the next page.

The ports and welded inserts of the head plate are listed in the table below:

Port #	Description
A1 (Luer Lock)	Sparger Pipe
B1 (Luer Lock)	Addition or Overlay Pipe
B2 (Luer Lock)	Addition or Overlay Pipe
B3 (Luer Lock)	Addition or Overlay Pipe
B4 (Luer Lock)	Addition or Overlay Pipe
C1 (Luer Lock)	Fixed Sample Pipe
D1	Temperature Pocket
E1	Contact Bus for Level Sensor
E2	Contact Bus for Level Sensor
F1	Sensor / Condenser Port M12 x 1
F2	Sensor / Condenser Port M12 x 1
F3	Sensor / Condenser Port M12 x 1
F4	Sensor / Condenser Port M12 x 1
G1	Universal Port M8x1
G2	Universal Port M8x1
G3	Universal Port M8x1
G4	Universal Port M8x1
G5	Universal Port M8x1
H1	Stirrer Port



One of the four addition ports may be used for gas overlay.
For Microbial applications, the lower end of the sparger pipe is left open or a jet sparger tip is used:
Z813180252 Jet Sparger Tip
For Cell Culture applications, the lower end of the sparger pipe may be equipped with a porous sparger tip:
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Assembling instructions

Make sure that an O-ring is present between the auxiliary and the head plate so as to ensure sterility.
Use the tools that come with the MiniBio reactor. Do not apply excessive force.

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- Stirrer assembly
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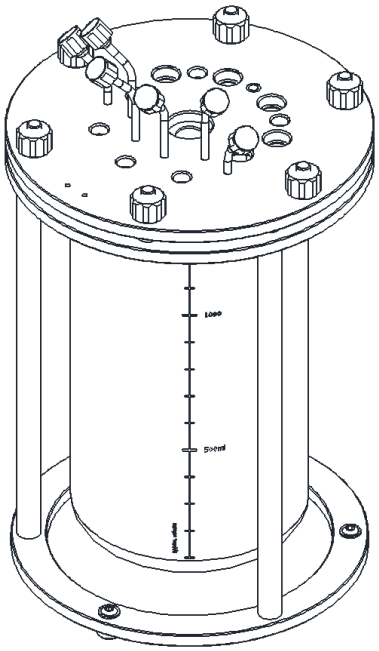
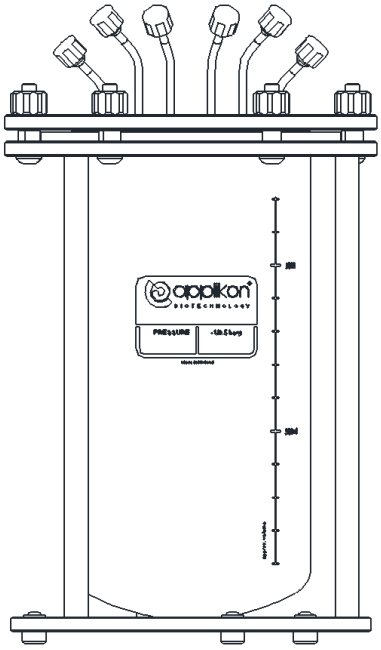
  - the pH sensor must be calibrated before it is mounted in the head plate (refer to section 4.2),
  - the optical LumiSens sensor for dO_2 measurements consists of two parts that stick together through a magnetic coupling. The Glass Tube with Sensor Tip and Threaded Magnetic Coupling must be mounted in the M12 x 1 port Head Plate port and be covered with the End Cap to prevent moisture entering the Glass Tube during autoclaving. The Sensor Head with electronics and Optical Fiber Tube is mounted later (after autoclaving). Refer to section 7.3.
- The M12 x 1 hose barb insert in the condenser port
- The temperature sensor in the thermometer pocket

3.2.3 1000 ML REACTOR

The "stand" for the 1000 ml MiniBio Reactor consists of an upper and lower support ring that are separated by three uprights.



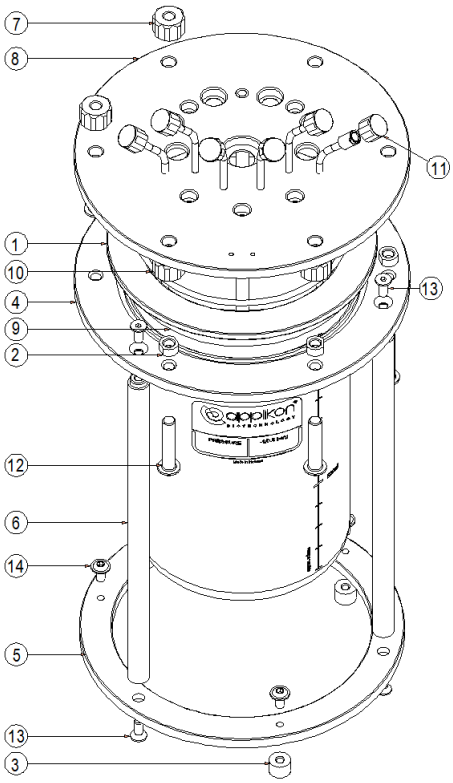
MiniBio Reactor 1000 ml
Isometric View



MiniBio Reactor 1000 ml
Side View

The table and exploded reactor view below show the different parts and pieces:

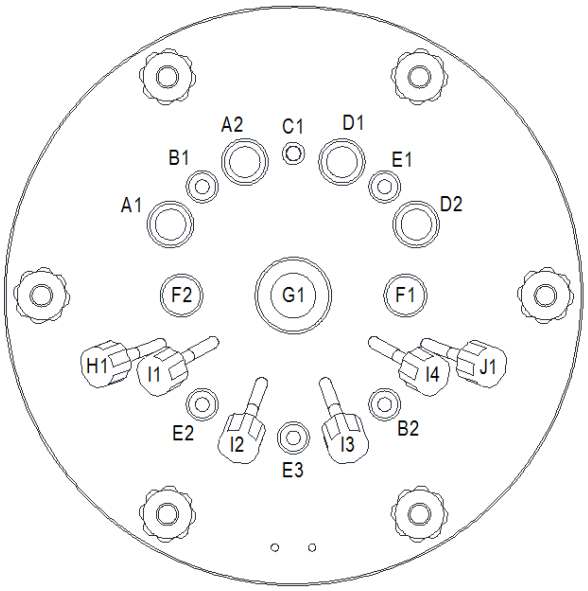
Pos.	Part#	Description
1	V3MP075021	Vessel Dished Bottom 1000 ml WV
2	V3MP072201	Clamping Ring – Top Plate Spacer M6
3	V3MP072191	Foot OD10 for 3-Stand Bottom Ring
4	V3MP070991	Clamping Ring ID105 SS316
5	V3MP070981	Bottom Ring ID120 for 3-Stand AISI316
6	V3MP070971	Stand for 3-Stand OD10 L=195
7	V3MA010111	Ribbed Nut D15 H10 M6
8	V3KP070181	Top Plate 1000 ml Reactor with Luer Locks
9	V1S4104X30	O-ring ID104.20X3.00 Silicone
10	V1S4095X26	O-ring ID94.92 x 2.62 Silicone
11	V0W0030100	Male Cap for Female Luer Lock PC
12	V0N0625130	M6X30 Hex. Sock. Button HD Cap Screw
13	V0N0433010	M4X10 Hex Sock. Countersunk Head Screw
14	V0N0425008	M4X08 Hex. Sock. Button HD Cap Screw



When assembling the 1000 ml MiniBio Reactor, the “stand” with the vessel must be prepared first.
Assemble the stand.
Make sure that O-ring 94.92 x 2.62 (position 10 in the exploded view image) is positioned around the vessel before it is placed in the stand. The O-ring will prevent the vessel from being damaged.
Assemble the reactor top plate according to the table and image below.

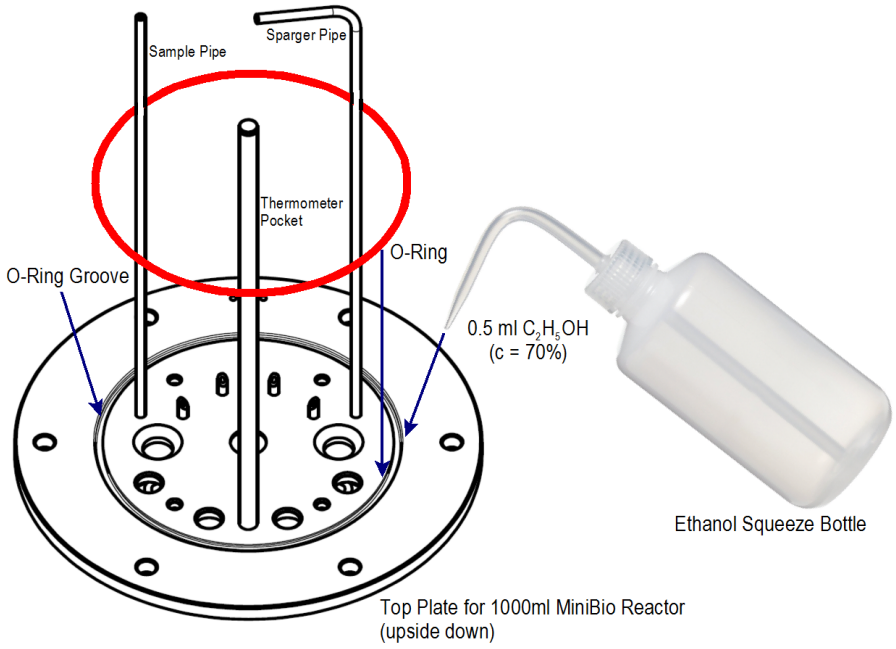
Layout and top view of the head plate:

Port #	Description
A1	Universal Port M12
A2	Universal Port M12
B1	Universal Port M8
B2	Universal Port M8
C1	Fixed Thermometer Pocket
D1	Universal Port M12
D2	Universal Port M12
E1	Universal Port M8
E2	Universal Port M8
E3	Universal Port M8
F1	Heat Exchanger Port
F2	Heat Exchanger Port
G1	Stirrer Port
H1 Luer Lock	Fixed Sample Port
I1 Luer Lock	Addition / Overlay Port
I2 Luer Lock	Addition / Overlay Port
I3 Luer Lock	Addition / Overlay Port
I4 Luer Lock	Addition / Overlay Port
J1 Luer Lock	Stirrer Port



Mounting instruction for the O-ring in the bottom of the top plate:
If the O-ring in the bottom of the top plate has to be replaced, it requires a special trick to mount it without twisting.

Use an ethanol squeeze bottle to add approx. 0.5 ml ethanol (c = 70%) to the O-ring groove.
Insert the O-ring in the groove and use your thumbs to force the ring into the groove.
Since the O-ring is wetted by the ethanol, it will not pop out any longer.



One of the four addition ports may be used for gas overlay.
For Microbial applications, the lower end of the sparger pipe is left open or a jet sparger tip is used:
Z813180252 Jet Sparger Tip
For Cell Culture applications, the lower end of the sparger pipe may be equipped with a porous sparger tip:
Z813180251 Porous Sparger Tip

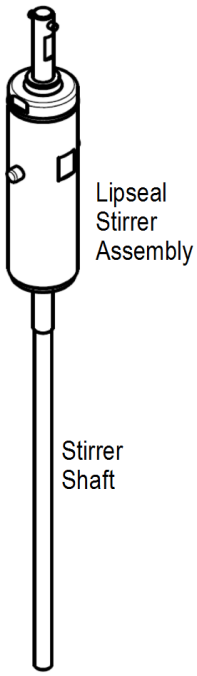
Assembling instructions

Make sure that an O-ring is present between the auxiliary and the head plate so as to ensure sterility.
Use the tools that come with the MiniBio reactor. Do not apply excessive force.

Mount the auxiliaries in the following order:

- Stirrer assembly
- Auxiliaries in the Universal M8 x 1 ports
- Sensors or sensor parts in the M12 x 1 sensor ports:
  - the pH sensor must be calibrated before it is mounted in the head plate (refer to section 4.2),
  - the optical LumiSens sensor for dO_2 measurements consists of two parts that stick together through a magnetic coupling. The Glass Tube with Sensor Tip and Threaded Magnetic Coupling must be mounted in the M12 x 1 port Head Plate port and be covered with the End Cap to prevent moisture entering the Glass Tube during autoclaving. The Sensor Head with electronics and Optical Fiber Tube is mounted later (after autoclaving). Refer to section 7.3.
- The M12 x 1 hose barb insert in the condenser port
- The temperature sensor in the thermometer pocket

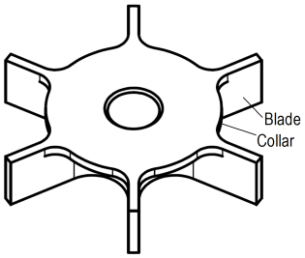
3.3 MOUNTING THE STIRRER SHAFT AND IMPELLERS



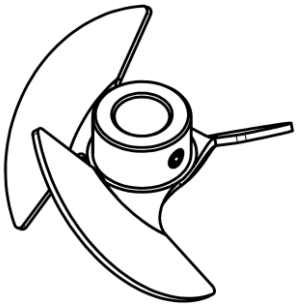
Loosen the Top Plate Ring in order to remove the glass reactor.

Insert the Stirrer Assembly in the head plate.
Before fixing the Stirrer Assembly, mount the impeller(s) on the Stirrer Shaft by using the Allen Key from the Start-Up Kit

Description	Part Number 250ml Reactor	Part number 500ml Reactor	Part number 500ml Reactor
Lipseal Stirrer Assembly	Z813150255	Z813150505	Z813151001
Marine Impeller("V"-bent blades)	Z813140261	Z813140511	Z813141011
Flat Disc Impeller (6 blades)	Z813130261	Z813130511	Z813131011



Flat Disc Impeller



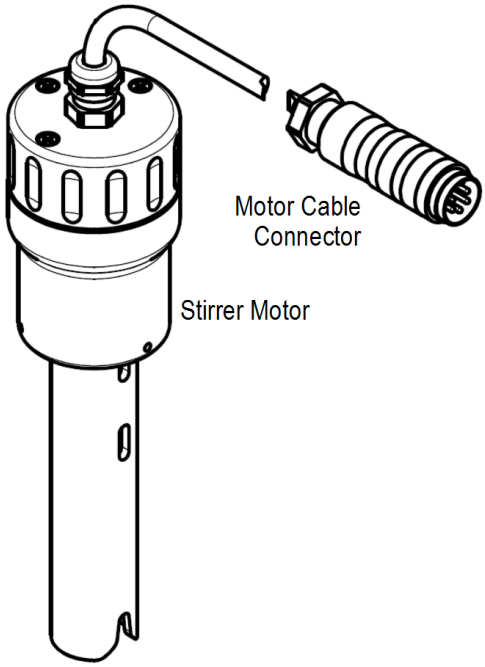
Marine Impeller

It is advisable to mount the Flat Disc Impeller with the collar facing upwards. In this way, the retention time of gas bubbles is increased, thereby improving the K_La-value.

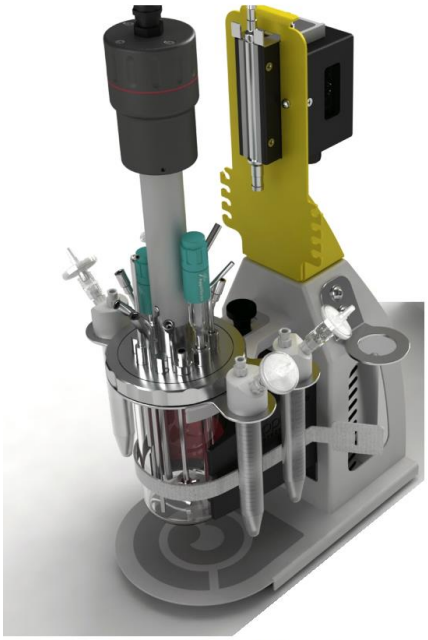
Experimental Oxygen Transfer Rate data can be found in the Hardware Manual, section 6.3.2.
The optimum Impeller Configuration is described in the Hardware Manual, section 6.3.3.

3.4 MOUNTING THE STIRRER DRIVE

When the Stirrer Assembly is mounted in the central port of the reactor head plate, the Stirrer Motor can be put on top of it. As soon as the motor starts turning, the fork coupling on the motor shaft will fit on the stirrer shaft.



When the bioreactor is not in operation, the motor can be fitted in the holder at the right hand side of the stand:



Stirrer Motor on top of
Stirrer Assembly

Stirrer Motor in Holder

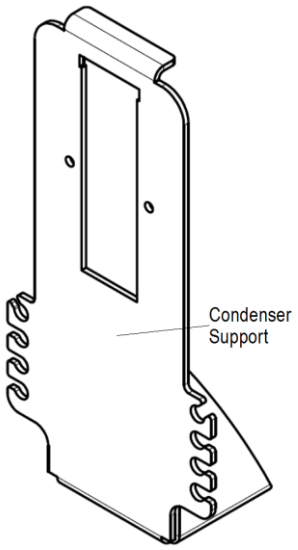
3.5 THERMO ELECTRIC CONDENSER

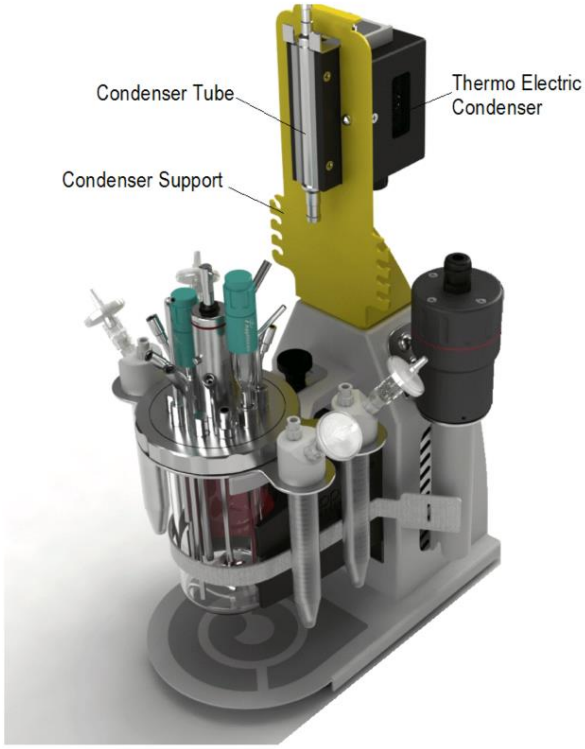
For microbial fermentations, a relatively large gas flow is used to aerate the bioreactor.
A Thermo Electric Condenser is therefore used to prevent excessive evaporation of the medium.
The condenser is connected to an analog output of the ***my***-Control.

Before starting the aeration of the medium, switch on the corresponding analog output manually. Advised output % = 70%!

3.5.1 CONDENSER MOUNTING FOR THE 250 AND 500 ML REACTOR

For the 250 and 500 ml Reactor, the Condenser is mounted in the Condenser Support that fits on top of the reactor Stand.





Condenser and Yellow Condenser Support mounted on Reactor Stand

Use a hose to connect the hose barb insert in the head plate to the lower end of the condenser tube.

Make sure that the length of the connecting tube is long enough to slide the condenser downwards (in order to remove it from the condenser holder).

Connect a length of tubing (size 25; ID = 3/16” or 4.6 mm) fitted with an Off-gas filter to the condenser outlet.

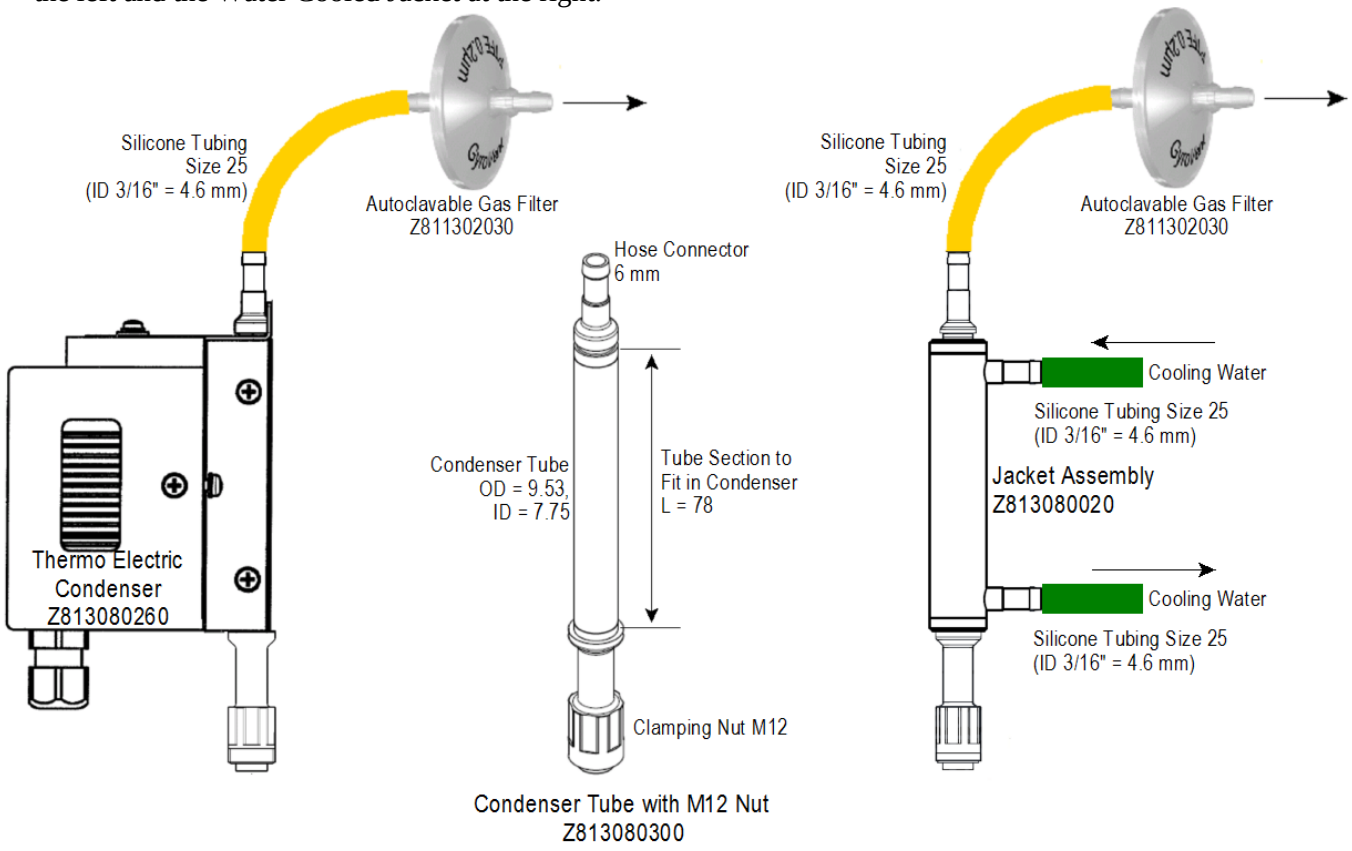
When autoclaving the bioreactor, the condenser tube must be removed from the tube holder. The condenser tube is a fragile part, so handle with care!

3.5.2 CONDENSER MOUNTING FOR THE 1000 ML REACTOR

The 1000 ml MiniBio Reactor is not equipped with a reactor stand.
Therefore, the Condenser Tube is equipped with a M12 clamping nut that can be mounted directly in the M12 Off-gas Port of the reactor.

The Condenser Tube can be cooled with either a Thermo Electric Condenser or with a Water Cooled Jacket.

The image below shows the Condenser Tube (center of the image) with the Thermo Electric Condenser at the left and the Water Cooled Jacket at the right.



The outlet side of the Condenser Tube is connected to an autoclavable gas filter by silicone Tubing size 25 (ID 3/16” = 4.6 mm).

4 pH AND dO_2 SENSOR PREPARATION

4.1 GENERAL

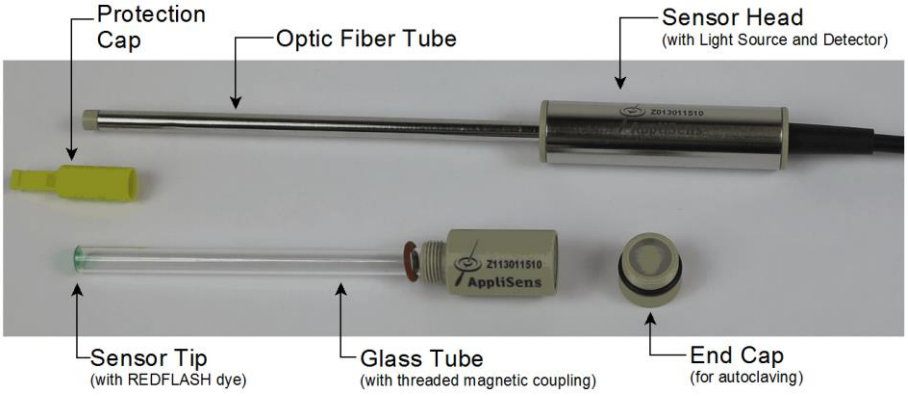
To measure the pH and dO_2 concentration during cultivation / fermentation, an invasive pH sensor and LumiSens sensor tip are used. As a result, these parts are mounted in the reactor head plate during autoclaving.

Since the sensitivity of the **pH sensor** must be calibrated with buffer solutions, this calibration routine must be performed before the sensor is mounted in the reactor head plate and the reactor is autoclaved.
The pH sensor calibration procedure is described in section 4.2.
Prepare the sensor in the following manner:

1. Remove the Watering Cap from the sensor.
2. Rinse the sensor with de-ionized water and blot it dry with a tissue.
3. Connect the pH sensor to the front of the ***my*-**Control.

The optical **LumiSens dO_2 sensor** consists of two parts:
The glass sensor tip with threaded magnet coupling (mounted in the reactor head plate and covered with the End Cap before autoclaving the bioreactor) and
The sensor head with optic fiber tube and sensor cable (stays outside the autoclave)

Parts of the LumiSens Optical dO_2 Sensor Assembly:



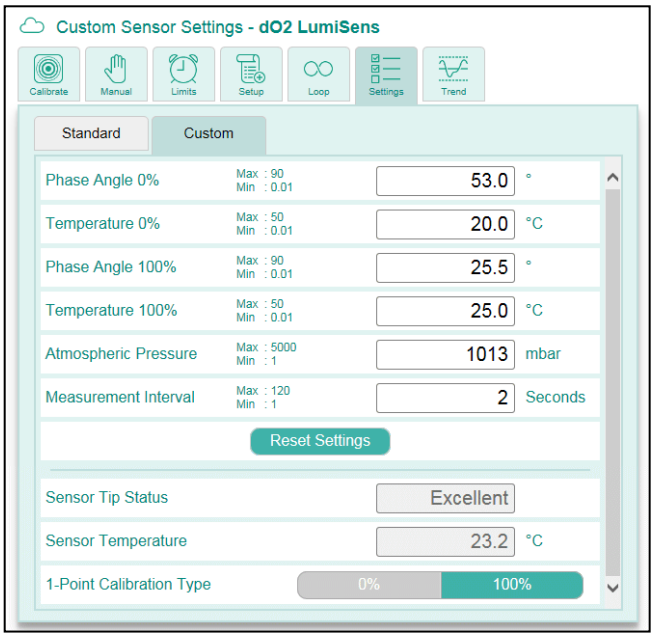
To the LumiSens sensor tip, a thin film of REDFLAH dye is applied. Exposing this dye to environmental light will slightly reduce the lifetime of the sensor tip.
The expected operational lifetime of the sensor tip = approx. 12 months.
In order to prevent unnecessary wear of the sensor tip, follow the instructions below:

- Store the LumiSens sensor / spare sensor tip in its box (with the protection cap on top of the sensor tip).
- In between runs (cultivations), the sensor tip must be covered with the protection cap.
- When the LumiSens sensor is connected to the controller, but not yet in use, the Sampling parameter must be disabled. As a result, the light source in the sensor head is not activated an the dye at the sensor tip is not irradiated.
  Before starting the dO_2 Control loop, the “Sampling” parameter must be enabled.

The LumiSens optical dO_2 sensor comes with a yellow plastic protection cap on the sensor tip at the bottom part of the glass tube. This cap protects the dye at the sensor tip (surrounding light will slightly reduce the lifetime of the sensor tip).
The glass tube is equipped with a threaded magnetically coupling that can be separated from the sensor head.
The sensor cable has a USB connector to fit in the USB port of the Applikon Controller.

4.1.1 LUMISENS CUSTOM SENSOR SETTINGS

The LumiSens Custom Sensor Settings screen contains the calibration data and the presentation of the sensor quality:

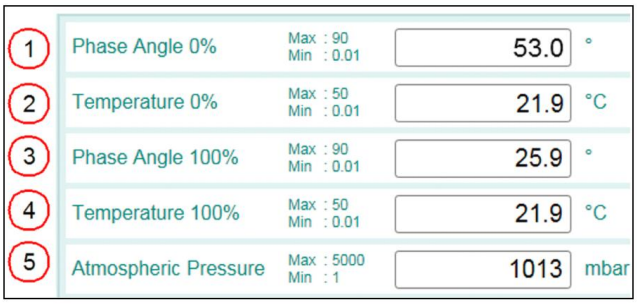


The Sensor Calibration settings are described in the next section.

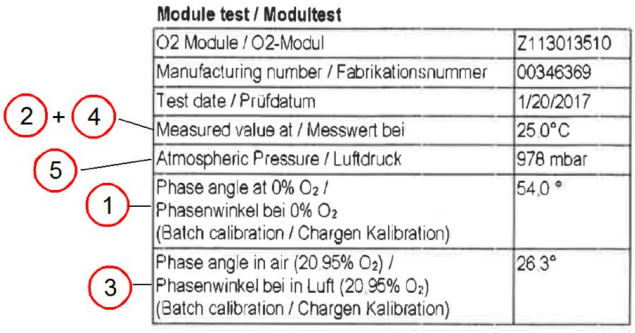
4.1.2 FACTORY CALIBRATION DATA FOR THE LUMISENS SENSOR TIP

The LumiSens sensor comes with its factory calibration data. These data (including the Atmospheric Pressure value) must be copied to the parameters at the Custom Sensor Settings tab.

The images below show the upper part of the Custom Settings list (left) and an example of the LumiSens test data sheet (right):



Sensor Calibration Settings



Part of the LumiSens Test Data Sheet

Make sure to enter the factory calibration data to the LumiSens Custom Sensor Settings at the my-Control. Basically, the LumiSens dO_2 sensor tip is now calibrated for the rest of its lifetime.

The sensitivity of the LumiSens sensor tip is not affected by autoclaving. It is therefore not necessary to calibrate the LumiSens sensor after autoclaving.

4.1.3 STATUS AND LIFETIME OF THE LUMISENS SENSOR TIP

The expected lifetime of the LumiSens sensor tip is approx. 12 months. During operation, the quality of the sensor tip is being reported in the LumiSens Custom Sensor Settings window. The sensor tip quality may be presented as:

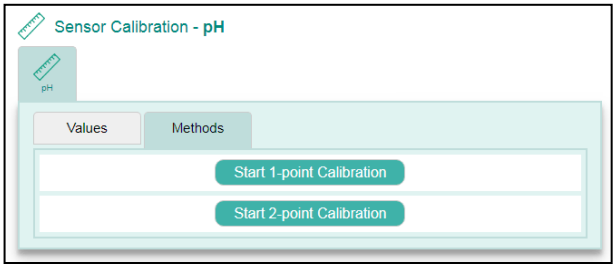
- Excellent,
- Good,
- Moderate or
- Bad.

If the sensor status is presented as “Moderate”, it is advised to prepare for sensor tip replacement (make sure that a new tip is available).
If the sensor status is presented as “Bad”, it is strongly advised to replace the sensor tip.

For additional information concerning the LumiSens sensor, refer to the LumiSens Optical dO2 Sensor Manual that comes with the ***my***-Control (part of the USB Sensor Manuals).

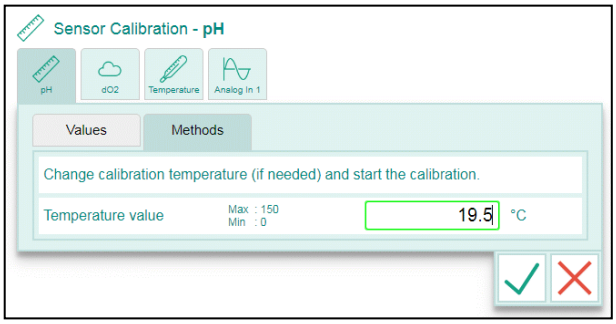
4.2 pH SENSOR CALIBRATION ROUTINE

For more details regarding the pH sensor calibration procedure, refer to the ***my*-Control** Software Reference Manual. It is advised to calibrate the sensor with two buffer solutions (pH = 7.00 and pH = 4.00). This means that a 2 point calibration is performed. The sequence of the buffer solutions is not relevant. It does not matter if buffer pH 4 is used as first or second buffer solution.



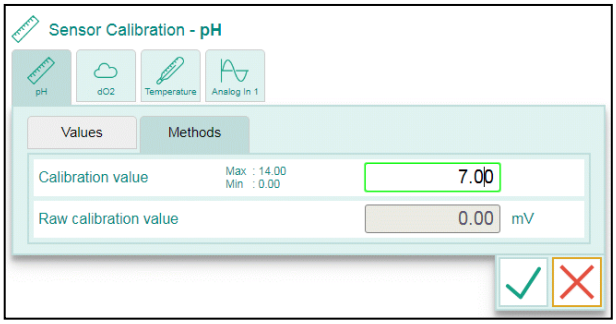
Step 1:
Open the pH Sensor Calibration window and select the Methods tab.

Press the Start 2-point Calibration button.

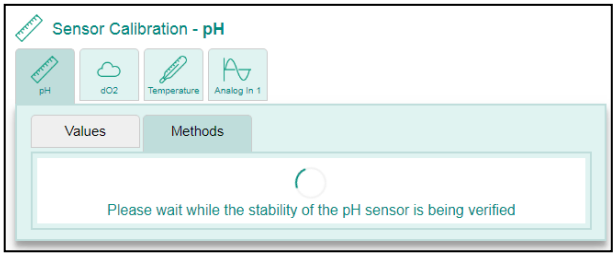


Step 2:
Enter the temperature of the first buffer solution.
The readings of the pH sensor are temperature dependent. Therefore, the temperature of the buffer solutions must be entered.

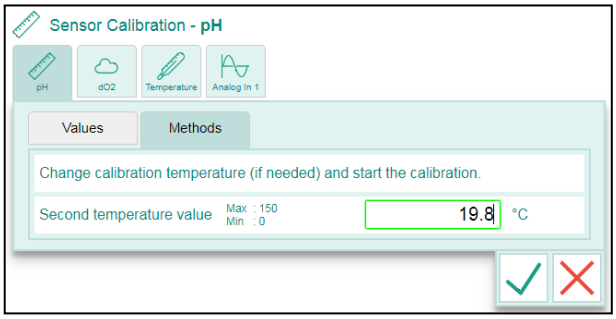
Type the numeric temperature value in the presented data field and press the ✓ button.



Step 3:
Enter the pH value of the first buffer solution (e.g. 7.00).
Rinse the pH sensor and carefully wipe it dry (do not rub). Immerse the pH sensor in the first buffer solution.
Type the numeric pH value of the first buffer solution in the presented data field and press the ✓ button.



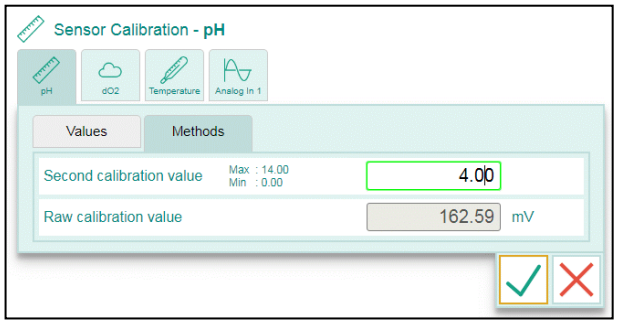
The sensor value will now be monitored to verify the stability.
As soon as a stable value has been obtained, the calibration routine will be resumed.



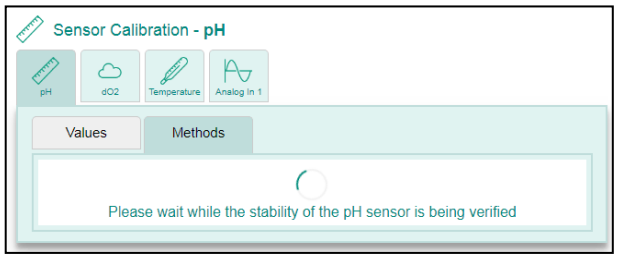
Step 4:
Enter the temperature of the second buffer solution.

Type the numeric temperature value in the presented data field and press the ✓ button.

Step 5:
Enter the pH value of the second buffer solution (e.g. 4.00).
Rinse the pH sensor and carefully wipe it dry (do not rub). Immerse the pH sensor in the second buffer solution.
Type the numeric pH value of the second buffer solution in the presented data field and press the  button.



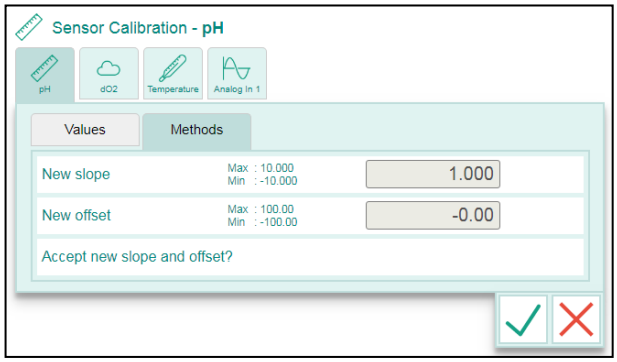
The sensor value will now be monitored to verify the stability.
As soon as a stable value has been obtained, the calibration routine will be resumed.



Step 6:
Finalizing the pH sensor calibration.
The calculated values for Slope and Offset are presented:

Slope = 1.000 *
Offset = -0.00 **

Press the  button to accept the presented calibration data.



* The presented Slope is the calculated Slope value divided by the theoretical Slope value (Nernst potential). Expected Slope value range: 0.95 . . . 1.05. If the calculated slope exceeds this range, the sensor must either be cleaned or replaced. Refer to the instructions in the sensor manual.
** The presented Offset is the calculated deviation at pH=7. Expected Offset range: -0.3 . . . +0.3. If the calculated offset exceeds this range, the sensor must either be cleaned or replaced. Refer to the instructions in the sensor manual.

The calibration routine can be cancelled at any time by pressing the  button.

Mounting the pH sensor
Mount the calibrated pH sensor in the head plate.

5 DOSE MONITOR INITIALIZATION

5.1 GENERAL

The actuators that are used for the addition of liquids and/or gasses can be monitored, using the Dose Monitor function. The Actuator Factor of the configured actuators (pumps, valves or mass flow controllers) need to be calibrated in order to convert their “On time” or “controller output %” into “volume”.

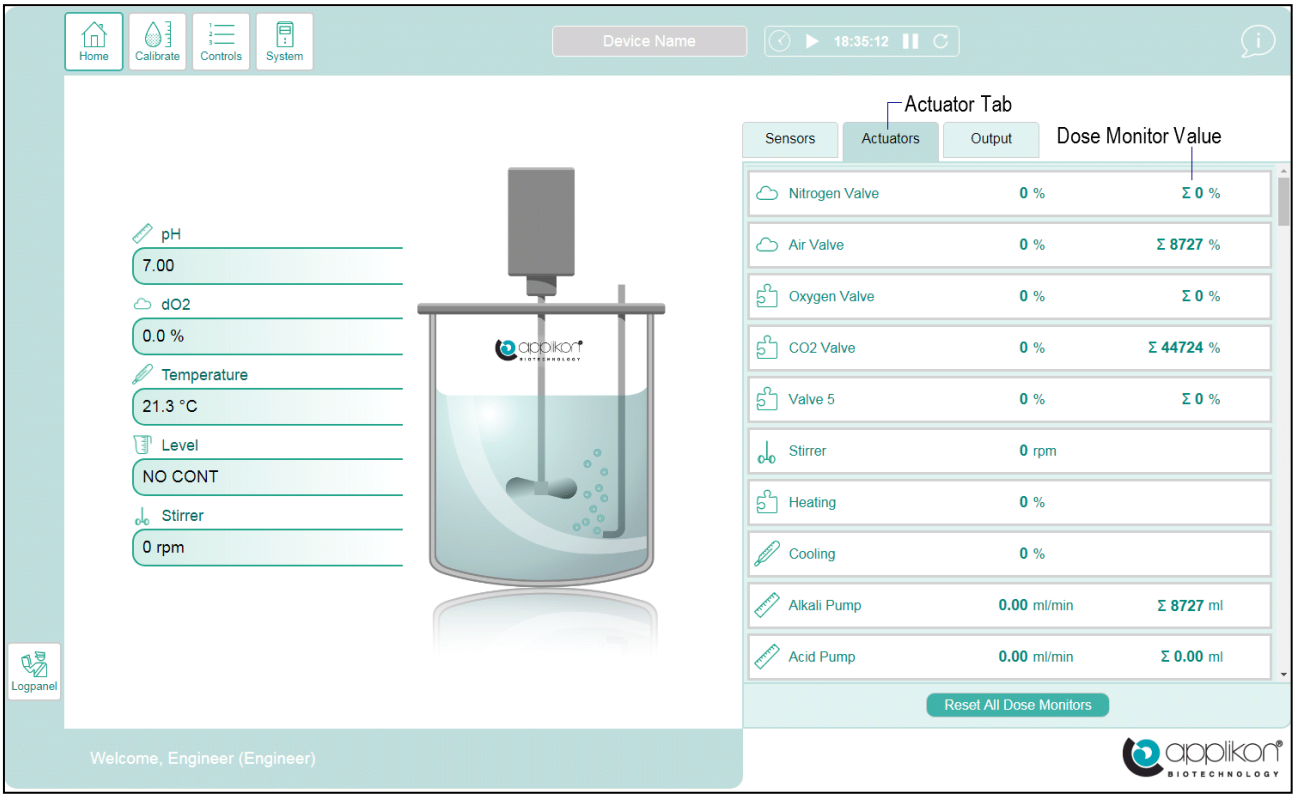
This procedure needs to be carried out once after changing the actuator hardware (like pump tubing, etc.). When a configured actuator has not yet been calibrated, the corresponding actuator factor is 100% or 1 ml/min (depending on the type of actuator).

Calibrating the actuator factor is described in the Software Reference Manual, section 5.3.1.

Also, make sure that the Dose Monitor function has been enabled for the individual actuators. Refer to the Software Reference Manual, section 5.3.2.

5.2 DISPLAY VALUES

Press the Actuators tab in the Home screen. The actuator status including the integrated Dose Monitor values are displayed:



Press the Reset All Dose Monitors button at the bottom of the Actuator section to set all Dose Monitor values to 0.

6 AUTOCLAVING

The assembled Bioreactor should be autoclaved before cultivation in order to create a sterile environment inside the reactor.

Filling the bioreactor

- Fill the bioreactor with culture medium.
  Do not exceed the working volume of the reactor. See the ***my***-Control Hardware Manual, Chapter 6,"Reactors and Auxiliaries". Be sure to leave enough space for additions after sterilization (e.g. inoculum, separately sterilized nutrients, etc.).

Proper Thermo-Conductivity when the bioreactor is autoclaved without medium

In case the reactor is autoclaved without medium, add a small amount of water to the reactor before it is autoclaved (this will improve the heat transfer inside the reactor).
Advised volumes: 5 ml in a 250 ml reactor, 10 ml in a 500 ml reactor.
Even when a small amount of water is added to the empty reactor, it is strongly advised to extend the autoclaving interval to make sure that the inside surface of the empty reactor is autoclaved properly!

Sensors

- Insert the Level sensor as far as possible into the vessel and fasten it. After autoclaving, the position of this probe can be adjusted upward to the desired height.
- Disconnect the pH sensor cable. Cover the sensor connector with the corresponding screw cap. Make sure that the rubber gasket is in place between the sensor connector and the cap.
- Make sure to remove the LumiSens sensor head and optic fiber tube from the sensor tip and to cover the sensor tip with the End Cap (to prevent moisture getting in contact with the sensor tip during autoclaving).

Condenser

- Remove the condenser tube from the holder by releasing the spring grip and sliding the condenser downwards until it is removed from the holder.

Stirrer motor

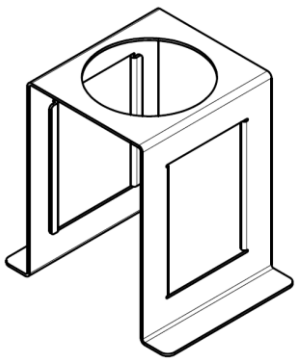
- Lift the Stepper Stirrer Motor from the Stirrer Assembly and place it in the holder at the right hand side of the Reactor Stand.

Pump tubing

- Remove the pump tubing from the pump heads. Open the cover of the pump head and remove the tubing from it. The liquid vials and tubing are autoclaved together with the reactor.

Bioreactor assembly (250 and 500 ml)

- Remove the bioreactor from the stand and place it, including the vials and tubing, in the Autoclave Frame that comes with the reactor.
- Place the autoclave frame together with the bioreactor in the autoclave.



Bioreactor assembly (1000 ml)

- The 1000 ml reactor, including the feed bottle that is fixed to the bottle holder at the stand, is autoclavable.
- Place the bioreactor including Feed Bottle(s) in the autoclave.

Autoclaving considerations

- Use appropriate tubing and filters for both air inlet and outlet. To avoid wetting of the inlet filter during autoclaving, use a clamp to close the tubing between the filter and the head plate.
- Close all other connections (except the air outlet) air-tight with a hose and a hose clamp.
- Close all open tubing ends with cotton and cover the ends with autoclavable foil or paper.
- Make sure that the air outlet of the bioreactor is open, because pressure differences during autoclaving may damage the reactor or the sensors.
  Use the air outlet filter to maintain pressure equilibrium inside and outside the reactor.

Autoclaving

- Place the autoclave support with the bioreactor(s) and all accessories in an autoclave.

CAUTION
Do not put the stirrer motor or the heating blanket in the autoclave.

- After the reactor is heated up, the autoclave should stay at 121 °C for at least 20 minutes in order to kill all organisms and thermo-resistant spores. In case of autoclaving an empty reactor, it is advised to extend the 20 minutes interval considerably.
- Do not open the autoclave.
  Let the autoclave cool down until the temperature in the autoclave has dropped below 90 °C.
  After reaching that temperature, it can be opened to allow it to cool down further.

  This cooling procedure should be performed to avoid low pressure in any part of the reactor system. Low pressure in tubing might result in contamination when the tube is connected to a peripheral device.

- After cooling down and removing the reactor from the autoclave, the Luer lock connectors at the head plate of the reactor must be tightened (they may have come loose by heating up).

Luer lock connectors are available in a wide range of price and quality. The Luer lock connectors that are supplied by Applikon, have been selected on low expansion coefficient. Therefore, during autoclaving, the Luer lock connectors that have been supplied by Applikon will hardly come loose.

6.1 RELATION BETWEEN STERILIZATION TEMPERATURE AND TIME

The relation between sterilization temperature and time interval is given by the **F_0**-equation.
This equation describes **F_0** that is defined as **the equivalent exposure time at 121 ºC at variable temperatures, calculated for an ideal micro-organism with a temperature coefficient of destruction equal to 10**.

Equation:

$$F_0 = \Delta t \times 10^{\frac{T - 121}{10}}$$

Where : F_0 = the equivalent exposure time in minutes
Δt = reference sterilization time (e.g. 20 minutes)
T = temperature variable
121 = sterilization reference temperature
10 = temperature coefficient of destruction

Using this equation, the equivalent exposure time at other temperatures can be calculated.
Example: Autoclaving 20 minutes at 121 °C corresponds with autoclaving 8 minutes at 125 °C:
The F_0 value for sterilizing 20 minutes at 121 °C = 20 ($F_0 = 20 \times 10^{0/10} = 20 \times 1 = 20$).
For sterilizing the medium at 125°C, the Δt value can be calculated according to the expression:

$$\Delta t = \frac{F_0}{10^{\left[\frac{(125 - 121)}{10}\right]}}$$

Since the F_0 value = 20, the expression gives a Δt value of approx. 8 minutes.

7 PREPARING CULTIVATION

After autoclaving the Bioreactor (and its liquid addition bottles, etc.), the system must be reassembled.

7.1 INSTALLATION

- Mount and assemble the MiniBio reactor in its reactor stand, alongside the ***my*-**Control. Refer to the assembly instructions in chapter 3 of this manual.

- In the case of microbial fermentations, the thermo electric condenser will be used in order to decrease evaporation of the medium.
  Slide the condenser tube upwards in the tube holder of the condenser assembly and secure the condenser with the spring.

- Connect the pH sensor cable to the corresponding sensor.

- Remove the End Cap from the LumiSens Sensor Tip and insert the LumiSens Optic Fiber Tube into its Sensor Tip that has already been mounted in the Head Plate.

- Make sure that the ***my*-**Control is switched on, but all control loops are switched off.

The dO_2 measurement is based on the polarographic principle (Clark-cell). Therefore, the sensor must be polarized for at least 4 hours before it can be calibrated (as described in the following sections.

- If used, wrap the heating blanket around the reactor.

- Hook up the aeration outlet(s) (sparger / overlay) of the ***my*-**Control to the air inlet filter(s) of the reactor.

Use soft tubing (e.g. silicone or norprene) for this connection.
Hard tubing like PUN that is often used for air is not flexible enough and may lead to loose connections.

- Mount the pump tubing of the different additions in the pump head at the front of the ***my*-**Control.

- Insert the Pt-100 sensor into the thermometer pocket.
  In order to improve heat transfer between the thermometer pocket and the Pt-100 sensor, fill the thermometer pocket with water or silicone oil. This will decrease the dead time of the sensor, making temperature control more accurate. When operating at higher temperature, silicone oil has the advantage over water of a lower vapour pressure (less evaporation).

- Connect the level control cable with the jacked end to the level sensor and the other end to one of the two 2-mm connection holes in the reactor stand. Remember (previous section) that the sensor was pushed all the way into the reactor. Now the level sensor must be carefully and slowly pulled up until its lower (sensing) tip reaches the desired height for level detection. Do not push the level sensor back into the reactor since this may cause contamination!

7.2 PREPARING FOR OPERATION

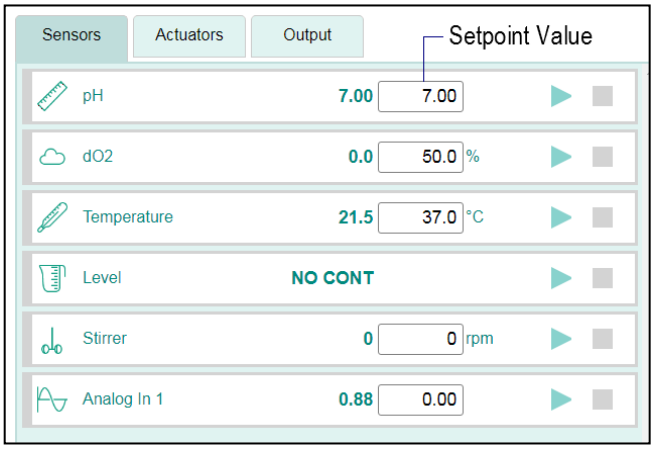
After connecting all cables and tubing, the set-points of the pH, temperature and stirrer speed control loops should be adjusted to the desired values.

Set points:

- Press the 

button of the ***my***-Control to display the Home screen and select the Sensor tab at the right side of the screen.

- Press the setpoint value field of a control loop. Enter the setpoint value that is required for this control loop for the new fermentation / cultivation.



- Repeat this step for all other configured control loops.

- Switch on the Temperature control loop by pressing its  button. The horizontal Process Parameter bar will turn green (

).

- When medium temperature has reached setpoint, start the pH control loop. The pH parameter bar will turn green as well.

- When temperature and pH value are stabilized at set-point and the dO_2 sensor has been polarized for at least 4 hours, the dO_2 sensor can be calibrated: see Section 0.

  Note: polarization of the dO_2 sensor starts as soon as the sensor is connected and the controller is switched on.

7.3 CALIBRATING THE dO_2 SENSOR (ONLY FOR CLASSIC SENSORS)

If the optical LumiSens dO2 sensor is used for measuring the dissolved oxygen concentration, this section can be omitted (the sensor sensitivity is not affected by autoclaving).

If a classic (polarographic) dO_2 sensor is used, the sensor must be calibrated before starting a new cultivation or fermentation (autoclaving may affect the characteristics of the gas-permeable membrane of the sensor).

Before starting the calibration of the classic dO_2 sensor, make sure that it has been polarized for at least 4 hours. Polarizing the sensor will start as soon as the sensor is connected to a powered up my-Control.

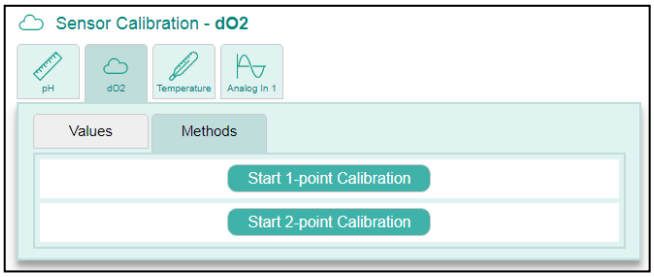
The calibration procedure below describes a 1-point calibration on air saturation (commonly used). If a 2-point sensor calibration is required, refer to the Software Reference Manual.

The following procedure uses air for calibrating the dO_2 sensor.
For highly aerobic cultivations, the dO_2 sensor can be calibrated with oxygen instead of air. In this case, the measurement range (dO_2 Sensor Setting) must be set to oxygen. The calibration routine remains the same. However, in this case the reactor must be saturated with oxygen.

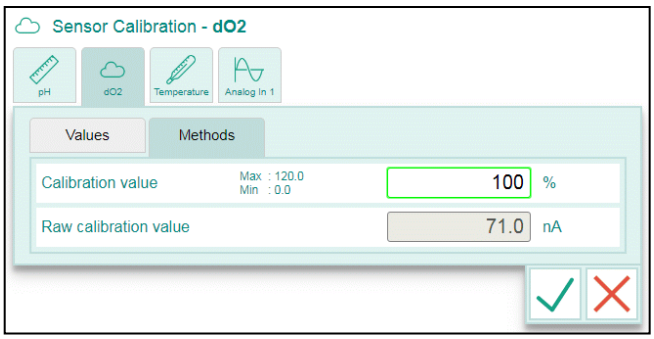
Since the dO_2 sensor calibration is performed at process temperature, the sensor temperature compensation (also a dO_2 Sensor Setting) can be disabled.

Step 1:
Open the dO_2 Calibration routine and select the Methods tab.

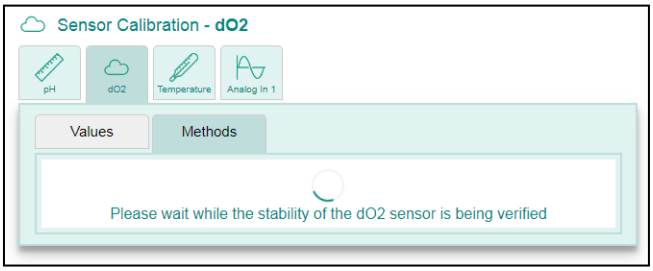
Press the Start 1-point Calibration button.



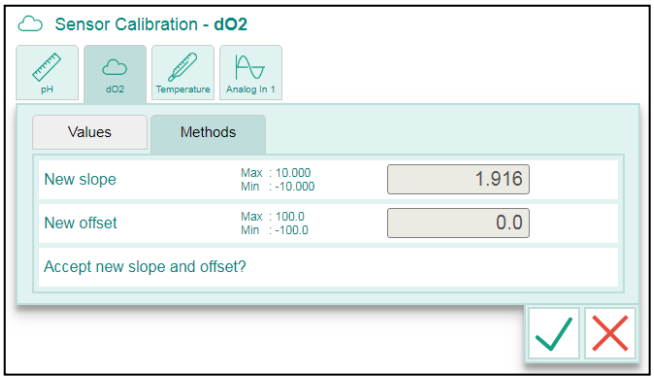
Step 2:
The calibration value can be entered.
If the medium is saturated with air (or oxygen, depending on the application), enter the value 100.



The sensor value is monitored to verify the stability. As soon as a stable value has been obtained, the calibration routine will be resumed.



Step 3:
The obtained slope value for the dO_2 sensor is presented (the offset will be set to 0.00).



Press the ✓ button to accept the presented calibration data (or press the ✗ button to reject the values).

The 1-point calibration routine is now completed.

The calibration routine can be aborted at any time by pressing the ✗ button.

The expected slope value of the sensor (for measurement range for air) is**:**
2.0 to 4.0 at 25 °C.
1.6 to 3.3 at 37 °C,

Verification of the quality of the dO_2-sensor
If nitrogen gas is available, it is advised to perform a dO_2 sensor quality check.

- Close the air inlet valve and purge the bioreactor with nitrogen for approx. 20 – 30 minutes. All oxygen will be driven out of the bioreactor.
- Monitor the value of the dO_2 sensor.
- If this value does not drop below 0.5% air saturation, the sensor requires maintenance before the next fermentation / culture is started.
- It is advised to record the measured value for future reference (in this way, preventative maintenance can be anticipated).

After finalizing the dO_2 sensor verification, close the N_2 valve and start the dO_2 control in order to create optimum process conditions.

When all control loops are at set point, the reactor system is ready for cultivation (inoculation).

7.4 RESETTING DOSE MONITOR VALUES

All Dose Monitor values should be reset to 0 ml.

Return to the Home screen and press the Actuator tab. Press the Reset All Dose Monitors button at the bottom of the Actuator section to set all Dose Monitor values to 0.

8 CULTIVATION

After proper preparations, the reactor is ready for cultivation. The first step is creating an optimum environment in the reactor.
In case the control loops are not running yet, return to the Home screen and start the relevant control loops.

8.1 FIXED PID CONTROL VERSUS ADAPTIVE CONTROL

The pH, Temperature and dO_2 control loops of the ***my***-Control can be set to:

- Control with preset PID parameters
- Adaptive (automatic) control

For information concerning the controller settings and optimizing the control parameters, see the ***my***-Control PID Control Manual.

8.2 INOCULATION

Inoculation can be performed in several ways. Two commonly used methods are described here.

1. Fill a sterile flask, to which a sterile hose is connected, aseptically with inoculum.
   The other end of the hose should be connected to a sterile needle.
   Turn off aeration, pierce the septum and transfer the inoculum to the reactor by gravity feed or by using a pump.

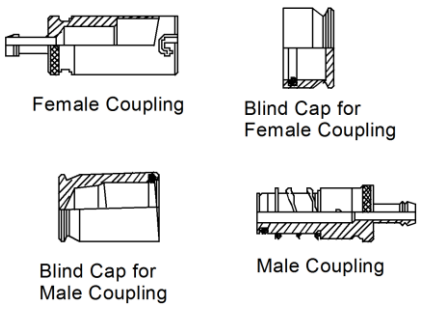
   Just before inoculation, the septum must be disinfected with ethanol. The needle must be sterilized in a flame.

2. Fill a sterile syringe with inoculum.
   Pierce the septum and inject inoculum into the reactor (this method is suitable for inoculum volumes smaller than 100 ml).

Other methods of inoculation (like discussed in the next section) can be used as long as they are performed aseptically.

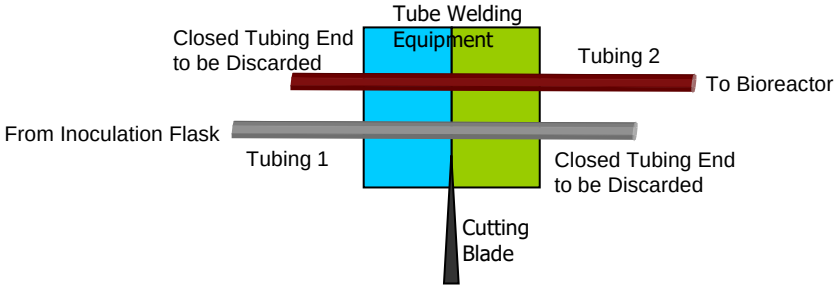
The autoclavable Rapi-Loks are a fast and reliable way to make or break tubing connections during a fermentation or cell culture process.

Refer to the ***my***-Control Hardware Manual, section “Rapi-Loks Sterile Connector”.

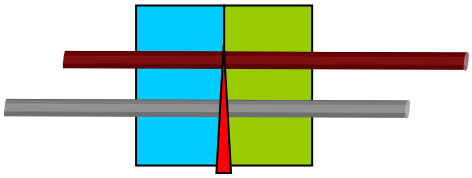


8.2.1 TUBE WELDING

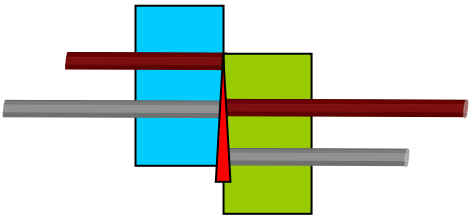
Like other additions, adding the inoculum must be carried out aseptically. An easy and safe way to make an aseptic connection of tube ends is the so-called “Tube Welding” technique.
This technique is explained schematically below.



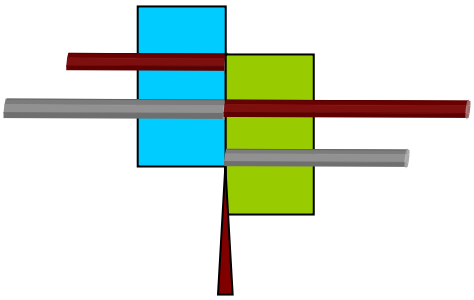
Step 1: Installation of tubing in welding equipment



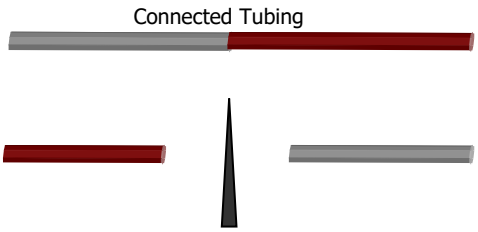
Step 2: Blade heats up and cuts both pieces



Step 3: Alignment of tubing; blade is still hot

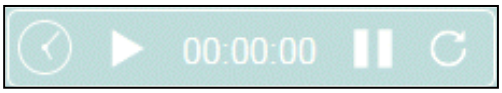


Step 4: Blade returns to start position; connection is made



Step 5: Discard blade and tubing ends

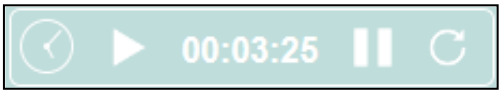
8.3 STARTING THE PROCESS TIMER



To be able to keep track of the process duration, the Process Timer must be started immediately after inoculating the bioreactor. Return to the Home screen and press the  button of the Process Timer.

Pressing the Run icon () will start the timer. Pressing the Reset icon () will reset the presented Process Time.

Example of a running Process Timer:



Pressing the Pause icon () will hold the running Process Timer.

8.4 SAMPLING

Two types of sample systems can be used with the MiniBio Reactors:

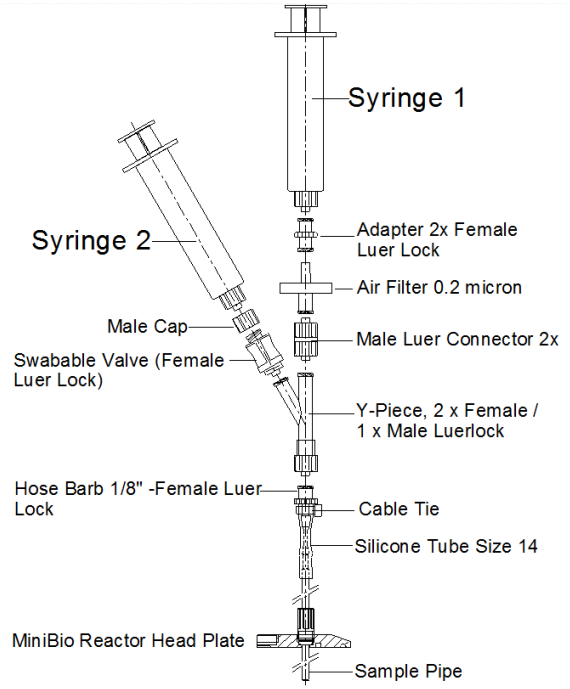
- Single Use Sample System and
- Reusable Sample System.

The **Single Use Sample System** is fixed on top of the Sample Pipe. Before sampling, a sterile Syringe 2 is mounted on top of the swabable valve.

Before mounting Syringe 2, clean the syringe and swabable valve with 70% ethanol in order to create a sterile connection!

Syringe 1 is mounted (with withdrawn plunger) on top of the air filter. Push the plunger of Syringe 1 to remove medium from the sample pipe.
Withdraw the plunger of Syringe 2 to extract a sample from the reactor.
Remove Syringe 2 from the Sample System and clean the Syringe and swabable valve with 70% ethanol.

For information concerning the parts of the Single Use Sample System, refer to the Hardware Manual.



The **Reusable Sample System** uses exchangeable sample vials.

Make sure to cut-off the sample tube (approx. 1 cm below the bottom of the cap).

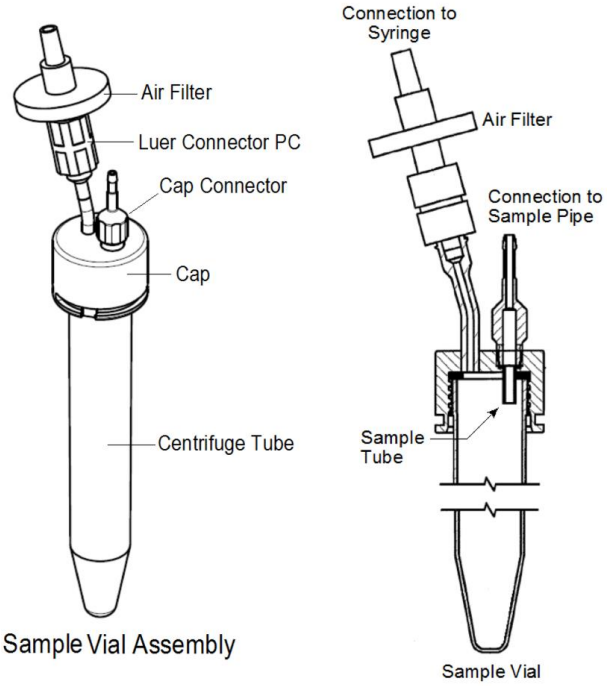
Connect a syringe to the air filter (using a piece of additional tubing).
Connect the sample tube side of the assembly with the sample pipe in the MiniBio Reactor.

During cultivation, the sampling system can be operated using the syringe:

- Air from the syringe can be forced though the filter to empty the sample pipe and tubing.
- Sample can be drawn through the sample pipe and tubing into the sample vial.
- The sampling action must be completed by using the syringe to force the content of the sample pipe and tubing back into the reactor.

After sampling, the filled sample vial can be replaced by a (sterile) new one in a sanitary manner.

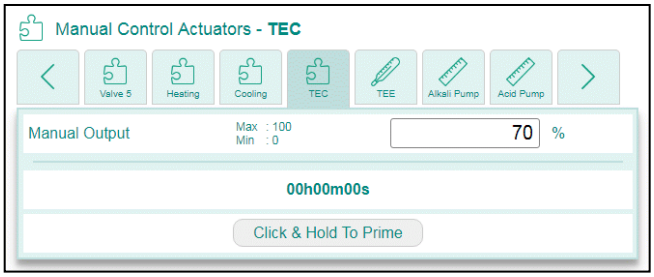
For information concerning the parts of the Single Use Sample System, refer to the Hardware Manual.



8.5 SWITCHING OFF THE THERMO ELECTRIC CONDENSER

For microbial fermentations, the Thermo Electric Condenser may be used.
This condenser can best be switched off before the bioreactor is dismantled.

Open the Manual Actuator Control window (Controls, followed by Manual) and select the TE Condenser.
Set the output % to 0.



Press the button to confirm the setting.

8.6 DECONTAMINATION AND HARVESTING

Depending on the type of organism in the broth, it may be necessary to decontaminate the bioreactor and its auxiliaries (e.g. by sterilizing it again). Refer to the relevant Standard Operating Procedures.

After decontamination, the reactor can be harvested. Refer to the relevant Standard Operating Procedures.

9 CLEANING

After finishing the fermentation or cultivation process, the glass and stainless steel parts of the reactor should be cleaned thoroughly as follows:

Cleaning the sensors

- pH sensor:
  Remove pH sensor from the reactor head plate. Detach the sensor cable and rinse the sensor with warm tap water (max. temp = 60°C). Make sure that all broth residue is removed. After cleaning, the sensor can be stored in a KCl (c=3M) solution.
  For sensor maintenance instructions (in case of sulphide or protein contamination), refer to the User Manual that comes with the sensor.
- Optical LumiSens dO_2 sensor (standard dO_2 sensor):
  Remove the LumiSens sensor head from the sensor tip and leave it near the my-Control. Remove the sensor tip from the reactor head plate and cover the tip with the End Cap (make sure that no moisture can enter the glass tube). Clean the outside of the sensor tip with warm tap water (max. temp = 60°C).
  Do not rub the sensitive tip. Carefully dip the sensor tip dry with a soft tissue. Cover the sensor tip with the yellow protection cap or store it in the closed sensor box.
- Classic dO_2 sensor (optional dO_2 sensor):
  The classic dO_2 sensor must be removed from the reactor head plate. Detach the sensor cable and rinse the sensor with warm tap water (max. temp = 60°C). Make sure that all broth residue is removed. After cleaning, the sensor can be stored dry.
  Cleaning the optical LumiSens dO_2 sensor

Cleaning the reactor

1. Fill the reactor with a NaOH (c = 0.1M) solution.
2. Activate the stirrer. Visually check for the dissolution of foam, debris and other contamination in the reactor. This cleaning takes about 30 minutes.
3. Drain the reactor.

Disassembling the reactor

Disassemble the MiniBio reactor according to the instructions that are given in chapter 3 (but in reversed order).

Cleaning all parts

Clean all parts by using hot water, 70% ethanol or other suitable cleaners. Allow all parts to dry.

Cleaning the porous sparger tip

Depending on the type of medium that is used (presence of proteins and/or peptides), the cleaning of the porous sparger tip may require a special procedure.

1. Remove the sparger tip from the air inlet pipe.
2. Soak the sparger overnight in a solution of pepsin (c = 10 mg/ml) / HCl (c = 0.01M).
3. Use ultrasonic cleaning with water and / or ethanol.
4. Store the sparger tip dry until the next run.

Reassembling the reactor

1. Assemble and mount the head plate (see chapter 3).

Take care not to damage or forget any O-rings, since this can cause contamination during the next run. It is advisable to replace the O-rings of all auxiliaries twice a year.