



my-Control for the AppliFlex ST Single Use Reactor 3L



OPERATOR MANUAL

Software Version mE.2.9.X; Document Version 1.2

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1 SAFETY

1.1 SAFETY SYMBOLS

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

**WARNING**

Important issue. 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 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 the fuse rating is appropriate for the line voltage (for fuse details, refer to section 3.2 of the Hardware Manual).
- This equipment is 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 apparatus may still be charged, even if the apparatus has been disconnected from all voltage sources.
- Any adjustment, maintenance and repair of the opened device under voltage must be avoided as far as possible and, if inevitable, the activities must only be carried out by qualified personnel.
- Make sure that only fuses with the required rated current and of the specified type (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 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 1.0mm² wires (for the USA, the power cord complies with 18AWG which is equal to 3 x 0.82mm² 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.
- the same (or better) specifications than the original.

**WARNING**
Do not use flammable substances in the vicinity 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 vicinity 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.

**Standard Operating Procedures**

- Preparing and operating the AppliFlex ST Single Use Bioreactor requires aseptic procedures: working in a Biological Safety Cabinet (BSC), use of Luer Lock couplings or C-Flex tubing plus welding equipment, etc.
- For specific information concerning this subject, refer to this Operator Manual or to the Standard Operation Procedures that are applicable in your lab.

**WARNING**

Sensor is fragile. **Handle with care!**

- For measuring the dissolved oxygen concentration in the bioreactor, the LumiSens Optical dO₂ sensor is used. This sensor is fragile; the replaceable LumiSens sensor tip is made of glass and can break easily.

**CAUTION**

Single Use Bioreactor

- The AppliFlex ST Single Use Bioreactor is intended for “single use” only. Applikon is not liable in case of damage caused by multiple use of the reactor!

2 GENERAL

2.1 GENERAL INTRODUCTION

The AppliFlex ST Single Use Reactor Systems basically consist of the following parts:

- an AppliFlex Stirred Tank Single Use Reactor with the appropriate auxiliaries like a stirrer assembly, sensors, an aeration assembly, etc.
- a *my-Control* control console for measurement and control of process variables (pH, temperature, dO₂, 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.

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:

HMI 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 calling its IP-address.

Start the Internet Browser at the HMI-device and surf to the following address: <http://IP-address/>. The *my-Control* HMI will be displayed.

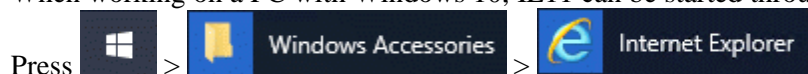
***The IP-address of the *my-Control*. The *my-Control* comes with a preset IP-address. This address can easily be customized. Refer to section 1.3 and Appendix A of the Software Reference Manual.**

Refer to Appendix D of the software manual in order to prevent instabilities in the network connection when the *my-Control* is the only controller directly connected to its controlling PC.



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.



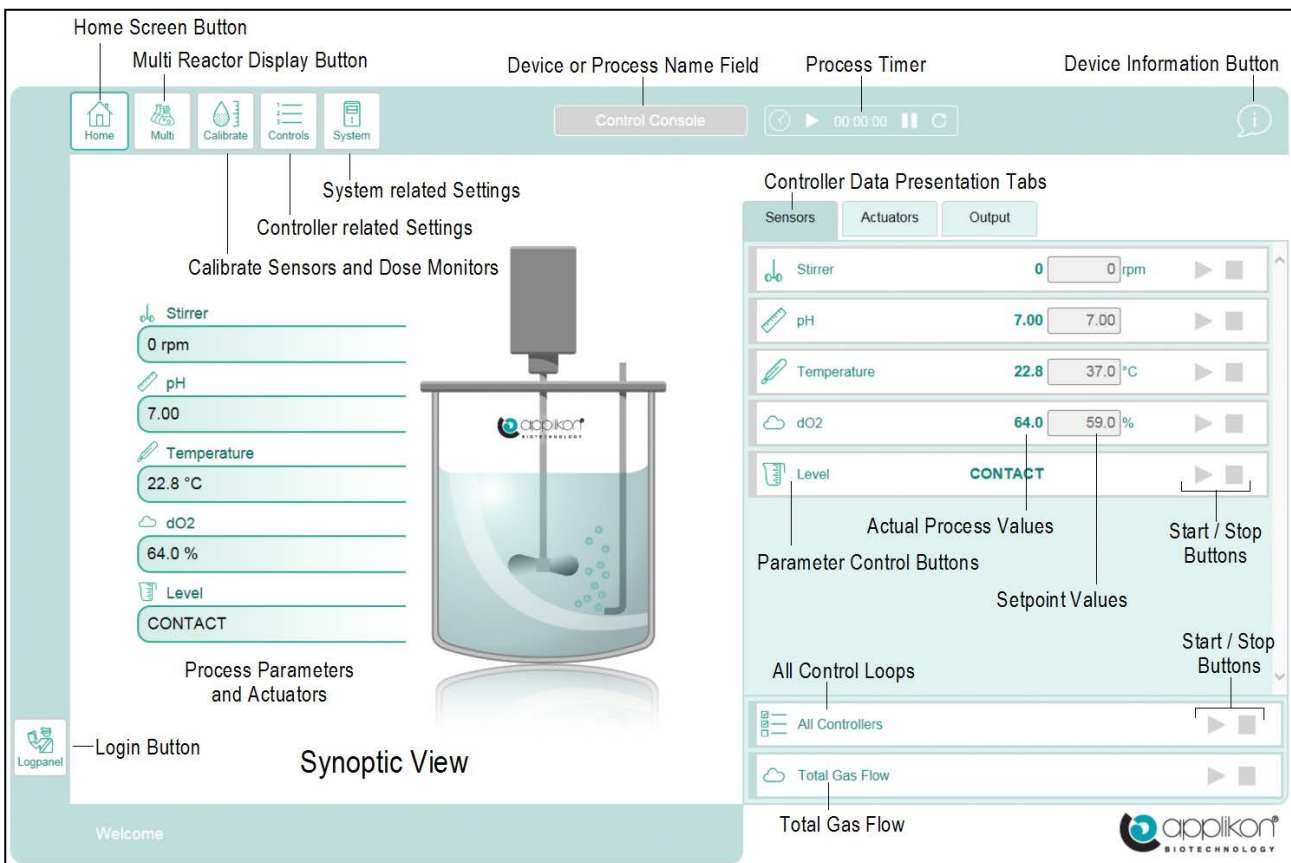
After switching on the power of the *my-Control* and invoking the Web Interface through the Internet browser on the PC, the *my-Control* displays its Home Screen (see next page).

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.

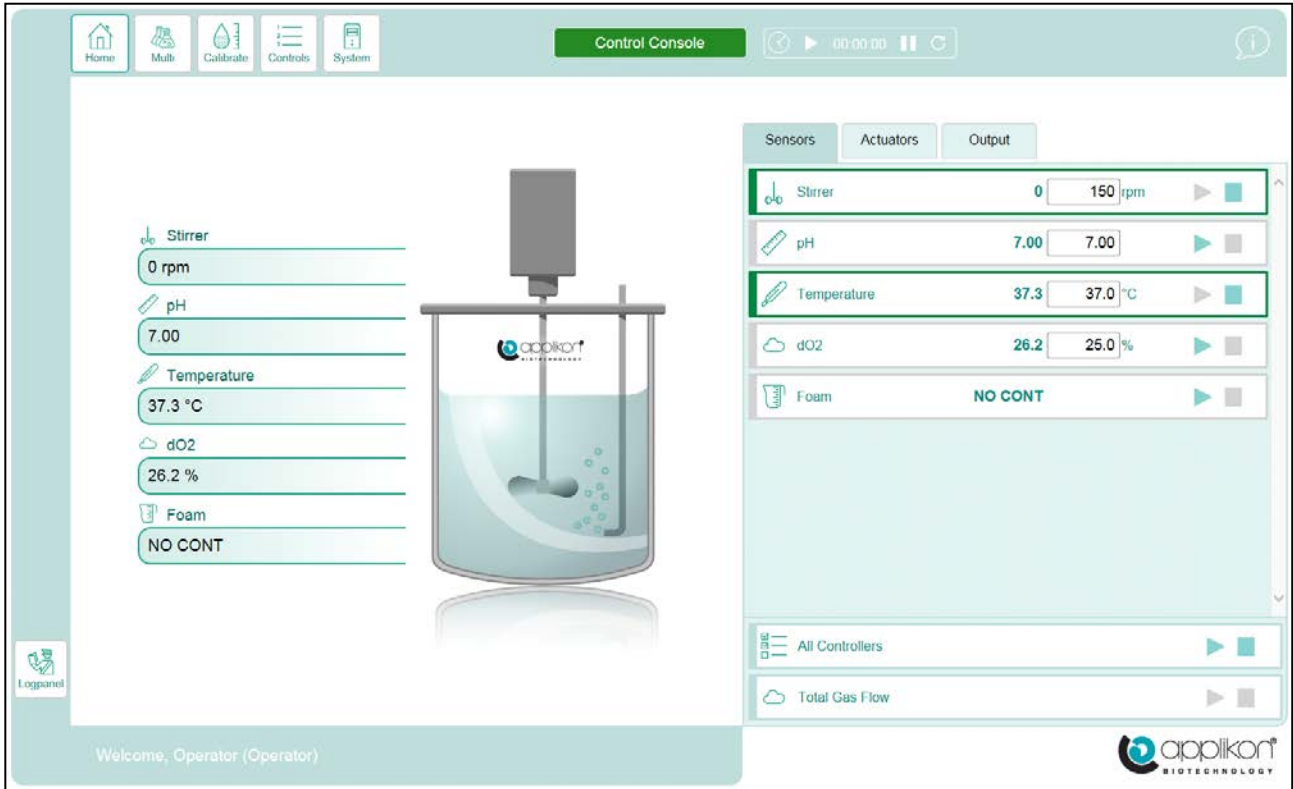
Default password for Operator and System Engineer: 0000.

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:




i 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 $\geq 100\%$.

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.

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



2.4 CONTROL LOOPS



the  button to enter the control loop configuration window.

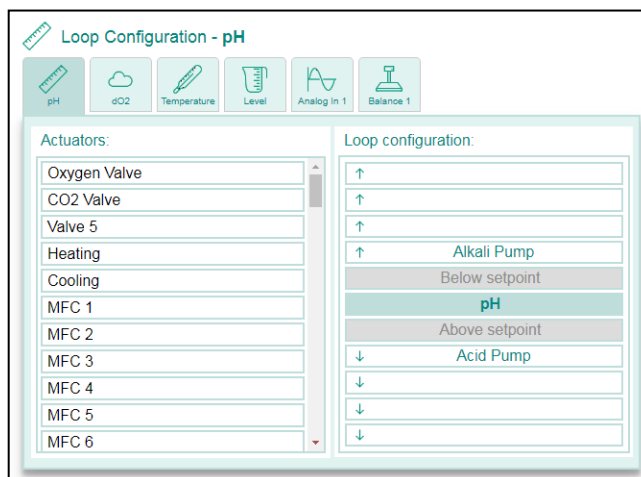
The tabs at the top of the screen present the configured control loops.

When the number of configured loops exceeds the

available space, the scroll buttons ( and ) are introduced.

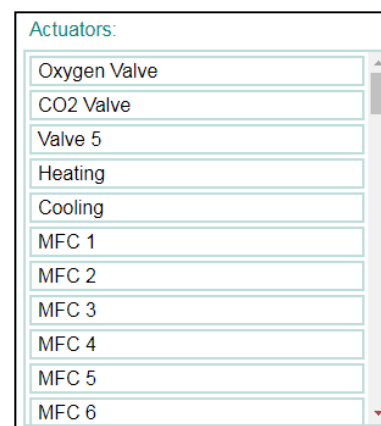
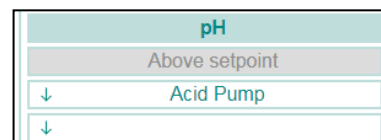
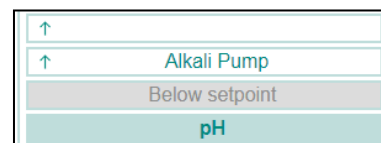
The keyboard cursor keys of the device that is used as WebUI can also be used as scroll tabs!

Use these tabs to view and / or edit the control loops.



The control loops can be configured by assigning actuators to it. Procedure:

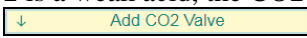
- Select a control by clicking on one of the tabs at the top of the screen. In this example, the pH Control loop is selected.
- The actuators that are currently presented to act Below Setpoint are presented as:
 Actuators that are used below setpoint are used for Upward Control.
- The actuators that are currently presented to act Above Setpoint are presented as:
 Actuators that are used above setpoint are used for Downward Control.
- At the left side of the screen, the list of available actuators is presented. Use the Scrollbar to examine all selectable actuators. An actuator can be selected by clicking on it. The selected actuator will be highlighted and will be projected as both actuator “Below” and “Above” setpoint.(light yellow color).



- The presented image shows an example of adding the CO2 valve as actuator to a pH Control loop where the alkali pump (for upward control) and an acid pump (for downward control) have already been assigned as actuators.

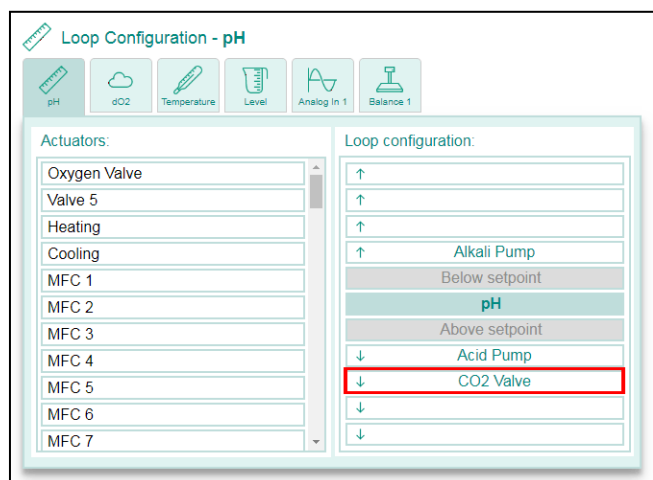
In the actuator list at the left side of the screen, the CO2 Valve has been selected.

In the Loop Configuration section at the right side of the screen, the CO2 Valve is projected (in light yellow) as actuator for both upward and downward control.


- Since CO2 is a weak acid, the CO2 valve is an actuator for downward pH control. Press the  button for downward control.

- The CO2 valve will be added as actuator for downward control to the pH Control loop. See image (the added actuator is indicated with a red rectangle).

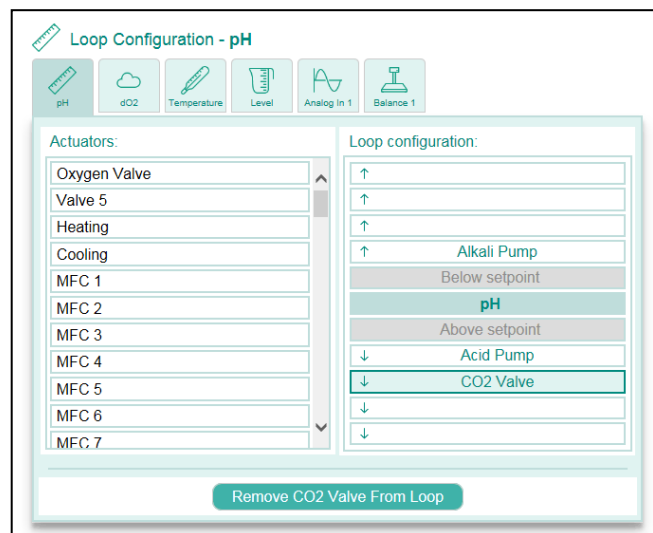
Press the  button to confirm.



An actuator can be removed from the control loop configuration:

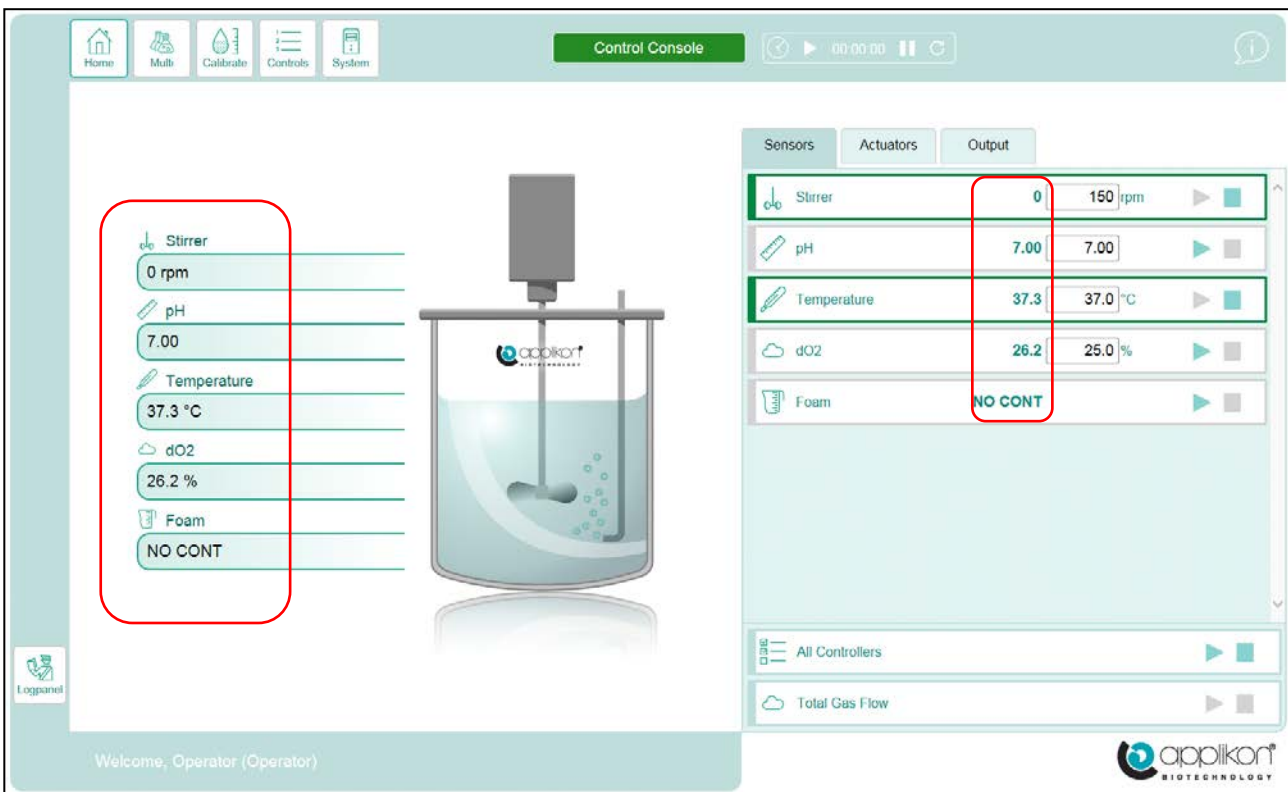
- Click on the actuator that needs to be removed from the loop.
- The selected actuator is highlighted and the  button is presented at the bottom of the Loop Configuration window. Press this button to remove the CO2 valve as actuator from the loop.

See the *my-Control Software Reference* for more examples.



2.5 PROCESS VALUE PRESENTATION

The main synoptic displays the primary process values next to the image of the bioreactor as well as in the sensors tab:



2.6 RECONFIGURING CONTROL LOOPS

By default, certain control loops will have been configured and actuators will have been assigned to these loops. The Control Loop configuration can be recalled and edited as shown in the previous control loops section.

2.7 RECONFIGURING ACTUATOR SETTINGS





The properties and settings of the Actuators that are (or can be) assigned to Control Loops can be recalled and edited by accessing the Actuators tab/

2.8 GRAPHICAL DISPLAY CONVENTIONS

The following Graphical Conventions (colors and shapes) are used for the data presentation.

Colors of the sensor button and bar

The table below lists the different colors that are used to present the process parameters:

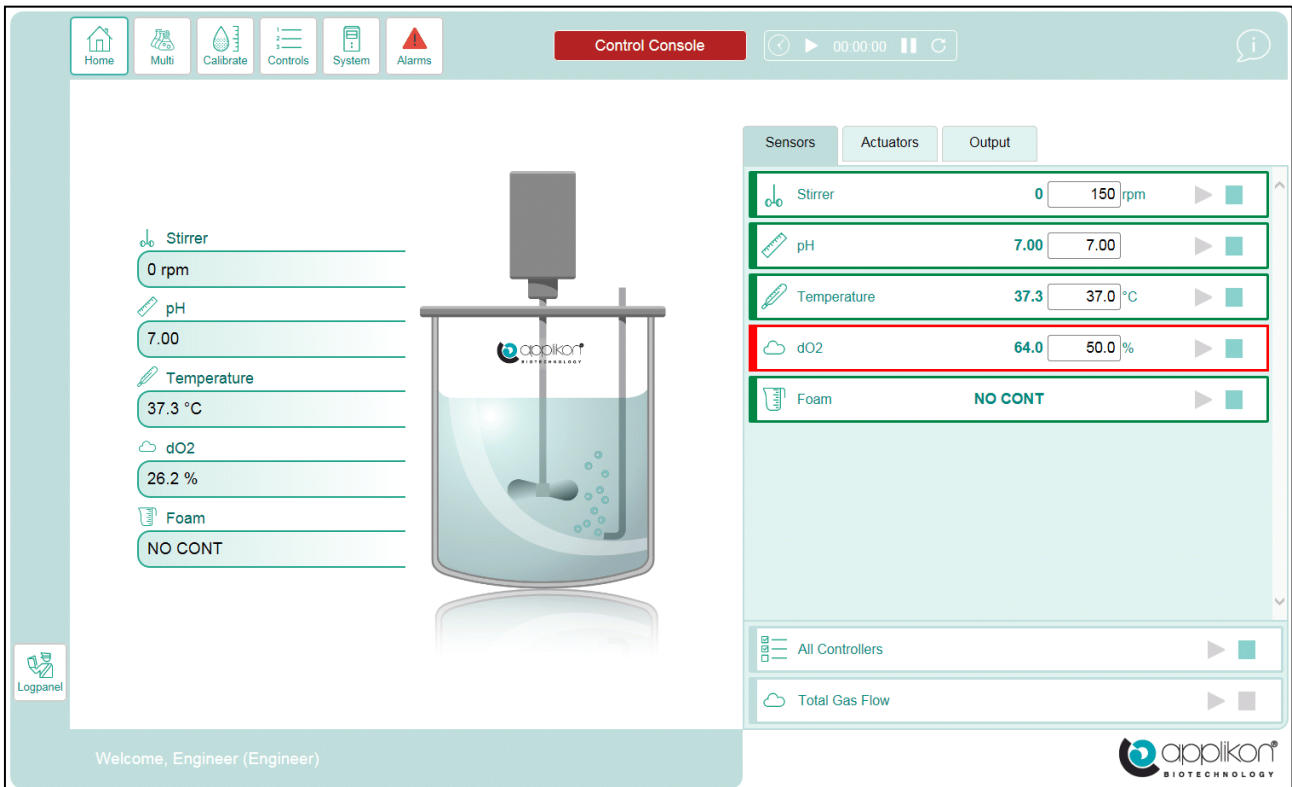
Sensor Button and Bar	Description
 pH 7.00 7.00	Controller not running Process Value within Alarm Limit range
 pH 7.00 7.00	Controller is running Process Value within Alarm Limit range
 Temperature 33.9 37.0 °C	Controller not running Process Value outside Alarm Limit range
 Temperature 34.3 37.0 °C	Controller is running Process Value outside Alarm Limit range



The alarms mentioned in the table above, are related to measured process values and are called Process Alarms or Deviation Alarms.




If a Deviation or System Alarm is detected, an Alarm button (Alarms) will be presented in the header of the screen. The Device Name will be presented with a red background. See image below.




Pressing this button will open the list with the active alarm events.






















By pressing the  button, a list with active alarm events will be displayed. An example of this list is presented below:











Press the  button to hide the presented Alarm list.




















2.9 ICONS

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

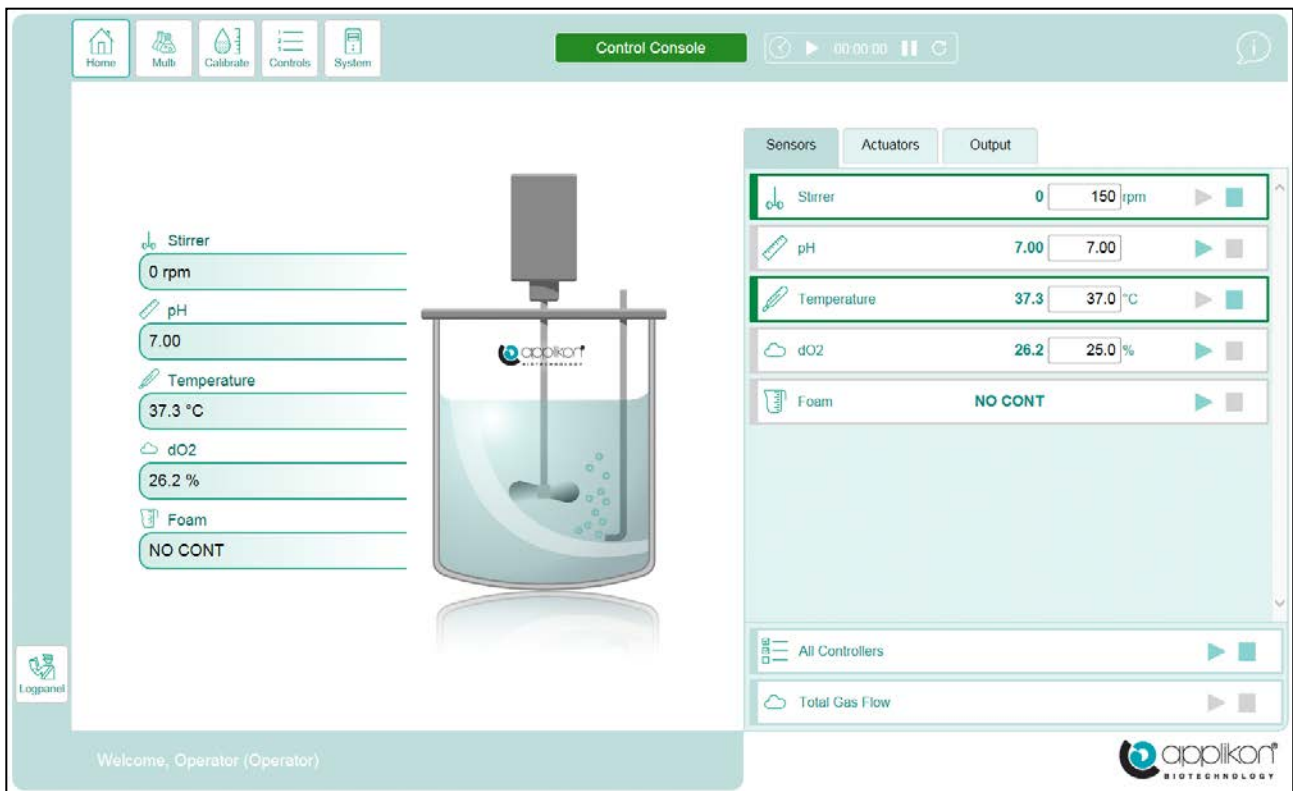
	Controller Configuration button (assign actuators per control loop)		Control Loop Tab, based on weight (balance input)
	Actuator Property button (edit actuator name, units, type and limits)		Control Loop Tab, based on a Biomass sensor (or Conductivity)
	Sensor Property button (edit sensor name, units 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)		

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






2.10 OPERATING THE CONTROL CONSOLE



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 $\geq 100\%$.

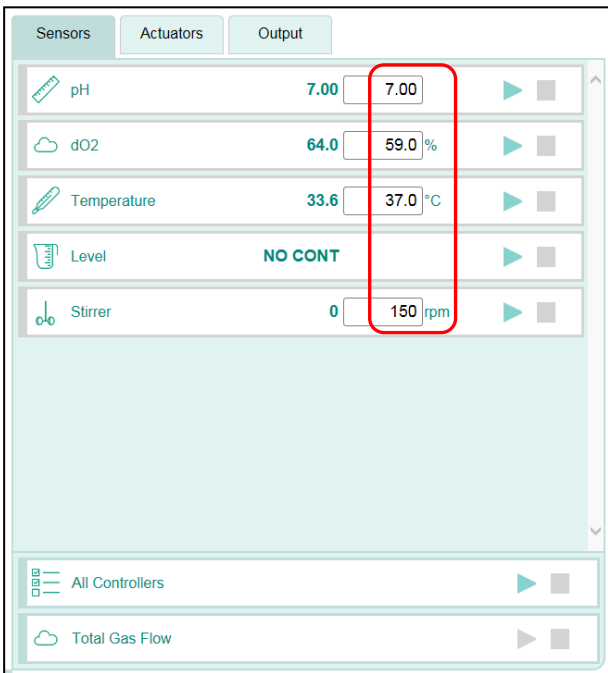
The User Interface shows buttons, bar and process information fields. For configuration purposes, use the buttons that are presented in the screen header:

	Return to the Home Screen
	Switch to the Multiple Reactor Display mode
	Access the calibration options
	View/edit controller related settings
	View/edit system related settings

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.

2.11 SETPOINTS

You can change the setpoints by entering new values in the input fields shown in the Sensors tab.





2.12 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 BIOREACTOR PREPARATION

3.1 GENERAL

The AppliFlex ST Single Use Bioreactor is delivered as a γ -irradiated (> 25 kGray) empty reactor and is ready to use.

However, before it can be put to use, several preparation steps have to be performed at different locations (refer to the table below). In this chapter, the reactor preparations are described.

The table below describes an overview of preparation steps and the corresponding locations

Step#	Operation	Location
1	Calibrating the reusable pH sensor.	Alongside the my-Control, in the Lab.
2	Autoclaving the reusable pH sensor and LumiSens sensor tip.	In the Autoclave.
3	Autoclaving the refilled Feed Bottle(s) including pump tubing and Luer Lock connector(s).	
4	Filling the AppliFlex ST reactor with medium.	In the BSC.
5	Mounting the reusable pH and LumiSens dO ₂ sensors.	
6	Making the hydraulic connections: feed bottle(s) such as medium, nutrient, inoculum, acid and alkali.	
7	Preparing the Heating Blanket as actuator for temperature control.	Alongside or at the my-Control, in the Lab.
8	Mounting the Off-gas tubing in the Condenser Cooling Groove (only if the Condenser is used).	
9	Mounting the Pt-100 Temperature sensor in the Thermometer Pocket.	
10	Connecting the Gas Supply tubing for aeration	
11	Connecting the detachable pH and dO ₂ sensor cables to the sensors.	
12	Connecting the sensor cables (pH and temperature) to the my-Control.	
13	Mounting the Pump tubing in the Pumps.	
14	Mounting the Stirrer Drive on top of the Stirrer Shaft.	



Standard Operating Procedures

- Preparing and operating the AppliFlex ST Single Use Bioreactor requires aseptic procedures, such as: working in a Biological Safety Cabinet (BSC), use of Luer Lock couplings or C-Flex tubing plus welding equipment, etc. For specific information concerning this subject, refer to the Standard Operation Procedures that are applicable in your lab.



CAUTION








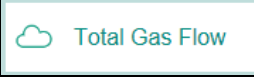
Single Use Bioreactor

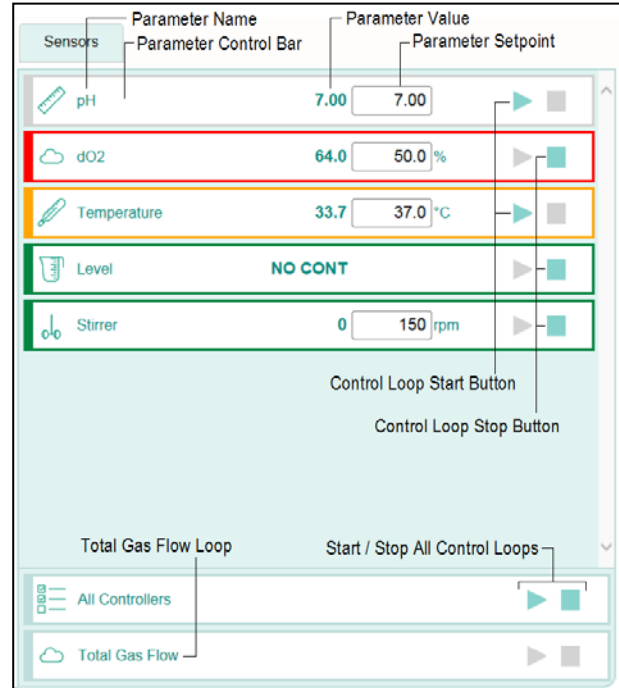
- The AppliFlex ST Single Use Bioreactor is intended for “single use” only. Applikon is not liable in case of damage caused by multiple use of the reactor!

3.2 ACCESSING SENSORS VIA THE INTERFACE

The Sensor Tab displays the control parameters and control status together with current process value and defined setpoint value.

Depending on the controller status and current process value in relation to setpoint, different colors are used to indicate the status (refer to [section 1.5.3](#)).

- The  or  buttons can be used to Start / Stop individual controllers.
- The  or  buttons in the  area can be used to start all idle controllers or to stop all running controllers.
- If the reactor is aerated through mass flow controllers, the total gas flow can be controlled. The  or  buttons in the  area at the bottom of the screen area can be used to start or stop Total Gas Flow Control.



Total Gas Flow control can only be achieved when all aeration gas flows are controlled through mass flow controllers.

3.3 SENSOR PREPARATION

3.3.1 GENERAL

To measure the pH and dO₂ concentration during cultivation / fermentation, an invasive pH sensor and LumiSens sensor tip are used. Therefore, these parts need to be autoclaved (preferably by using the autoclavable Sensor Holders or autoclavable bags; refer to [section 3.2.3](#)) before they are mounted in the Applikon ST reactor.

Prior to calibration, prepare the pH sensor in the following manner:

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


3.3.2 CALIBRATING THE ELECTROCHEMICAL pH SENSOR

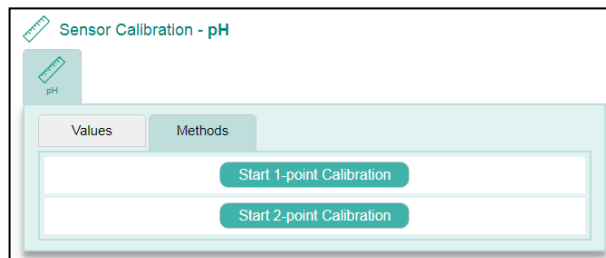
Connect the pH sensor to the Control Console (*my*-Control). For detailed information concerning the pH calibration procedure and its background, refer to the *my*-Control Software Reference Manual.

It is advised to calibrate the sensor with two buffer solutions (pH = 7.0 and pH = 4.0), resulting in a 2 point calibration. 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.

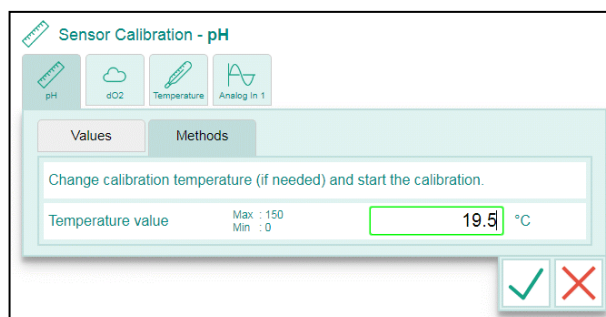
Press the  button.



Step 2:

Enter the temperature of the first buffer solution. The readings of the pH sensor are temperature dependent. Therefore, the Pt-100 temperature sensor from the Thermometer Pocket can be inserted in the buffer solution. The measured temperature will be displayed.


Press the  button.

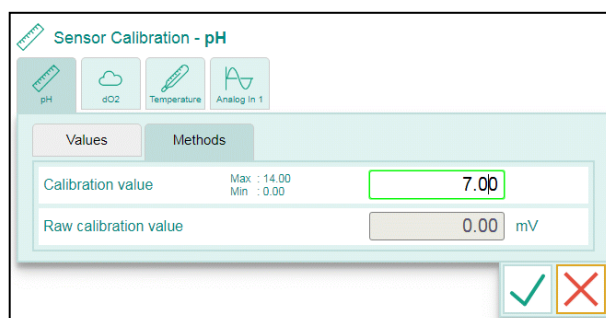


Step 3:

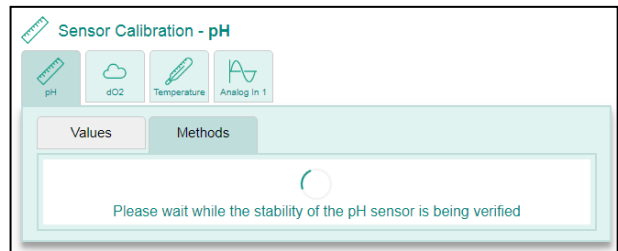
Enter the pH value of the first buffer solution (e.g. 7.00).


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

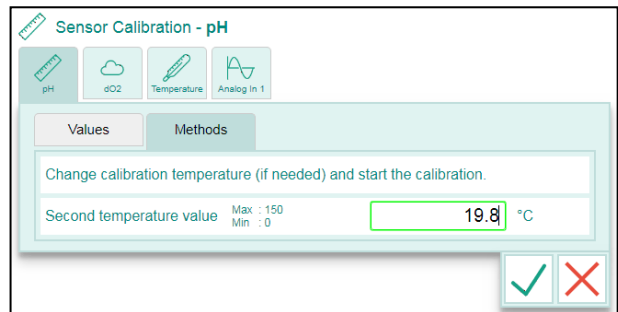
in the presented data field and press the  button.




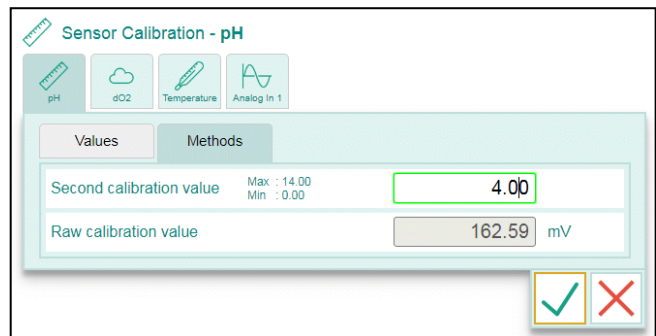
The sensor value will now be monitored to verify the stability.
 As soon as a stable value has been obtained, the calibration routine will be resumed.



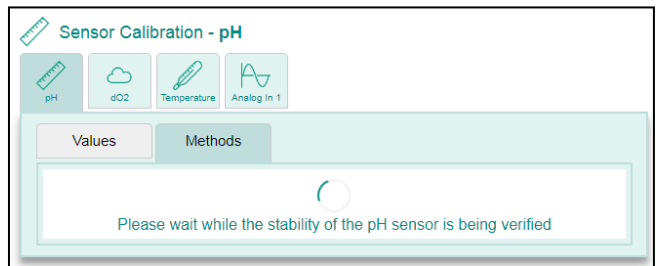
Step 4:
 Immerse the Pt-100 temperature sensor in the second buffer solution. Wait until the value is stable and press the  button.



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.




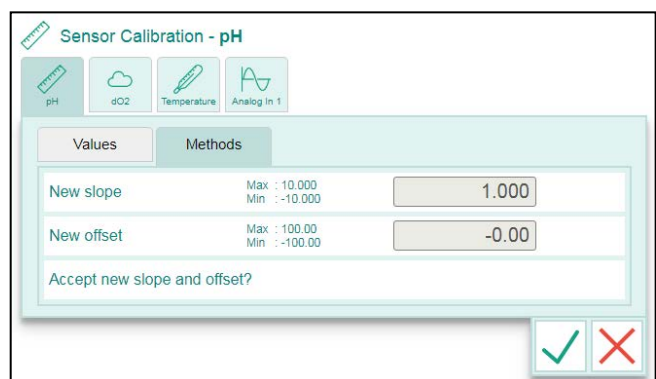
The sensor value will now be monitored to verify the stability.
 As soon as a stable value has been obtained, the calibration routine will be resumed.



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 expected slope value of the pH-sensor is: 0.95 – 1.05 (at any temperature*)

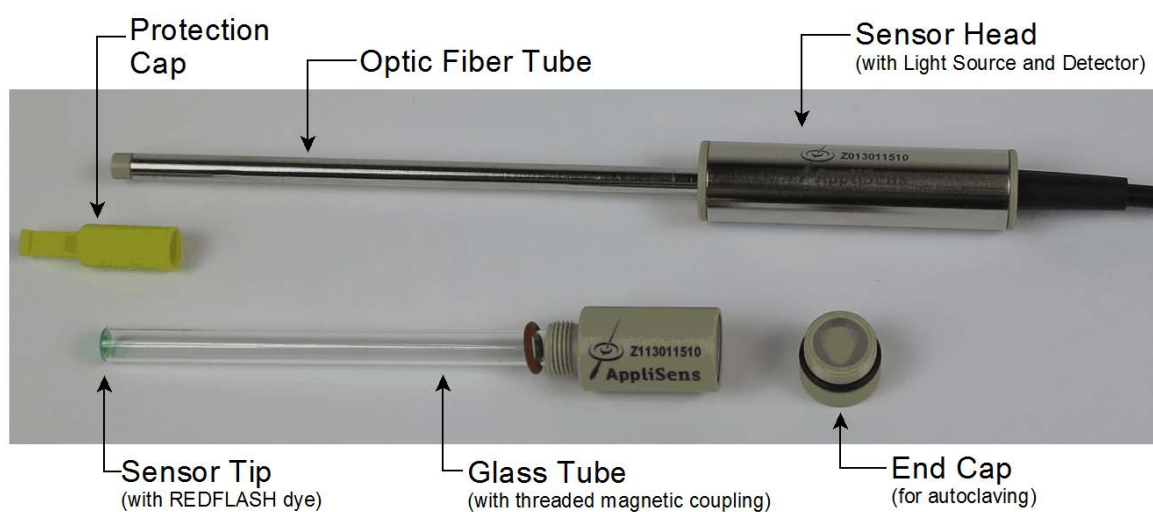
*Reported pH measurements are corrected for the current temperature. Therefore, the calculated slope (sensitivity) of the pH sensor valid for the temperature range of 0 – 70°C.

3.3.3 ABOUT THE DO₂ SENSOR

The optical **LumiSens dO₂ 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 dO₂ Sensor Assembly:



To the LumiSens sensor tip, a thin film of REDFLASH 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 and the dye at the sensor tip is not irradiated.

Before starting the dO₂ Control loop, the “Sampling” parameter must be enabled.

The LumiSens optical dO₂ 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.

Lifetime of the LumiSens Sensor Tip

The typical lifetime of the LumiSens sensor tip is approx. 12 months of light exposure or 50 autoclaving cycles. 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
- 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.

3.3.4 CALIBRATING THE LUMISENS SENSOR TIP USING FACTORY DATA

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):



1	Phase Angle 0% O ₂	Max : 90 Min : 0.01	54.0 °
2	Temperature 0% O ₂	Max : 50 Min : 0.01	25.0 °C
3	Phase Angle 100% Air	Max : 90 Min : 0.01	26.3 °
4	Temperature 100% Air	Max : 50 Min : 0.01	25.0 °C
5	Atmospheric Pressure	Max : 5000 Min : 1	978 mbar

Sensor Calibration Settings

Module test / Modultest	
O ₂ Module / O ₂ -Modul	Z113013510
Manufacturing number / Fabrikationsnummer	00346369
Test date / Prüfdatum	1/20/2017
Measured value at / Messwert bei	25.0 °C
Atmospheric Pressure / Luftdruck	978 mbar
Phase angle at 0% O ₂ / Phasenwinkel bei 0% O ₂ (Batch calibration / Chargen Kalibration)	54.0 °
Phase angle in air (20.95% O ₂) / Phasenwinkel bei in Luft (20.95% O ₂) (Batch calibration / Chargen Kalibration)	26.3 °

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 dO₂ sensor tip is now calibrated for the rest of its lifetime.



If the LumiSens sensor shows a structural deviation from 100% during measurement in air saturated medium, this deviation can be corrected by using the **Calibrate At 100% Air Saturation** button at the bottom of the Custom Settings screen. The procedure below describes the way how to do this.

Adjusting the LumiSens reading to 100% for air saturated medium:

- Aerate the bioreactor with medium at process temperature for at least 15 – 20 min (stirrer is running at moderate speed); air flow through the sparger must be approx. 1 VVM.
- The LumiSens sensor signal must be stable. If the signal is not stable, reduce the stirrer speed.
- Press the **Calibrate At 100% Air Saturation** in order to correct for any structural deviation in sensor sensitivity.

3.3.5 MANUALLY CALIBRATING THE LUMISENS OPTICAL dO₂ SENSOR

The Lumisens Optical dO₂ Sensor can also be calibrated manually by purging the medium with either air or nitrogen.

This manual dO₂ calibration procedure may be used as an alternative for entering the sensor data from the certificate.

Calibrate Ambient Air:


Purge the medium with **air** for at least 1 minute at a rate of about 30 L/min (at least 1 vvm, and always agitate at > 500 rpm!) The medium will be saturated with air.

Choose 1-point calibration and set the selection to  (default setting): the 1-point calibration is performed at 100% air saturation.

The currently measured dO₂ signal will be set to 100% dissolved oxygen.

0% Air Saturation

Purge the medium with **nitrogen** for at least 1 vvm (or about 10 minutes). The oxygen saturation of the medium will decrease to 0%.

Choose 1-point calibration and set the selection to  so that the 1-point calibration is performed at 0% air saturation.

The currently measured dO₂ signal will be set to 0% dissolved oxygen.

3.3.6 AUTOCLAVING THE pH SENSOR AND LUMISENS SENSOR TIP

The reusable pH sensor and LumiSens sensor tip must be autoclaved before mounting them in the AppliFlex ST Single Use Bioreactor.

- Cover the top of the LumiSens sensor tip with the End Cap (in order to prevent moisture entering the inner area of the glass tube).
- Remove the detachable cable from the pH sensor head and mount the protection cap.
- Mount the pH sensor in an Autoclavable Sensor Holder (Z850030320). The PG13.5 thread at the sensor fits in the thread of the sensor holder.



Before closing the sensor holder or bag, make sure to put a few ml of d.i.-water inside for heat-transfer during autoclaving. The sensor holder or autoclaving bag may be placed in a beaker inside the autoclave.

The vent outlet of the sensor holder prevents pressure build-up during autoclaving. It is equipped with a filter in order to keep the sensor sterile after autoclaving



When no Autoclavable Sensor Holders are available:

- Instead of mounting the pH sensor and LumiSens sensor tip in the Sensor Holder, an autoclavable (plastic) bag may also be used to protect the parts during autoclaving. Put the individual parts (detached cable and mounted protection cap) in the bag. Add a few ml's of d.i.-water for heat-transfer, remove as much air as possible and seal the autoclave the sensor bag.

Autoclaving the sensors is usually performed at 121 °C during a 20 minutes interval. After autoclaving is completed, the sensors must be kept inside the Sensor Holder or autoclavable bag to maintain sterility!



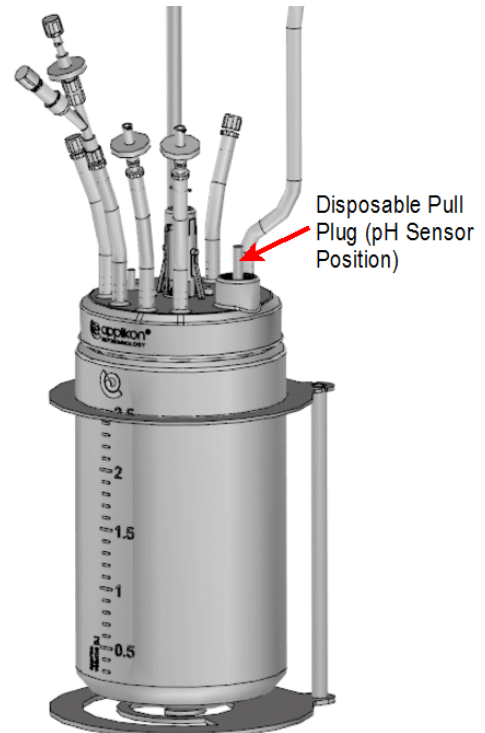
If, instead of a LumiSens dO₂ sensor, a classic (polarographic) dO₂ sensor is used, it must be autoclaved in the same way as the pH sensor. Calibrating the classic dO₂ sensor is done after mounting it in the reactor.

3.3.7 MOUNTING THE pH SENSOR AND LUMISENS dO₂ SENSOR TIP

- Remove the disposable Pull Plug from the pH sensor port in the top plate of the AppliFlex ST Single Use Bioreactor.

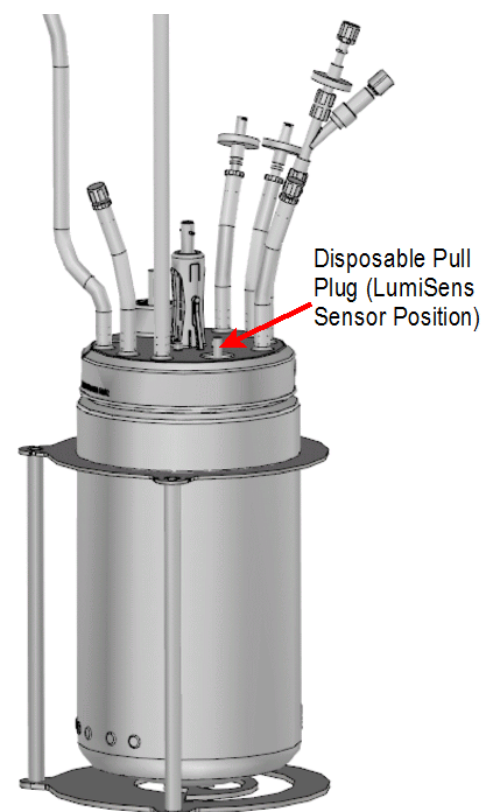
In the image, the Pull Plug at the pH sensor position is indicated by the red arrow.

- Remove the pH sensor from its autoclavable holder / autoclaving bag and mount it in the spare port of the reactor top plate. Turn the sensor clockwise until it is fixed hand tight (do not use a tool).

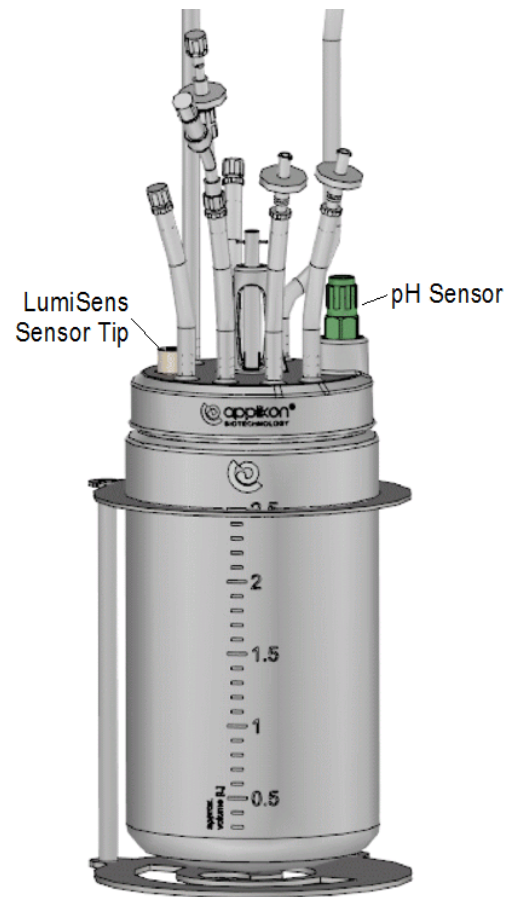


- Remove the other disposable Pull Plug from the LumiSens sensor port in the top plate of the AppliFlex ST Single Use Bioreactor.

- Remove the LumiSens sensor tip from its holder / autoclaving bag and mount it in its position at the reactor top plate. Turn the sensor tip clockwise until it is fixed hand tight (do not use a tool).



The image at right shows the AppliFlex ST Single Use Bioreactor with mounted pH sensor and LumiSens sensor tip.

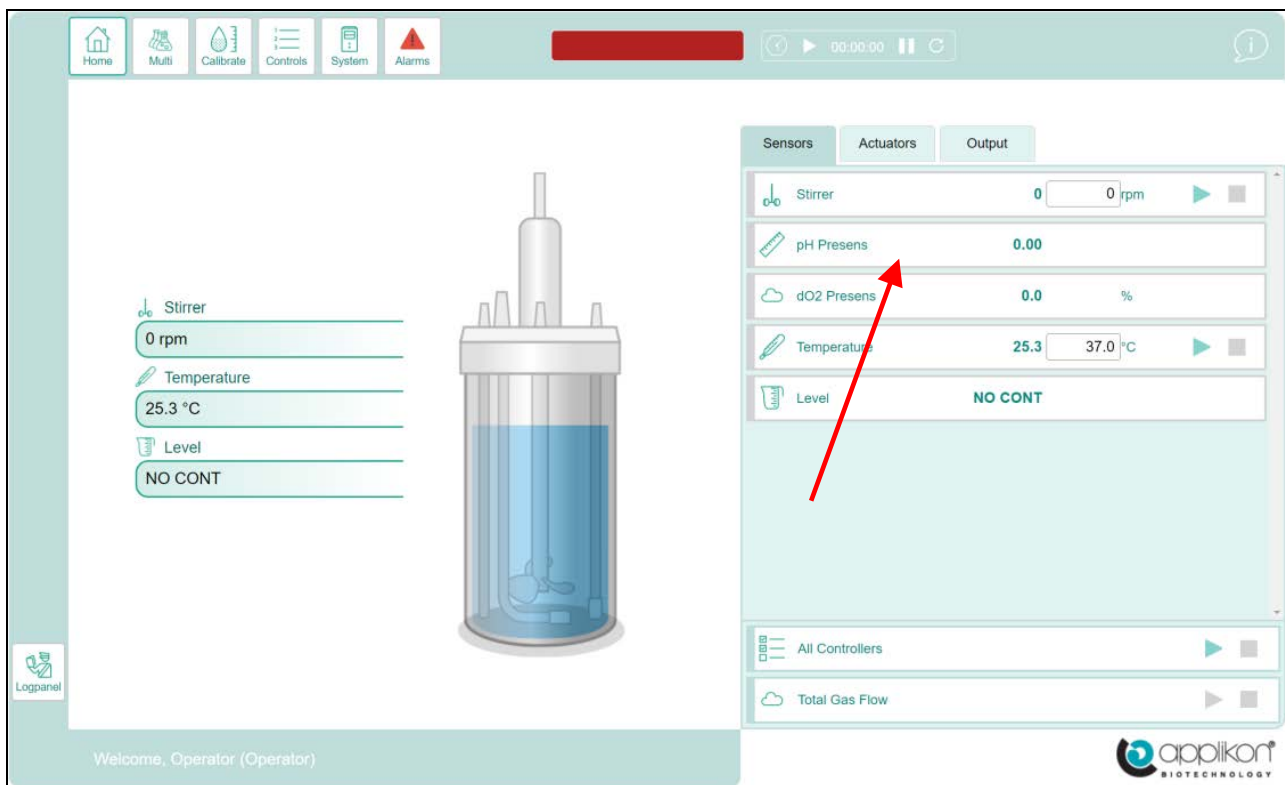


3.4 SINGLE-USE SENSOR PREPARATION

If you are working with the **single-use sensors** – the sensors incorporated in Appliflex vessels – then their preparation requires the entry of external calibration data in the controller system, as well as the execution of a calibration measurement. This section shows the preparation for a pH sensor followed by that for a dO₂ sensor.

3.4.1 PERFORMING THE SINGLE-USE PH SENSOR PREPARATION

Start by invoking the my-Control webUI interface:



Click on the pH Presens button (indicated) for this example. The buttons will display the name of the sensor actually being used.

This invokes the following:

Sensor Calibration - pH Presens

Calibrate Limits Loop Settings Trend

Actual Sample Value	Max : 14.00 Min : 0.00	<input type="text" value="0.00"/>
Correction Value	Max : 14.00 Min : -14.00	<input type="text" value="0.00"/>

Calculate Correction

Click on **Settings**:

Sensor Settings - pH Presens

Calibrate Limits Loop Settings Trend

Standard Custom

Sensor Name	<input type="text" value="pH Presens"/>
Engineering Unit	<input type="text"/>
Screen Priority	Max : 65 Min : 0 <input type="text" value="2"/>
Decimals	Max : 4 Min : 0 <input type="text" value="2"/>
Sample Correction	Enabled <input type="checkbox"/>
Dead Band	Enabled <input type="checkbox"/>
Sampling	Enabled <input checked="" type="checkbox"/>

Then select the **Custom** tab:

Custom Sensor Settings - pH Presens

Calibrate Limits Loop Settings Trend

Standard Custom

Imin Phase Signal Low pH	Max : 90 Min : 0.01	<input type="text" value="55.15"/> °
Imax Phase Signal High pH	Max : 90 Min : 0.01	<input type="text" value="19.00"/> °
pH0 Point Of Inflection	Max : 90 Min : 0.01	<input type="text" value="6.69"/>
dpH Slope In Dynamic Region	Max : 90 Min : 0.01	<input type="text" value="0.56"/>
Calibration Temperature	Max : 90 Min : 0.01	<input type="text" value="37.00"/> °C
Measurement Interval	Max : 120 Min : 1	<input type="text" value="60"/> Seconds

Reset Settings

Samples To Average	Max : 9 Min : 0	<input type="text" value="1"/>
LED Current	Max : 255 Min : 0	<input type="text" value="30"/>

In this settings screen, the supplied calibration can be entered.

You enter the desired values and save each separately (by clicking on the acceptance checkmark which appears).

Setting	Max	Min	Value	Unit
Imin Phase Signal Low pH	90	0.01	54.90	°
Imax Phase Signal High pH	90	0.01	30.25	°
pH0 Point Of Inflection	90	0.01	5.24	
dpH Slope In Dynamic Region	90	0.01	0.53	
Calibration Temperature	90	0.01	37.00	°C
Measurement Interval	120	1	30	Seconds
Samples To Average	9	0	1	
LED Current	255	0	30	

After entering this data, you need to take a sample from the reactor and measure the pH offline in order to subsequently be able to obtain the offset correction. Before doing so, make sure that the system has stabilized and that the bioreactor has reached its normal working temperature. A typical stabilization time is 30 minutes at 37°C. When the system has reached a steady state, click on the **Calibrate** option:

Enter the **Actual Sample Value** i.e. the value for a known buffer. In the case of a pH sample correction, you use a buffer with pH 7.1. Finally, press **Calculate Correction** to compute the offset.

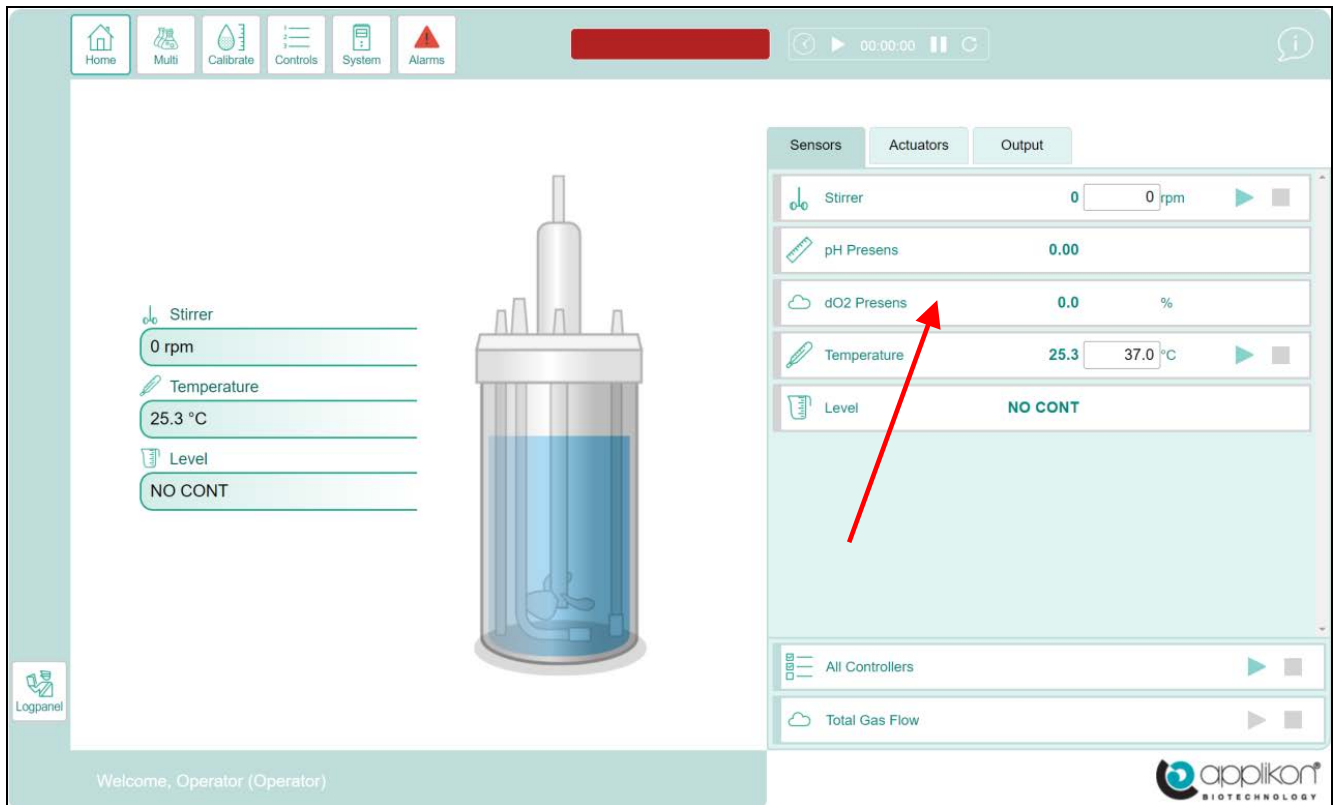
Actual Sample Value	Max : 14.00 Min : 0.00	7.10
Correction Value	Max : 14.00 Min : -14.00	0.00

The sensor is now fully calibrated. The bioreactor can be inoculated (if not already done), after which the pH control loop and the process can start.

For a large change in pH (> 0.5 pH), due to media addition or a pH step in the process, it is advisable to repeat the last procedure in order to obtain a new offset for the single-use pH sensor.

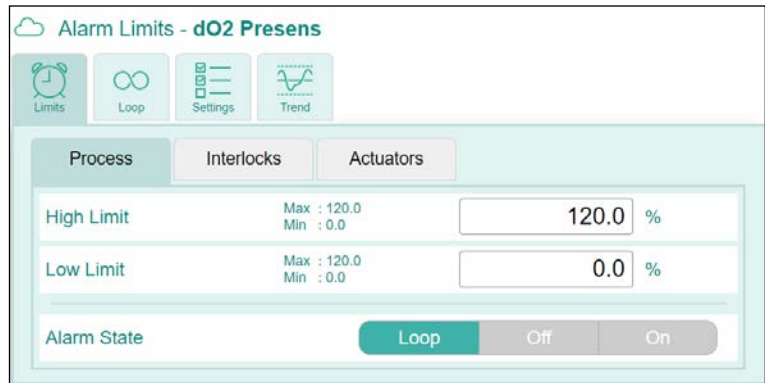
3.4.2 PERFORMING THE SINGLE-USE DO2 SENSOR PREPARATION

Start by invoking the my-Control webUI interface:



Click on the dO2 Presens button (indicated) for this example. The buttons will display the name of the sensor actually being used.

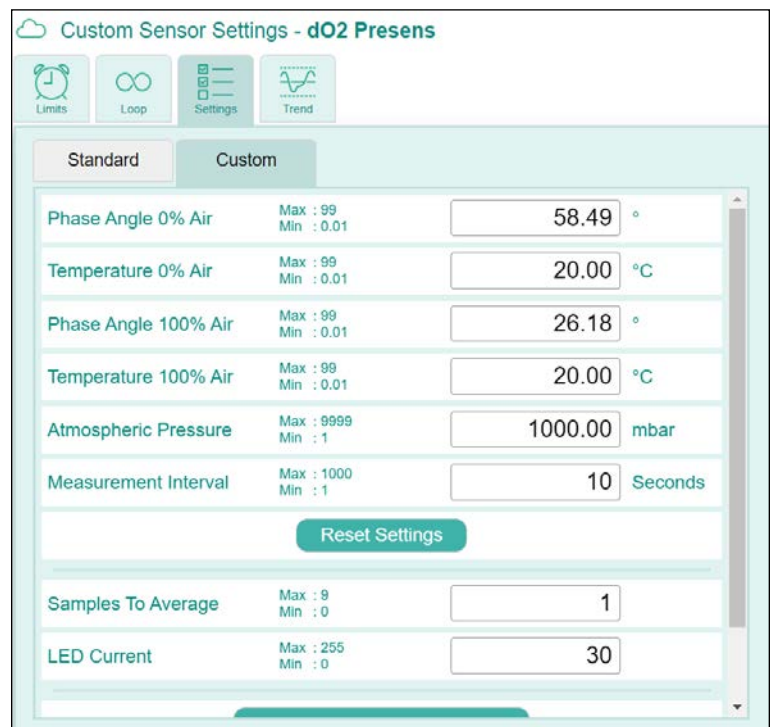
This invokes the following:



Click on **Settings**:

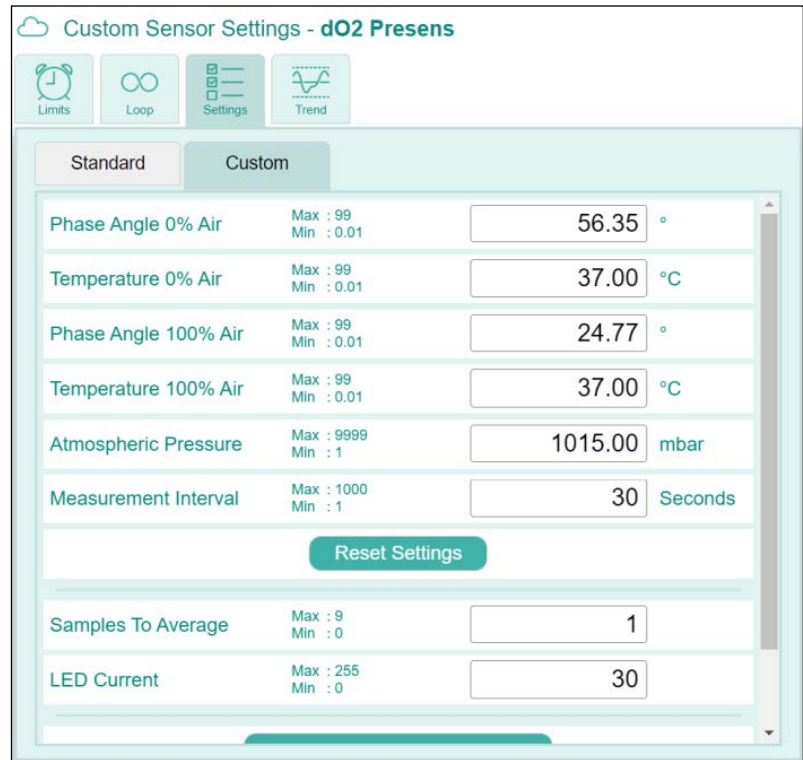


Then select the **Custom** tab:



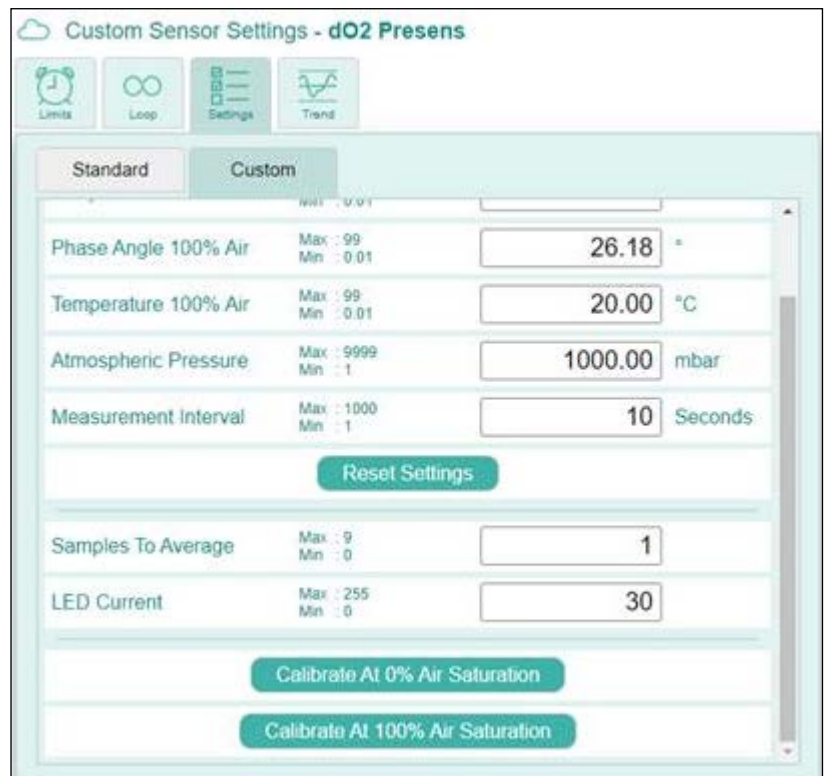
In this settings screen, the supplied information can be entered.

You enter the desired values and save each separately (by clicking on the acceptance checkmark that appears when you enter the field).



Technically, the procedure just outlined inputs the vendor-supplied calibration data and this can be considered to constitute the initial calibration of the sensor. The sensor is now fully calibrated. The bioreactor can be inoculated (if not already done), after which the dO2 control loop and the process can start.

If one wishes to perform an actual calibration, this can be done by clicking on the 0% Air Saturation or the 100% Air Saturation button at the bottom of the screen, take a reading, and enter the value in the appropriate field.



3.5 MOVING THE REACTOR TO A BSC

Inside the Biological Safety Cabinet (used as a sterile environment), the following actions must be performed:

- Filling the AppliFlex ST reactor with medium,
- Mounting the reusable pH and dO₂ sensors and
- Making the hydraulic connections (feed bottles, such as: medium, nutrient, inoculum, acid or alkali).

A possible procedure to create sterile conditions is described below. Also refer to the Standard Operating Procedures that are applicable in your lab.

1. Switch on the BSC. Wait approx. 30 minutes.
2. Spray the working surface of the BSC with 70% ethanol. Wipe the surface dry with tissue.
3. Spray the AppliFlex ST Single Use Bioreactor and the autoclavable Sensor Holders (or autoclavable bags) with the sterile reusable sensors with 70% ethanol and also place them inside the BSC.
4. Spray the liquid addition bottles (e.g. for medium, acid and alkali) and place them inside the BSC.

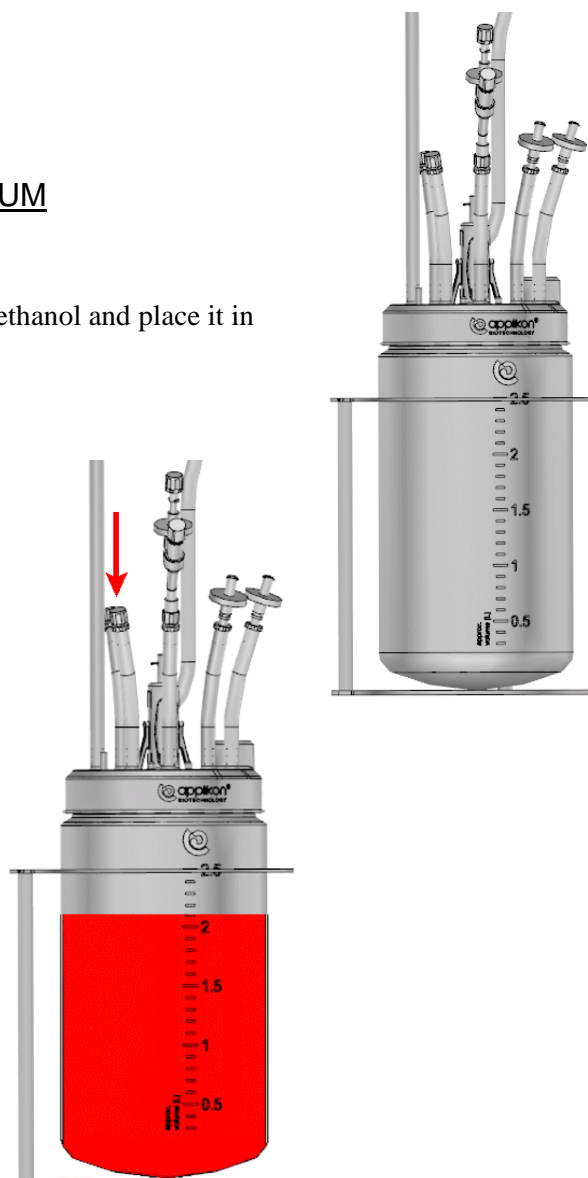
3.5.1 FILLING THE BIOREACTOR WITH MEDIUM


- Spray the AppliFlex ST reactor and its stand with 70% ethanol and place it in the BSC,

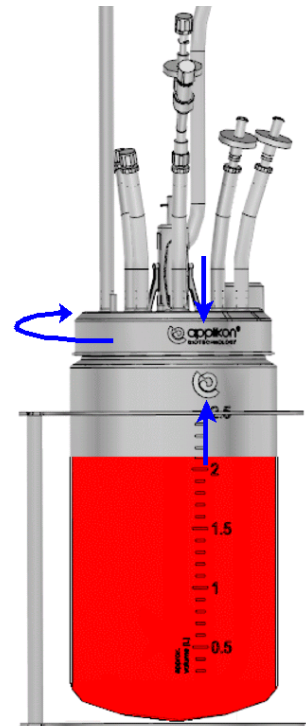
- Connect the medium addition bottle to one of the liquid addition lines in the top plate of the reactor and fill it up to working volume with sterile medium. In the adjacent image, the liquid addition is presented by the red arrow.

As an option, the addition line can be extended by using Tube Welding. Refer to [section 3.3.3](#). Material of the Head Plate tubing. For tubing type and lengths, refer to the Hardware Manual.

- As an alternative, you can remove the Top Plate from the reactor,
- Fill the reactor with sterile medium. Make sure to leave enough space for top plate auxiliaries (such as sensors, stirrer shaft, etc.) and any additional fluids,



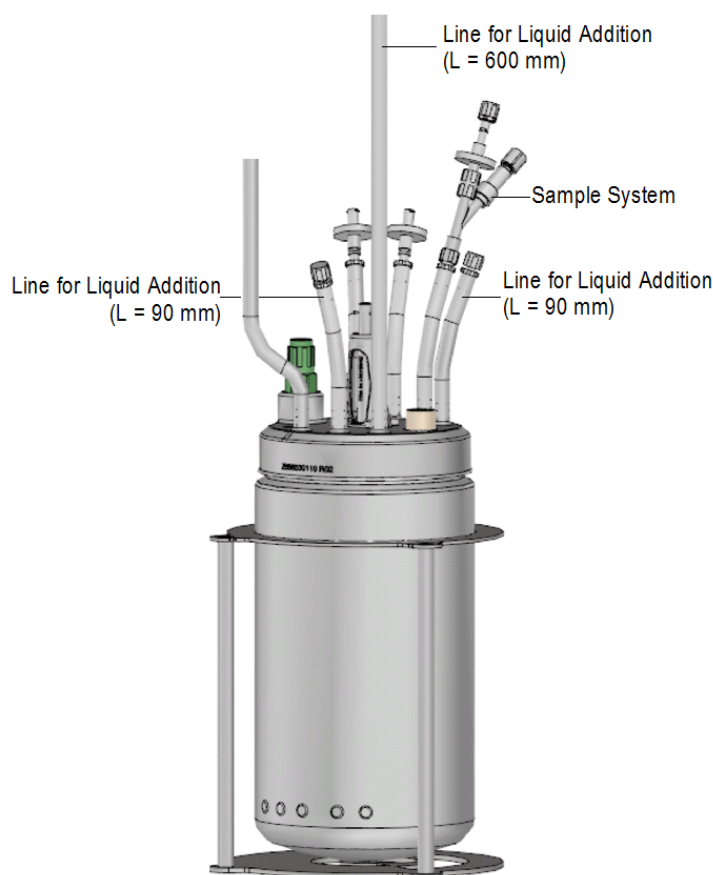
- If the Head Plate of the reactor has been removed, it must be remounted. To be gas tight, the Head Plate needs to be tightened with considerable force. Fasten it to such extent that the Applikon logo () at the top plate passes the alignment with the logo at the reactor. See the blue aligning and rotation arrows in the adjacent image.



3.5.2 HYDRAULIC CONNECTIONS

While the reactor is still located in the BSC, the hydraulic connections for medium, inoculum, acid / base addition (used for pH control) and sampling can be made as well.

In the adjacent image, the liquid addition lines and sample system are indicated by text.



Liquids that will be connected to the AppliFlex ST Single Use Bioreactor, need to be sterile.



The liquid containers can best be placed on a cart that is positioned in front of the BSC (only the connection lines have to be placed inside the cabinet).



CAUTION

In order to prevent infection (caused by cross-contamination), the tubing that is used for the hydraulic addition lines must be new.

1. Place the reagent storage bags / bottles for medium, the inoculum and any other liquid on a cart.
2. If applicable, move the cart in front of the BSC.
3. Spray the connecting tubing of medium, inoculum and other liquids with 70% ethanol.
4. Place the cleaned tubing ends inside the BSC.
5. Remove the blind cap of the individual addition lines (one at the time) and make aseptic connections by using Luer Lock and/or CPC (male and female) connectors.
Use pinch clamps or hose clips to close the connecting tubing between reagent container and the AppliFlex ST Single Use Bioreactor.

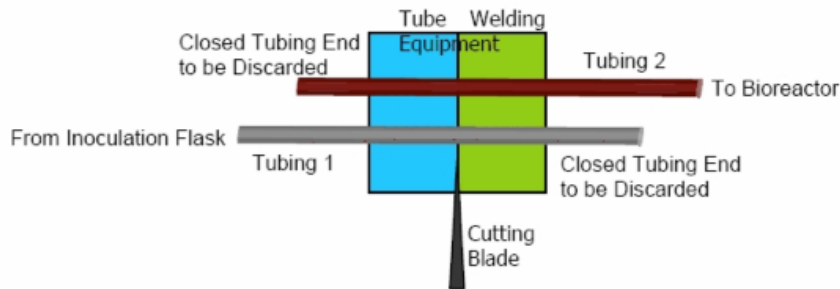
Alternative connection method: tube welding!

All hydraulic connection lines can be used for tube welding. Tube welding is used as sanitary tubing connection outside a BSC.

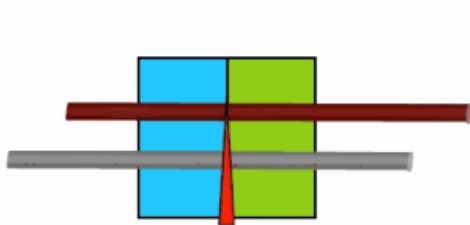
Example of the tube welding procedure is presented on the next page.

Example of the Tube Welding procedure:

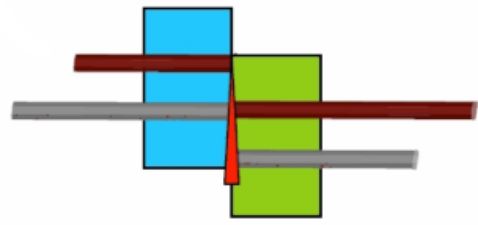
Tube welding is the traditional technique for joining tubes while maintaining sterility.



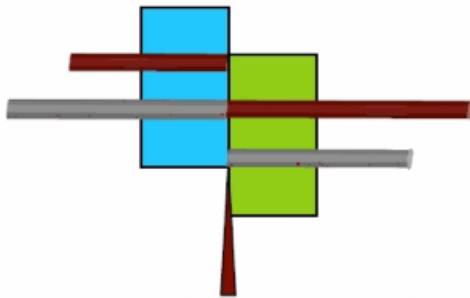
Step 1: Installation of tubing in welding equipment



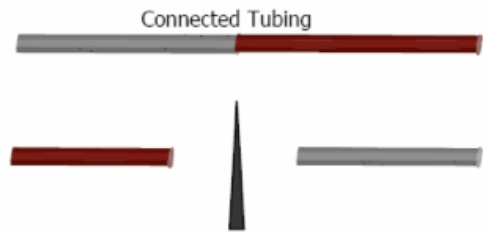
Step 2: Blade heats up and cuts both pieces



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

3.6 COUPLING THE BIOREACTOR AND THE my-CONTROL

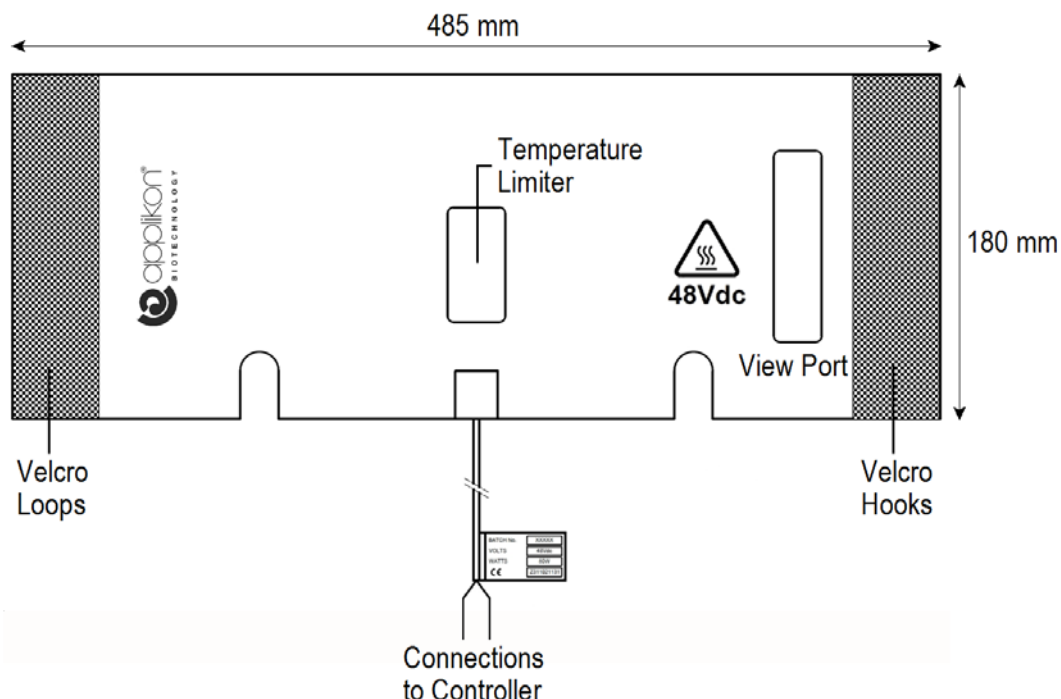
Now the sensors are mounted in the AppliFlex ST Single Use Bioreactor and the hydraulic connections have been established, the reactor can be moved to the stand, near the my-Control.

The actuators preparation for the relevant control loops are described in the sections below.

3.6.1 ACTUATORS FOR TEMPERATURE CONTROL

For temperature control, a Cold Water valve assembly and a Heating Blanket can be used.

The image below shows the Heating Blanket in stretched position. It must be wrapped around the reactor in such way that the Velcro strips on each other.



CAUTION

In order to prevent vessel deformation, the Actuator Output to the Heating Blanket must be limited. In the table below, the maximum Actuator Output value is presented, depending on the working volume.

Reactor Volume	Working Volume	Actuator	Maximum Actuator Output	Max. Working Temperature
3L	1.0 L	Heating Blanket 80W	50%	45 °C
	1.5 L		50%	45 °C
	2.0 L		70%	50 °C
	2.5 L		80%	55 °C



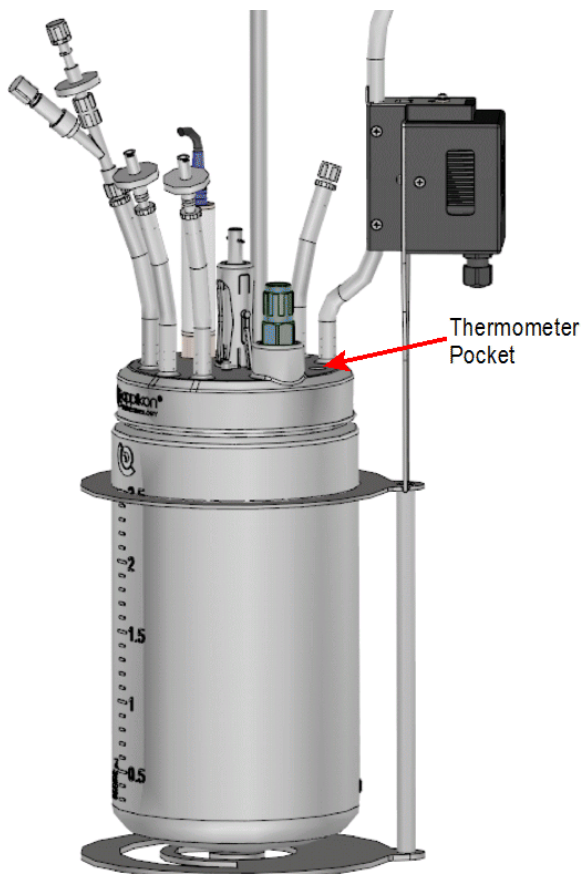
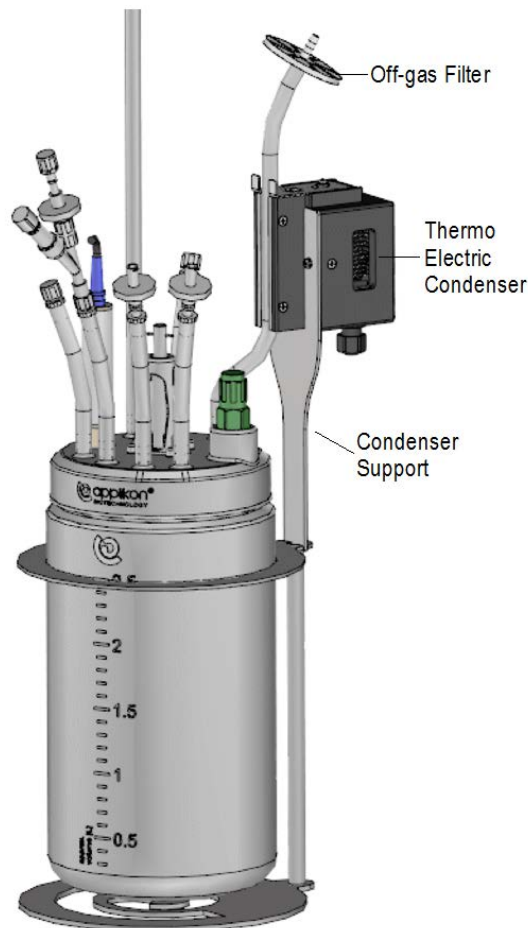
Limiting the Actuator Output can be achieved by navigating to Home > Controls > Actuators. Maximize the Limit High according to the value in the table above. Also refer to section 4.5.2 of the Software Reference Manual.

3.6.2 MOUNTING THE OFF-GAS TUBING IN THE CONDENSER

Depending on the aeration gas flow, it may be necessary to cool down the off-gas in order to prevent undesired loss of liquid through evaporation. For this purpose, the Thermo Electric Condenser can be used.

The adjacent image shows the reactor with Condenser Support (mounted at the upper ring of the reactor holder) and the Thermo Electric Condenser.

Nudge (gently squeeze) the Off-gas tubing into the Condenser Cooling Groove.



3.6.3 MOUNTING THE TEMPERATURE SENSOR

The top plate of the reactor is equipped with a Thermometer Pocket (ID = 7.5 mm).

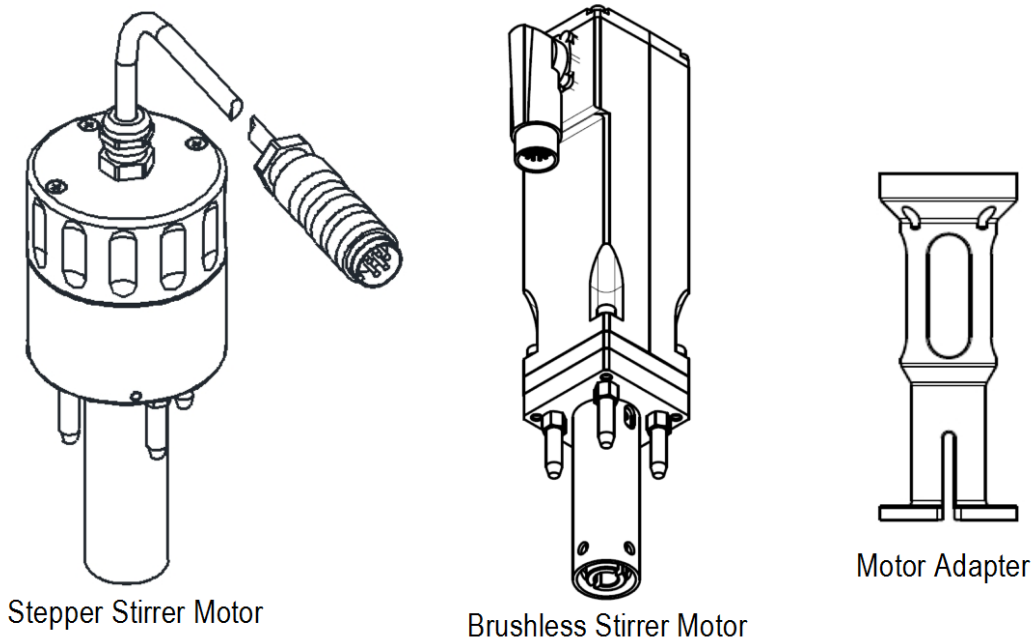
Make sure to fill the Thermometer Pocket with a bit of water in order to improve the heat transfer between the medium and the sensor.

Insert the Pt100 sensor (OD = 6 mm) in the thermometer pocket.

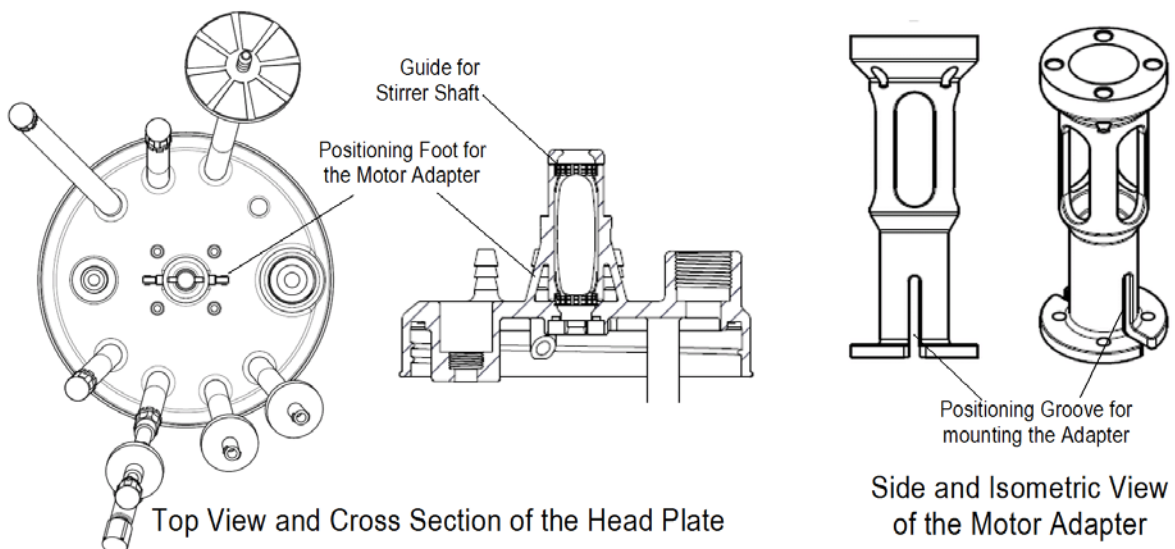
In the image, the Thermometer Pocket is indicated with a red arrow.

3.6.4 MOUNTING THE STIRRER MOTOR

In most cases, the Stepper Stirrer Motor is used in combination with the AppliFlex ST Single Use 3L Bioreactor. If more stirrer power is required, the brushless DC Stirrer Motor M10 can be used. Both motors fit in the Motor Adapter that can be mounted on top of the reactor head plate. The image below shows both motor types and the Motor Adapter:

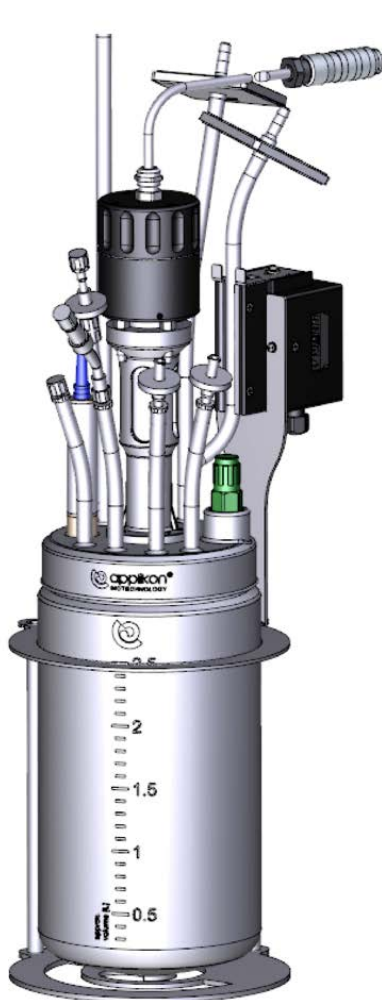


The Stirrer Adapter can be fitted on the Guide for the Stirrer Shaft (see the image below). Mind the Positioning Feet and make sure that they fit in the Adapter Groove.



The four Motor Studs of the Stirrer Motor assembly fit in the four holes of the upper ring of the Motor Adapter. The flexible fork of the Stirrer Motor fits on the top of the Stirrer Shaft.

The image below shows the assembled AppliFlex ST 3L bioreactor with two types of stirrer motors:



Bioreactor Assembly
with Stepper Stirrer



Bioreactor Assembly
with Brushless Stirrer

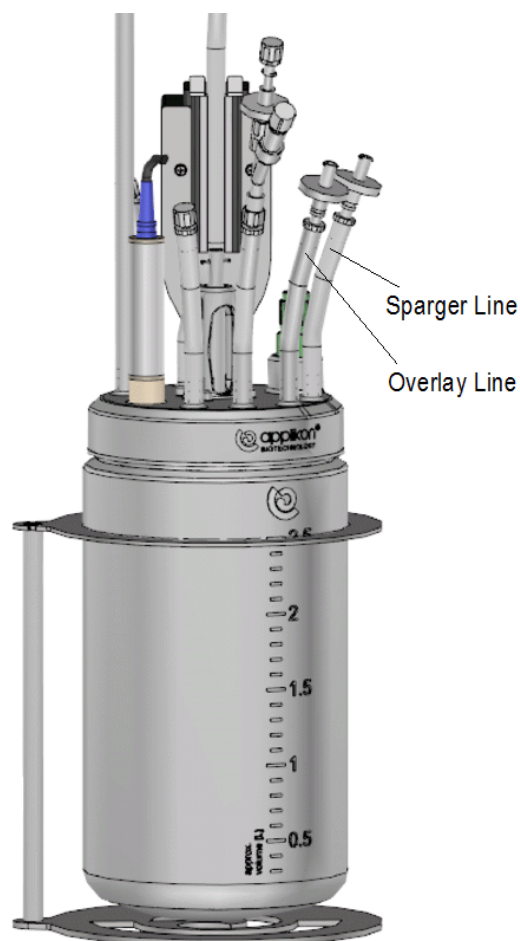
3.6.5 MOUNTING THE AERATION TUBING

Connect the sparger outlet at the front of the *my*-Control cabinet with the sparger inlet of the bioreactor.

If present, connect the overlay outlet at the front of the *my*-Control cabinet with the overlay inlet of the bioreactor. The aeration connection can best be made using flexible tubing.



If one of the aeration lines remains unused, it must be closed with a tubing clamp (e.g. from the Start-up Kit)!



3.6.6 CONNECTING THE CABLES AND THE TUBING TO THE CONTROLLER

- Connect the pH and Temperature sensor cables to the front of the *my*-Control cabinet.
- Connect the USB-connector of the LumiSens sensor to the rear of the *my*-Control cabinet.
- Insert the pump tubing of the liquid addition lines in the pump heads at the front of the *my*-Control cabinet.

3.6.7 CONNECTING THE LEVEL SENSORS TO THE CONTROLLER

If the AppliFlex ST reactor is used for a microbial application, the Level Foam sensors must be connected to the controller.

The Level sensors operate in pairs. One of the sensors is inserted in the medium to such extent that it is always in contact with the medium. The insertion depth of the other sensor can be adjusted to the required level.

Connect the Level sensor Audio connector to the Level sensor input of the controller. Connect the alligator clips at the other end of the cable to the Level sensors in the reactor.

3.6.8 CONNECTING THE COLD WATER ASSEMBLY

Recall the cold-water assembly:



This is a general-purpose assembly and for the AppliFlex ST 3L bioreactor, the condenser valve and ports are not used; only the reactor ports are used.

Connect the **Water from Reactor** and **Water to Reactor** ports to the heat exchanger.

3.7 SETTING UP PROCESS CONDITIONS

When all (electric and hydraulic) connections have been made, the control loops at the my-Control can be started in order to create appropriate process conditions (temperature, pH and dO₂).

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



If, instead of the LumiSens dO₂ sensor, a classic (polarographic) dO₂ sensor is used, this sensor must be calibrated before the dissolved oxygen control is started. This calibration procedure is presented below.

3.7.1 PREPARING FOR ELECTROCHEMICAL dO₂ SENSOR CALIBRATION

Make sure that the power to the *my*-Control cabinet has been switched on and prepare the system for calibration the dO₂ sensor. Procedure:

- Start the Temperature Control loop (make sure that the temperature setpoint and the temperature control parameters are set at the proper value)
- Start the pH Control loop (make sure that the pH setpoint and the pH control parameters are set to the proper value).
- Wait until both the measured temperature and pH values are at setpoint.
- Aerate the bioreactor for at least 20 minutes at maximum air flow while activating the stirrer. Monitor the measured dO₂ value to make sure that the medium is saturated with oxygen (dO₂ sensor reading must reach a stable value).

3.7.2 PERFORMING THE ELECTROCHEMICAL dO₂ SENSOR CALIBRATION

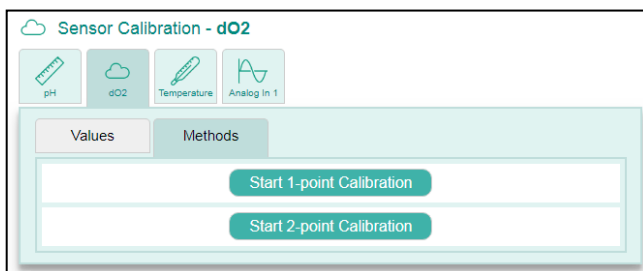
The calibration procedure below describes a 1-point calibration on air saturation (commonly used). If a 2-point sensor calibration is required, refer to the User manual.



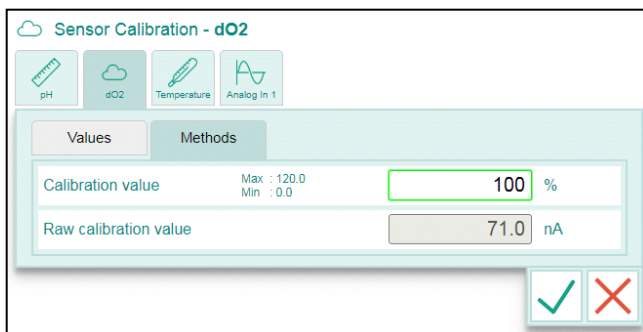
The procedure below uses air for calibrating the dO₂ sensor.
For highly aerobic cultivations, the dO₂ sensor can be calibrated with oxygen instead of air. If so, the measurement range must be set to oxygen. The calibration routine remains the same.

Step 1:
Open the dO₂ Calibration routine and select the Methods tab.

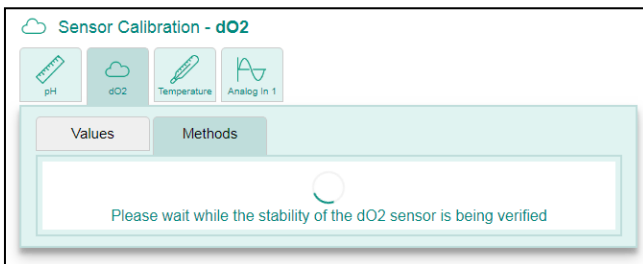
Press the **Start 1-point Calibration** button.





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.



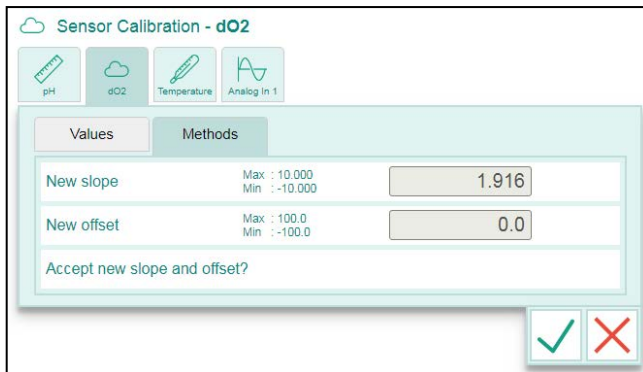
The sensor value is monitored to verify the stability.
As soon as a stable value has been obtained, the calibration routine will be resumed.




Step 3:
The obtained slope value for the dO₂ 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 dO₂-sensor quality

It is advised to measure the sensor signal at 0% dO₂ saturation in order to verify the sensor performance.

Use the procedure below:

- Purge the bioreactor with nitrogen for 20 to 30 minutes until all oxygen is driven off.
- Monitor the dO₂ value, shown on the dO₂ Process Value button.
 In case the dO₂ value does not drop below 0.5%, the sensor requires maintenance after this cultivation run. Refer to the User Manual of the dO₂ sensor.




It is good practice to record the verification measurements of the dO₂ sensor (measured value at 0% air saturation); in this way, preventive maintenance of the dO₂ sensor can be anticipated.

- After finalizing the dO₂ sensor verification, close the N₂ valve and start the dO₂ control in order to create optimum process conditions.

3.8 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  button at the bottom of the Actuator section to set all Dose Monitor values to 0.

4 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.

4.1 FIXED PID CONTROL VERSUS ADAPTIVE CONTROL

The pH, Temperature and dO₂ 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.



4.2 INOCULATION

The bioreactor needs to be inoculated in order to start the cultivation. Different procedures can be used to do so. One option is described below as an example.

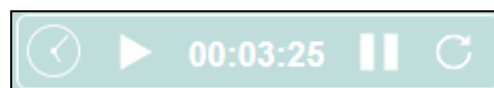
- Switch off all control loops at the *my-Control*,
- Disconnect the pH and LumiSens sensor cables from the sensors,
- Remove the Pt-100 temperature sensor from the thermometer pocket,
- Remove the pump tubing from the pumps at the *my-Control* cabinet,
- Remove the off-gas tubing from the Thermo Electric condenser,
- Close the sparger tubing with a pinch valve and disconnect the sparger tubing at the gas inlet filter,
- Spray the AppliFlex ST Single Use Bioreactor with 70% ethanol and move it to the BSC,
- Use one of the addition lines to add the inoculum,
- Put the bioreactor back alongside the *my-Control* cabinet,
- Redo all cable and tubing connections, put the stirrer motor in place and insert the Pt-100 temperature sensor in the thermometer pocket.
- Start all configured control loops at the *my-Control* and start the Process Timer




Immediately after inoculating the bioreactor, the Process Timer must be started in order to keep track of the process duration. 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:

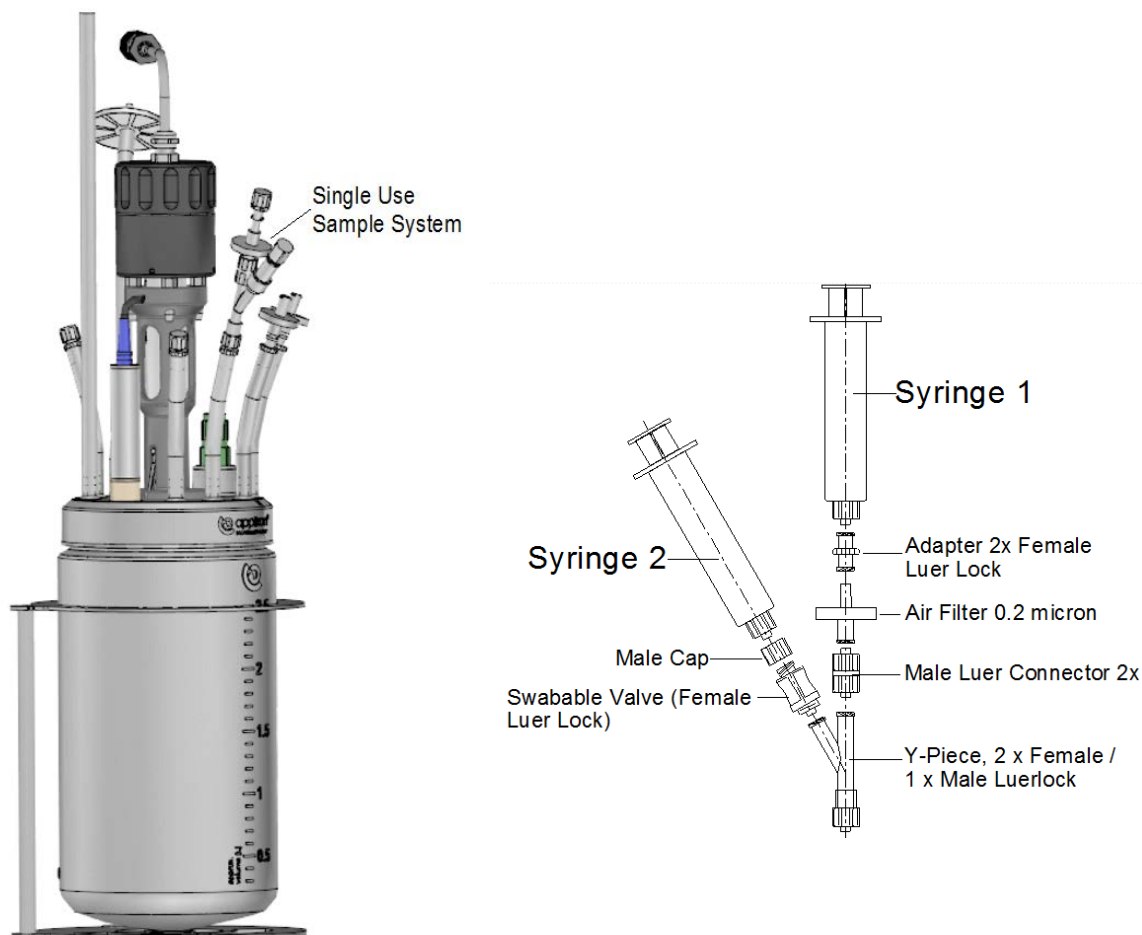


Pressing the Pause icon () will hold the running Process Timer.

The cultivation has now been started.

4.3 SAMPLING

The Single Use Sample System is fixed on top of the Sample Pipe. It consists of a Y-piece with two Luer Lock syringe connections.



Syringe 1 is used to remove medium from the Sample Pipe (in order to prevent taking “old” sample). Syringe 2 is used to withdraw sample from the Single Use Bioreactor.

Sampling Procedure:

- 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.

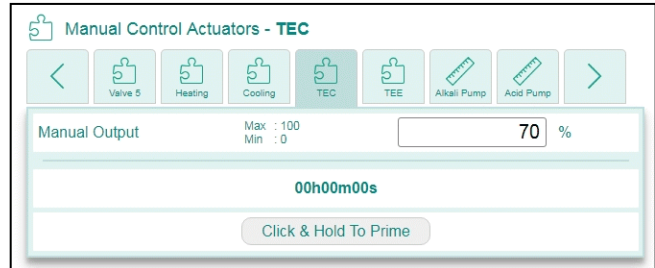
4.4 SWITCHING OFF THE THERMO ELECTRIC CONDENSER

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 (),

followed by  and select the TE Condenser.
Set the output % to 0.

Press the  button to confirm the setting.



5 HARVESTING, DEACTIVATION, CLEANING

After finishing the fermentation or cultivation process, the reactor content may be harvested via the Sample Pipe. Next, the micro-organisms in the broth must be deactivated and the reusable sensors must be cleaned. These actions are described in the sections below.

It is also possible to replace the reactor top plate by a blind cap and keep it in a refrigerator at 4°C (or at -20°C or even at -80°C).

5.1 DEACTIVATION

Before the broth can be disposed of, it may be necessary to deactivate the micro-organisms. One of the possible options to do this, is autoclaving the AppliFlex ST reactor. Procedure:

- Disconnect the pH sensor cable from the sensor head and cover the sensor head by mounting the sensor cap.
- Remove the LumiSens sensor head and fiber optic from the sensor tip. Cover the sensor tip with its End Cap.
- Disconnect the Stirrer Motor from its shaft and remove it from the reactor.
- If a Thermo Electric Condenser is used, remove the Off-gas tubing from the Cooling Groove in the condenser.
- Disconnect all hydraulic Luer Lock connections at the top plate of the reactor and mount the corresponding counter parts (cap nuts).
- Remove the temperature sensor from the temperature pocket.
- Remove the pH sensor and LumiSens sensor tip from the top plate, put them in an autoclavable sensor holder or bag and cover the sensor ports in the top plate with the disposable plugs.
- Put the reactor (with closed sensor ports) in another autoclavable bag.
- Remove as much air as possible from the autoclavable bag(s) and seal it/them.
- Place the sealed autoclavable bags and sterilize it at a temperature of 121 °C for 20 minutes. Depending on the type of organism, a lower temperature may do; refer to the applicable Standard Operating Procedures.
- After cooling down the autoclave, the sensors and the reactor can be removed from the sealed autoclavable bags.



Take care: due to thermal deformation of the Applikon ST reactor, it will probably be leaking!

- The sensors must be cleaned according to the instructions below. The reactor content and the reactor itself may be disposed of according to the relevant S.O.P.s.

5.2 SENSOR CLEANING

The pH and LumiSens sensors, that have been removed from the deactivated AppliFlex ST reactor, must be cleaned thoroughly. Refer to the cleaning instructions below.

5.2.1 CLEANING THE pH SENSOR

It is very important to clean the pH-sensor thoroughly. Make sure that no broth-residue remains on the membrane surface or diaphragm. Rinse the sensor body thoroughly with tap- or de-ionized water to clean the sensor before storage.

Store the sensor in a KCl (c = 3M) solution using the watering cap. In this way the pH membrane is kept wet and the diaphragm is conditioned. Do not allow the sensor (pH membrane & diaphragm) to dry out, as this will compromise the performance of the sensor.

Bad performance of the sensor may be the result of repetitive autoclaving and/or contamination of the reference diaphragm by either protein or sulphide precipitation. In this may be the case, the pH sensor can be cleaned with either a Protein Cleaning Solution (containing pepsin / HCl) or with a Sulphide Cleaning Solution (containing thiourea / HCl).

Decreased general performance of the pH sensor may be reversed by treating it with an Etching Solution (containing HF / HCl).

For information concerning these cleaning solutions, refer to the User Manual that comes with the pH sensor.

5.2.2 CLEANING THE LUMISENS dO₂ SENSOR

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.

5.2.3 CLEANING THE ELECTROCHEMICAL dO₂ SENSOR (OPTIONAL SENSOR)

Clean the sensor body and membrane of the dO₂ sensor. Make sure that no broth-residue remains on the membrane surface. Rinse the sensor body thoroughly with tap- or de-ionized water to clean the sensor before storage. For short intervals (a few days), the dO₂ sensor can be stored dry (membrane module is filled with electrolyte).

Storage instructions for longer intervals:

- For intervals up to 6 months, the sensor can be stored with the protective cap on the membrane module (the membrane module is filled with electrolyte).
- For storage longer than 6 months, the sensor should be stored dry: empty the membrane module of electrolyte and rinse the membrane module & inner body of the sensor with d.i. water. The membrane module can best be screwed loosely on the sensor so the membrane is not under tension.

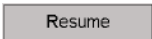

In case the sensor has been stored dry, do not forget to refill the membrane module with electrolyte before use.

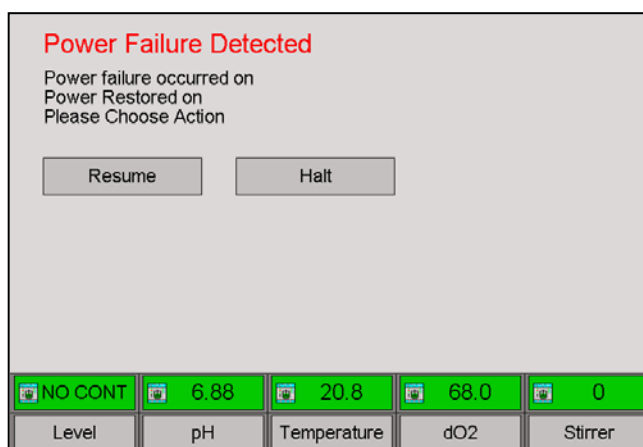
For general information concerning cleaning and maintenance of the dO₂ sensor (including replacing and refilling the membrane module), refer to the User Manual that comes with the dO₂ sensor.

6 SYSTEM WARNINGS





6.1 POWER FAILURE DURING OPERATION

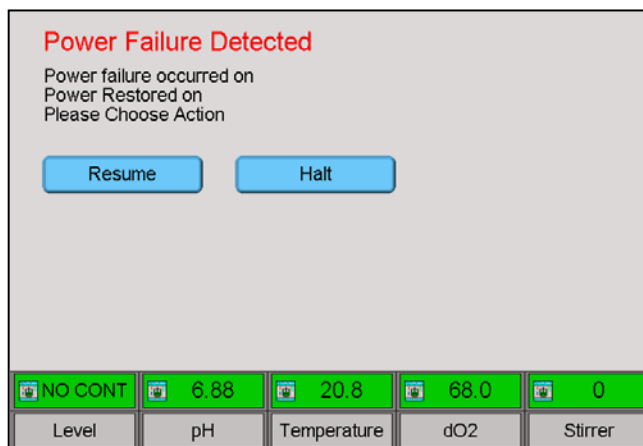
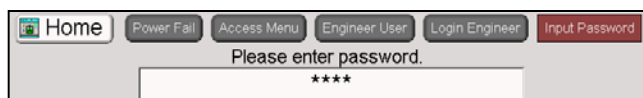
In case during a running process (running controllers) a power failure occurs that takes longer than the brown out time, the ez2-Control will detect this event. It will restart with a Power Fail message.

The  and  buttons are disabled (Engineer rights are required to resume).



Restart after power failure

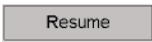

1. Log in as Engineer.
2. The  and  buttons are now enabled.
3. Before pressing the  button, make sure that the bioreactor is in a safe condition to restart the process.
4. Pressing the  button will turn the ez2-Control into an idle mode (no running control loops).



6.2 EMERGENCY STOP DURING OPERATION

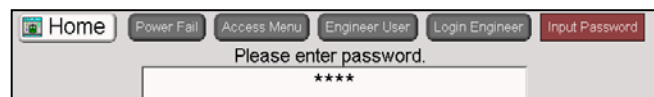
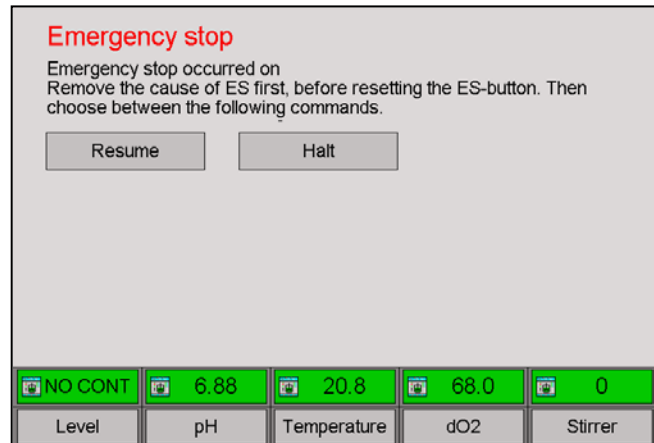
In case during operation the Emergency Stop button was pressed, control is stopped immediately. However, measuring will be continued.





The Emergency Stop screen will be presented.

The  and  buttons are disabled.

Restart after emergency stop

1. Remove the cause of the Emergency Stop in order to return to a stable process.
2. Now turn the Emergency Stop button clockwise until it resets itself to the normal position.
3. Log in as Engineer.



5. The  and  buttons are now enabled.
6. Before pressing the  button, make sure that the bioreactor is in a safe condition to restart the process.
7. Pressing the  button will turn the ez2-Control into an idle mode (no running control loops).

