

SOP: Applikon ez-Control Bioreactor Controller Operation

Approvals

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1. Purpose

- 1.1. Cultivate a cell culture using an Applikon ez-Control bioreactor controller and a 3-liter glass autoclavable bioreactor.

2. Scope and Applicability

- 2.1. A bioreactor controller is used to measure and control process variables (temperature, pH, dissolved oxygen, stirrer speed, and so on) within a bioreactor vessel such as a glass autoclavable bioreactor, a single use bioreactor, or a single use wave bag. This SOP provides the basic steps required to cultivate a cell culture using the Applikon ez-Control and a 3-liter glass autoclavable bioreactor. Other process SOPs are intended to provide additional details such as culture medium composition and volume, control process settings, and run time.

3. Summary of Method

- 3.1. Preparing the controller
 - 3.1.1. Power up the controller and login
 - 3.1.2. Enter the project name (optional)
 - 3.1.3. Emergency stop and resume (emergency only)
- 3.2. Preparing the bioreactor
 - 3.2.1. Fill the bioreactor with culture medium
 - 3.2.2. Calibrate the pH sensor
 - 3.2.3. Mount sensors to the bioreactor
 - 3.2.4. Prepare the liquid addition bottles
 - 3.2.5. Mount connections to the bioreactor
 - 3.2.6. Autoclave the bioreactor and addition bottles
- 3.3. Connecting the bioreactor to the controller
 - 3.3.1. Verify that all control loops are switched off
 - 3.3.2. Connect the sensors
 - 3.3.3. Connect the heating blanket
 - 3.3.4. Connect the aeration
 - 3.3.5. Connect the stirrer motor
 - 3.3.6. Connect the alkaline bottle
 - 3.3.7. Enter process parameter settings
 - 3.3.8. Start all control loops
 - 3.3.9. Calibrate the DO sensor
 - 3.3.10. Reset dose monitor values
- 3.4. Starting the cultivation
 - 3.4.1. Start process data acquisition
 - 3.4.2. Inoculate the bioreactor
- 3.5. Monitoring the cell culture
 - 3.5.1. View process data

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- 3.5.2. Sample the cell culture
- 3.6. Harvesting the cell culture
 - 3.6.1. Stop the controllers
 - 3.6.2. Save process data
 - 3.6.3. Disconnect the bioreactor
 - 3.6.4. Decant the cell culture
- 3.7. Cleaning the bioreactor
 - 3.7.1. Clean the sensors
 - 3.7.2. Pre-clean the bioreactor in place
 - 3.7.3. Disassemble the bioreactor and clean all parts
 - 3.7.4. Clean the porous sparger tip (optional)
- 4. References**
 - 4.1. ez-Control Hardware Manual for Autoclavable Bioreactors, Applikon Biotechnology, Hardware version 2, Documentation version 1.10.
 - 4.2. ez-Control Operator Manual for Autoclavable Bioreactors, Applikon Biotechnology, Software version 1.6X, Documentation version 1.0.
 - 4.3. ez-Control Software Reference Manual, ez-Control for Autoclavable and Single Use Applications, Applikon Biotechnology, Software version 1.6X, Documentation version 1.0.
 - 4.4. BioXpert Lite Data Acquisition Program User Manual, Applikon Biotechnology, Software version 1.1X, February 2010.
 - 4.5. Tuttnauer 3850 ELV Autoclave Operation, document number 14.02.01, effective date November 25, 2013
 - 4.6. Labconco Purifier Class 2 Biosafety Cabinet SOP
- 5. Precautions**
 - 5.1. Alkaline solutions used for controlling pH are caustic. Read the Material Safety Data Sheet (MSDS) for hazards, handling and storage information. Wear personal protection equipment (PPE).
 - 5.2. Do not put the stirrer motor or the heating blanket in the autoclave.
- 6. Responsibilities**
 - 6.1. It is the responsibility of the course instructor/lab assistant to ensure that this SOP is performed as described and to update the procedure when necessary.
 - 6.2. It is the responsibility of the students/technician to follow the SOP as described and to inform the instructor about any deviations or problems that may occur while performing the procedure.
- 7. Equipment and Materials**
 - 7.1. Applikon 3-liter glass autoclavable bioreactor:
 - 7.1.1. Vessel
 - 7.1.2. Head plate
 - 7.1.3. Gas sparger (pre-mounted on the head plate)
 - 7.1.4. Impeller (pre-mounted on the head plate)
 - 7.1.5. Air outlet condenser (pre-mounted on the head plate)
 - 7.1.6. Septum (pre-mounted on the head plate)
 - 7.1.7. Sample pipe (pre-mounted on the head plate)
 - 7.1.8. pH sensor

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- 7.1.9. DO sensor
 - 7.1.10. Temperature sensor
 - 7.1.11. Stirrer motor
 - 7.1.12. Heating blanket
 - 7.2. Applikon ez-Control bioreactor controller
 - 7.3. Laboratory gasses:
 - 7.3.1. Air compressor
 - 7.3.2. O₂ (optional)
 - 7.3.3. CO₂
 - 7.4. pH 4.0 and pH 7.0 buffer standards
 - 7.5. O₂ Electrolyte solution for DO probe
 - 7.6. 10X PBS
 - 7.7. Liquid addition bottles
 - 7.8. Alkaline solution (generally 1M sodium bicarbonate)
 - 7.9. Gas filters, 0.2 µm
 - 7.10. Autoclavable silicone tubing, size 14 (1.6 mm interior diameter)
 - 7.11. Autoclavable silicone tubing, size 16 (3.1 mm interior diameter)
 - 7.12. Autoclavable silicone tubing, size 25 (4.8 mm interior diameter)
 - 7.13. Tubing clamps
 - 7.14. Autoclavable Male and female connectors
 - 7.15. aluminum foil (for autoclaving)
 - 7.16. Autoclave indicator tape
 - 7.17. Culture medium
 - 7.18. Culture inoculum
 - 7.19. Autoclave (such as a Tuttnauer 3850 ELV)
 - 7.20. Computer system with BioXpert Lite installed
- 8. Procedure**
- 8.1. *Preparing the controller*
 - 8.1.1. **Power up the controller and login**

The controller is used to measure and control process variables (temperature, pH, dissolved oxygen, and stirrer speed) within a bioreactor. After switching on the power of the controller, it presents itself (after initialization) on the touch screen display with its Home screen, which is generally configured as the Synoptic View shown in Figure 2. The operator uses the touch screen display to monitor the bioreactor conditions and to enter process control parameters. Four authorization (login) levels allow access to various controller capabilities: View (initial level), Operator, System engineer, and Service engineer level. The View authorization level only allows process values to be monitored. To set process control parameters, the user needs to login as Operator. For an example of how to navigate the controller screens, see Example 1.

 - 8.1.1.1. Power up the controller using the green power switch located on the back of the controller (upper right).
 - 8.1.1.2. The touch screen display located on the front shows that the controller is initializing. Once initialization completes, the display switches to the Home screen.
 - 8.1.1.3. Login as Operator using the touch screen display:

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- 8.1.1.3.1. Home > View> Operator > Login
 - 8.1.1.3.2. Enter the Operator password: 0000
- 8.1.1.4. To logout:
Home > login button (top middle) > View
Note: logout is automatic after a period of inactivity (generally 10 minutes).
- 8.1.1.5. The touch screen backlight is switched off automatically after a period of inactivity (generally 30 minutes) and the touch screen goes dark. To switch on the backlight, touch the screen.
- 8.1.2. Enter the project name (optional)**
The project name is displayed on the top of all controller screens in order to identify the bioreactor system and cell culture being cultivated.
- 8.1.2.1. Login as Operator per section 8.1.1.3 if not already logged in.
 - 8.1.2.2. Home > project name button (top left)
 - 8.1.2.3. Enter a descriptive name
- 8.1.3. Emergency stop and resume (emergency only)**
The Emergency Stop button on the front of the control shuts down all control loops immediately and displays the Emergency Stop screen. Measurements continue to be collected.
- 8.1.3.1. In the event of an emergency requiring the bioreactor process to stop immediately, press the red Emergency Stop button located on the front of the controller.
 - 8.1.3.2. To resume the bioreactor process:
 - 8.1.3.2.1. Remove the cause of the Emergency Stop.
 - 8.1.3.2.2. Turn the Emergency Stop button clockwise until it resets itself to the normal position.
 - 8.1.3.2.3. Login as System Engineer.
 - 8.1.3.2.4. Verify that the bioreactor is in a safe condition.
 - 8.1.3.2.5. Select Resume on the Emergency Stop screen to restart the control loops.
- 8.2. Preparing the bioreactor**
- 8.2.1. Fill the bioreactor with culture medium**
The bioreactor is filled with culture medium before it is autoclaved (if it will not damage the media) so that the medium is sterilized along with the bioreactor. Do not exceed the working volume of the bioreactor (2.4 liters for a 3-liter bioreactor). Leave enough space for inoculation and nutrients to be added during cultivation. For mammalian cell culture the media is added to the bioreactor after autoclaving. 100 ml of 1X PBS is added to the bioreactor before autoclaving.
- 8.2.1.1. A process SOP should provide details regarding culture medium composition and volume. For example refer to section 8.5 of Batch culture of Recombinant tPA Secreting CHO cells.
 - 8.2.1.2. Loosen the six mill nuts that fasten the head plate on the bioreactor vessel and remove the head plate.
 - 8.2.1.3. Add 100 ml of 1X PBS to the vessel.
 - 8.2.1.4. Verify that the sample pipe, sparger pipe and thermowell are attached to the underside of the Headplate .

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8.2.1.5. Mount the head plate on top of the vessel and fasten with the six mill nuts finger-tight.

8.2.2. *Calibrate the pH sensor*

Before the bioreactor is autoclaved, the pH (acidity) sensor needs to be calibrated with pH 4.0 and pH 7.0 buffer standards in order to obtain accurate measurement values during cultivation.

8.2.2.1. Measure the temperature of the pH buffer standards using a thermometer. Record the temperature in the Batch Record.

8.2.2.2. Remove the protective cap from the bottom of the pH sensor. Rinse the pH sensor with de-ionized water and pat it dry with a clean lint-free laboratory wipe.

8.2.2.3. Remove the pH sensor screw cap. Connect the pH sensor to the pH sensor cable on the right side of the controller. Verify that the pH sensor cable is plugged into the controller correctly.

8.2.2.4. Login as Operator per section 8.1.1.3 if not already logged in.

8.2.2.5. Go to the controller pH Settings screen: Home > pH (bottom)

8.2.2.6. Verify that the pH control loop is off (i.e. the pH Process Value button is grey or yellow, not green). If it is on, touch the button Stop pH controller.

8.2.2.7. Touch the button Calibrate pH to go to the pH Calibration screen. The numerical data for Slope, Offset and any Sample correction are displayed.

8.2.2.8. Touch the button 2-point calibration.

8.2.2.9. Enter the temperature of the buffer solutions using the numeric keypad.

8.2.2.10. When prompted for the pH value of the first buffer solution, put the pH sensor in the pH 4.0 buffer standard and wait until the shown process value stabilizes (shown near the Cancel button). Enter the pH value using the numeric keypad.

8.2.2.11. Rinse the pH sensor and repeat using the pH 7.0 buffer standard. Again, wait for the shown process value to stabilize and enter the corresponding pH.

8.2.2.12. Return to the pH Calibration screen to verify the newly found calibration data (slope and offset). Record the slope and offset in the Batch Record.

8.2.3. *Mount sensors to the bioreactor*

pH and DO sensors are mounted to the bioreactor before it is autoclaved so that the sensors are sterilized along with the bioreactor. Some systems also use a foam / level sensor.

8.2.3.1. Disconnect the cable of the pH sensor

8.2.3.2. Cover the pH sensor connector with the pH sensor screw cap. Verify that the rubber gasket is in place between the sensor connector and the cap.

8.2.3.3. Insert the pH sensor into its port in the head plate and fasten it. See Figure 6 for the location of the pH sensor port.

8.2.3.4. Remove the protective cap from the bottom of the DO sensor.

8.2.3.5. Inspect the screen at the bottom of the probe tip. Replace if damaged.

8.2.3.6. Holding the probe in a vertical position, unscrew the membrane module from the bottom of the probe.

8.2.3.7. Inspect the integrity of the O-ring underneath the module and replace if worn or cracked.

8.2.3.8. Replenish DO electrolyte. There should be 1 ml of O₂ electrolyte solution in membrane module.

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8.2.3.9. Screw the membrane module to the probe.

8.2.3.10. Repeat steps 8.2.3.2 and 8.2.3.3 for the DO sensor.

8.2.4. Prepare the liquid addition bottles

Liquid addition bottles are used to add liquids to the bioreactor aseptically (see Figure 7).

For example, alkaline solution is added to raise pH and inoculum is added to begin cultivation. Addition bottles are sterilized along with the bioreactor.

8.2.4.1. A Batch Culture of the process SOP should provide details regarding alkaline solution composition and volume.

8.2.4.2. Fill a liquid 250ml addition bottle with 150 ml alkaline solution. Cap the alkaline bottle with a two-port top.

8.2.4.3. Connect the air inlet on the alkaline bottle to a gas filter using a short length (approx. 7 cm) of size 25 tubing. Do not clamp.

8.2.4.4. Add 5 mL laboratory grade water to a 500 ml addition bottle to be used for transferring inoculum. This will improve the heat transfer during sterilization in the autoclave. Cap the inoculum transfer bottle with a two-port top.

8.2.4.5. Connect the air inlet on the inoculum transfer bottle to a gas filter using a short length (approx. 7 cm) of size 25 tubing. Do not clamp.

8.2.4.6. Connect the liquid outlet on the inoculum transfer bottle to an autoclavable male connector using a long length (approx. 75 cm) of size 25 tubing.

8.2.4.7. Cover the gas filters and autoclavable male connector loosely with aluminum foil.

8.2.4.8. Repeat steps 8.2.4.4. to 8.2.4.7. with 1L media addition bottle.

8.2.5. Mount connections to the bioreactor

Gas filters and silicone tubing connections are mounted to the bioreactor before it is autoclaved so that they are sterilized along with the bioreactor. See section 7 for the silicone tubing size specifications.

8.2.5.1. Connect one of the medium inlet triplet nipples to a second triplet nipple using a short length (approx. 7 cm) of size 14 tubing. Connect a medium length (approx. 15 cm) of size 14 tubing to the third medium inlet triplet nipple. Clamp the tubing closed by folding the end onto itself.

8.2.5.2. Connect the addition pipe to the liquid outlet on the alkaline bottle using an extra-long length (approx. 75 cm) of size 25 tubing. Clamp the tubing closed.

8.2.5.3. Connect a medium length (approx. 15 cm) of size 25 tubing to the sample pipe. Attach a female connector to the tube. Clamp the tubing closed.

8.2.5.4. Connect the sparger inlet to a gas filter using an approx. 60 cm length of size 25 tubing. Do not clamp.

8.2.5.5. Connect the bottom condenser nipple on the middle condenser nipple using a medium length (approx. 15 cm) of size 25 tubing.

8.2.5.6. Connect the top condenser nipple to a gas filter using a medium length (approx. 50 cm) of size 25 tubing. Do not clamp.

8.2.5.7. Insert a septum into its holder in the head plate and fasten it.

8.2.5.8. Cover all the tubing and head plate with aluminum foil.

8.2.5.9. Verify that the gas filters are open to avoid pressure differences during autoclaving. Cover the gas filters loosely with aluminum foil.

8.2.6. Mount connections to the bioreactor

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Gas filters and silicone tubing connections are mounted to the bioreactor before it is autoclaved so that they are sterilized along with the bioreactor. See section 7 for the silicone tubing size specifications.

- 8.2.6.1. Connect one of the medium inlet triplet nipples to a second triplet nipple using a short length (approx. 7 cm) of size 14 tubing. Connect a medium length (approx. 15 cm) of size 14 tubing to the third medium inlet triplet nipple. Clamp the tubing closed by folding the end onto itself.
- 8.2.6.2. Connect the addition pipe to the liquid outlet on the alkaline bottle using an extra-long length (approx. 75 cm) of size 25 tubing. Clamp the tubing closed.
- 8.2.6.3. Connect a medium length (approx. 15 cm) of size 25 tubing to the sample pipe. Attach a female connector to the tube. Clamp the tubing closed.
- 8.2.6.4. Connect the sparger inlet to a gas filter using an approx. 60 cm length of size 25 tubing. Do not clamp.
- 8.2.6.5. Connect the bottom condenser nipple on the middle condenser nipple using a medium length (approx. 15 cm) of size 25 tubing.
- 8.2.6.6. Connect the top condenser nipple to a gas filter using a medium length (approx. 50 cm) of size 25 tubing. Do not clamp.
- 8.2.6.7. Insert a septum into its holder in the head plate and fasten it.
- 8.2.6.8. Cover all the tubing and head plate with aluminum foil.
- 8.2.6.9. Verify that the gas filters are open to avoid pressure differences during autoclaving. Cover the gas filters loosely with aluminum foil.

8.2.7. Autoclave the bioreactor, alkaline addition bottle and liquid addition bottles

The assembled bioreactor is autoclaved before cultivation in order to create a sterile environment inside the bioreactor. Do not autoclave the heating blanket or the stirrer motor. Single use bioreactors are not autoclaved; they are sterilized by the manufacturer.

- 8.2.7.1. Apply autoclave indicator tape to the aluminum foil on the alkaline bottle and the inoculum transfer bottle.
- 8.2.7.2. Place the assembled bioreactor and the alkaline bottle without disconnecting the tubing, the inoculum transfer bottle and 1L addition bottle in the autoclave.
- 8.2.7.3. Loosen the caps on the alkaline bottle, liquid addition bottle and the inoculum transfer bottle.
- 8.2.7.4. Close the autoclave and run it on the liquid cycle at 121°C for 20 minutes, using a slow exhaust cycle per the autoclave SOP.
- 8.2.7.5. When the cycle completes, allow the autoclave to cool gradually. Do not open the autoclave until the temperature in the autoclave has dropped below 90°C. After reaching that temperature, open the autoclave to allow it to cool down until the contents can be unloaded safely.
- 8.2.7.6. Tighten the caps on the alkaline bottle and the inoculum transfer bottle.
- 8.2.7.7. Remove the assembled bioreactor and the alkaline bottle together and place beside them on the right side of the controller without disconnecting the tubing.
- 8.2.7.8. Remove the inoculum transfer bottle and the liquid addition bottle and place it in a biological safety cabinet.
- 8.2.7.9. Allow to cool to room temperature.
- 8.2.7.10. Perform a visual inspection to verify that the autoclave indicator tape

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changed color and that the bioreactor is dry.

- 8.2.7.11. Remove aluminum foil from the gas filters on the bioreactor and alkaline bottle. Leave the foil on the inoculum transfer bottle and the addition bottle.
- 8.2.7.12. Place the bioreactor and alkaline bottle on the right side of the controller.

8.3. Connecting the bioreactor to the controller

After the assembled bioreactor is autoclaved, it must be connected to the controller.

8.3.1. Verify that all control loops are switched off

- 8.3.1.1. Login as Operator per section 8.1.1.3 if not already logged in.
- 8.3.1.2. Verify that all control loops are switched off. The Process Value buttons (bottom of Home Screen) should be gray or yellow. If necessary, stop controllers:
Home > Menu (top right) > Start/Stop all controllers > Stop all controllers

8.3.2. Addition of the media to the Bioreactor

If mammalian cells are used for the cell culture production bioreactor is autoclaved without addition of the media. The media is added to the bioreactor using the 1L addition bottle.

- 8.3.2.1. Refer to the Batch culture SOP for the details regarding the media composition
- 8.3.2.2. Spray or swab the required material with 70% ethanol, and place in the biological safety cabinet.
- 8.3.2.3. Aseptically add the media component to the prepared 1L addition bottle with the tubing and autoclavable male connector attached.
- 8.3.2.4. Be sure the cap is on tightly and remove the 1L addition bottle with tubing and autoclavable male connector attached and bring it over to applikon bioreactor.
- 8.3.2.5. Carefully remove the foil from the female connector on the sample port of the Applikon bioreactor.
- 8.3.2.6. Carefully remove the aluminum foil from the male connector on the 1 L addition bottle and connect the male connector to the female connector on the sample port of the bioreactor.
- 8.3.2.7. Remove the clamp on the female connector on the addition port of the Applikon bioreactor.
- 8.3.2.8. On the Applikon reactor touch screen select Menu. Manual Control> Acid Pump On
- 8.3.2.9. As the pump turns feed the tubing around it. Use care to avoid pinching fingers.
- 8.3.2.10. Once all of the media has been transferred into vessel turn off the acid pump.
On the Applikon screen select Menu>Manual control>Acid Pump Off
- 8.3.2.11. Disconnect the male connector of the addition bottle from the female connector on the sample port of the bioreactor. Bend the tubing of the addition port and reattach the clamp.

8.3.3. Connect the sensors

Sensors are connected electrically to the controller.

- 8.3.3.1. Place the bioreactor and alkaline bottle on the right side of the controller if they are not already.
- 8.3.3.2. Remove the pH sensor screw cap. Connect the pH sensor to the pH sensor cable on the right side of the controller. Verify that the pH sensor cable is plugged into the controller correctly.
- 8.3.3.3. Remove the DO sensor screw cap. Connect the DO sensor to the DO sensor cable on the right side of the controller. Verify that the DO sensor cable is plugged into the

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controller correctly.

- 8.3.3.4. Insert the temperature sensor into the thermometer pocket. Verify that the temperature sensor cable is plugged into the controller correctly. Fill the thermometer pocket with MilliQ water in order to decrease the dead time of the sensor and make temperature control more accurate.

8.3.4. Connect the heating blanket

An electric heating blanket is used for warming the bioreactor as needed. Some systems also use a thermo circulator and cooling water for cooling the bioreactor.

- 8.3.4.1. Wrap the heating blanket around the bioreactor vessel (around the glass and inside the support legs). Position the blanket so that the volume markings on the vessel are visible. Fasten the blanket in place using the Velcro ends of the blanket.

- 8.3.4.2. Verify that the heating blanket is plugged into the controller correctly.

8.3.5. Connect the aeration

Laboratory gasses (air, O₂, and CO₂) are added by the controller to the bioreactor sparger in order to control DO and to lower pH as needed.

- 8.3.5.1. Connect the aeration outlet of the controller to the gas filter on the bioreactor sparger inlet using size 16 tubing.

- 8.3.5.2. Open the CO₂ tank and set its regulator to 10 psi.

8.3.6. Connect the stirrer motor

The stirrer motor and the impeller are used to control agitation of the cell culture. When connecting the stirrer motor, it is helpful for the stirrer to be on.

- 8.3.6.1. Login as Operator per section 8.1.1.3 if not already logged in.

- 8.3.6.2. Go to the Stirrer Settings screen: Home > Stirrer (bottom left)

- 8.3.6.2 Touch the Alarm limit button>lower alarm limit>Enter 58.>Touch the stirrer set point and enter 60.

- 8.3.6.3. Start the stirrer: touch the button Start Stirrer Controller.

- 8.3.6.4. Position the stirrer motor vertically over the bioreactor head plate and slowly lower it into place. Verify that the impeller is turning.

- 8.3.6.5. Stop the stirrer: touch the button Start Stirrer Controller.

8.3.7. Connect the aeration

Laboratory gasses (air, O₂, and CO₂) are added by the controller to the bioreactor sparger in order to control DO and to lower pH as needed.

- 8.3.7.1. Connect the aeration outlet of the controller to the gas filter on the bioreactor sparger inlet using size 16 tubing.

- 8.3.7.2. Turn on the air compressor and set its regulator to 10 psi.

- 8.3.7.3. Open the CO₂ tank and set its regulator to 10 psi.

8.3.8. Connect the stirrer motor

The stirrer motor and the impeller are used to control agitation of the cell culture. When connecting the stirrer motor, it is helpful for the stirrer to be on.

- 8.3.8.1. Login as Operator per section 8.1.1.3 if not already logged in.

- 8.3.8.2. Go to the Stirrer Settings screen: Home > Stirrer (bottom left)

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- 8.3.6.2 Touch the Alaram limit button>lower alaram limit>Enter 58.>Touch the stirrer set point and enter 60..
- 8.3.8.3. Start the stirrer: touch the button Start Stirrer Controller.
- 8.3.8.4. Position the stirrer motor vertically over the bioreactor head plate and slowly lower it into place. Verify that the impeller is turning.
- 8.3.8.5. Stop the stirrer: touch the button Start Stirrer Controller.
- 8.3.9. Connect the alkaline bottle**
A peristaltic pump is used to dispense measured amounts of alkaline solution from an addition bottle to the bioreactor. The silicone tubing that connects the alkaline bottle to the bioreactor needs to be threaded through the pump. See Figure 8.
- 8.3.9.1. Login as Operator per section 8.1.1.3 if not already logged in. 8.3.8.2. Locate the alkaline pump on the right front panel of the controller. Open the pump cover.
- 8.3.8.3. Locate the tubing that connects the alkaline bottle to the bioreactor. Bend the middle of the tubing into a U shape and hold in one hand. Clip the tubing U into the lower pump clamp.
- 8.3.8.4. Turn the pump on manually:
Home > Menu > Manual control> Alkaline pump: On
- 8.3.8.5. Ease the tubing into the pump as the pump rotors are turning. Use care to avoid pinching fingers.
- 8.3.8.6. Turn the pump off.
- 8.3.8.7. Clip the tubing into the upper pump clamp.
- 8.3.8.8. Close the pump cover.
- 8.3.8.9. Turn the pump on manually and watch the solution being drawn from the bottle into the tubing. When the solution reaches the bioreactor, turn the pump off.
- 8.3.9. Enter process parameter settings**
The pH, temperature, DO, and stirrer control loops should be adjusted to desired process parameter settings. Each control loop has settings for upper alarm limit, lower alarm limit, and setpoint. Additional settings for PID controls may also be set at this time.
- 8.3.9.1. A process SOP should provide details regarding process setpoints and alarm limits.
- 8.3.9.2. Login as Operator per section 8.1.1.3 if not already logged in.
- 8.3.9.3. Go to the pH Settings screen: Home > pH (bottom)
- 8.3.9.4. Enter the process settings for the pH control loop:
- 8.3.9.4.1. Enter the pH upper alarm limit:
pH settings > Alarm limits > Upper alarm limit
Enter the pH upper alarm limit value provided by the process SOP.
- 8.3.9.4.2. Enter the pH lower alarm limit:
pH settings > Alarm limits > Lower alarm limit
Enter the pH lower alarm limit value provided by the process SOP.
- 8.3.9.4.3. Enter the pH setpoint: pH settings > pH setpoint
Enter the pH setpoint value provided by the process SOP.
- 8.3.9.4.4. Enter any additional pH PID controls provided by the process SOP:
pH settings > pH controller setup
- 8.3.9.5. Repeat for each of the temperature and stirrer control loops

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8.3.10. Start pH, Temperature and stirrer control loops

The DO sensor needs to be polarized for at least 6 hours and the temperature and pH need to be stabilized at setpoint before the DO sensor can be calibrated.

- 8.3.10.1. Login as Operator per section 8.1.1.3 if not already logged in.
- 8.3.10.2. Go to controller pH setting screen: Home> pH(bottom)
- 8.3.10.3. Touch button Start pH controller.
- 8.3.10.4. Go to controller temperature setting screen: Home> temp(bottom)
- 8.3.10.5. Touch button Start Temp Controller.
- 8.3.10.6. Go to controller stirrer setting screen: Home> Stirrer (bottom)
- 8.3.10.7. Touch button Start stirrer controller

The Process Value buttons (bottom of Home Screen) should change to green or red, the actuators (heat, pumps, valves, and stirrer) should begin activating, and the impeller should begin turning.

- 8.3.10.8. Allow the process to run for at least 6 hours.

8.3.11. Calibrate the DO sensor

The DO measurement is based on the polarographic principle (Clark-cell). Therefore, the sensor must be polarized for at least 6 hours before it can be calibrated.

- 8.3.11.1. Login as Operator per section 8.1.1.3 if not already logged in.
- 8.3.11.2. Turn on the air compressor and set its regulator to 10 psi.
- 8.3.11.3. Verify that the medium in the bioreactor is stable at process temperature.
- 8.3.11.4. Stop the DO control loop:
Home > DO (bottom) > Stop DO controller
- 8.3.11.5. Go to the DO Calibration screen: Home > DO > Calibrate DO
The numerical data for Slope and Offset are displayed.
- 8.3.11.6. Verify if the measuring range is set to Air. If not, set it for air: Calibrate DO > Set measurement range > Measurement range for air
- 8.3.11.7. Open the aeration valve manually:
Home>DO>Manual control > O2 Valve: ON
- 8.3.11.8. Continue aeration until DO reading is stable (15 to 20 minutes)
- 8.3.11.9. Close the aeration valve manually:
Home > DO > Manual Control > O2 Valve: OFF
- 8.3.11.10. Set the DO calibration value to 100%:
Home > DO > Calibrate DO > Calibrate > Enter Calibration Value > 100
- 8.3.11.11. Return to the DO Calibration screen to verify the newly found calibration data (slope and offset). The expected slope value of the sensor (for measurement range for air) is:
2.0 to 4.0 at 25°C
1.5 to 3.0 at 37 °C
- 8.3.11.12. Start the DO Control loop: DO (bottom) > Start DO controller.

8.3.12. Reset dose monitor values

When all control loops are at set-point, the bioreactor system is ready for 24 hrs media hold to check the contamination. All Dose Monitor values should be reset to 0ml.

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8.3.12.1. Go to the Home screen and verify that the control loops are on. Allow the process to run until all control loops are at set-point.

8.3.12.2. Reset all dose monitor values:

Home > Menu > Dose Monitor > Reset all dose monitors > Are you sure? >

Yes

8.4 Starting the cultivation

8.4.1. Inoculate the bioreactor

Once the process parameters in the bioreactor are at their setpoints, the inoculum is added to the bioreactor aseptically. This SOP makes use of a sterile addition bottle for this purpose.

8.4.1.1. Fill the sterile inoculum transfer bottle with inoculum using aseptic technique per the biosafety cabinet SOP. Do not remove the foil from the gas filter or the autoclavable male connector. Recap the bottle before removing it from the biosafety cabinet. Place the inoculum bottle on the right side of the controller.

8.4.1.2. Login is Operator per section 8.1.1.3 if not already logged in.

8.4.1.3. Stop all control loops.

Home > Menu > Start/Stop all controllers > Stop all controllers

8.4.1.4. Swab gloves with 70% Isopropanol. Spray the female connector on the sample port.

8.4.1.5. Remove the foil from the attached tubing connected to the male connector and the gas filter on the inoculum transfer bottle. Connect the female connector on the sample port of the bioreactor to the male connector of the inoculum bottle.

8.4.1.6. Remove the clamp on the female connector on the addition port of the Applikon bioreactor.

8.4.1.7. On the Applikon reactor touch screen select Menu. Manual Control> Acid Pump On

8.4.1.8. As the pump turns feed the tubing around it. Use care to avoid pinching fingers.

8.4.1.9. Once all of the cell suspension has been transferred into vessel turn off the acid pump. On the Applikon screen select Menu>Manual control>Acid Pump Off

8.4.1.10. Disconnect the male connector of the addition bottle from the female connector on the sample port of the bioreactor. Bend the tubing of the addition port and reattach the clamp.

8.4.1.11. Re-start all control loops:

Home > Menu > Start/Stop all controllers > Start all controllers

8.5. Monitoring the cell culture

8.5.1. View process data

Process values for the last 72 hours are stored in controller memory and can be displayed using the Trend View screen. Process values for the entire run are stored and displayed using BioXpert Lite.

8.5.1.1. To view process data for a specific process parameter using the Trend View:

8.5.1.1.1. Login as Operator per section 8.1.1.3 if not already logged in.

8.5.1.1.2. Home > process value button (2nd row from the bottom)

8.5.1.1.3. Touch the X-axis button (bottom left) in order to change the range of the time axis (between 1 and 72 hours).

8.5.1.1.4. Touch the Y-axis upper limit button (top right) or lower limit button (bottom

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right) in order to change the range of the process value axis.

8.5.2. Sample the cell culture

Periodically, the cell culture is sampled in order to measure cell concentration and perform product assays per the process SOP. Some bioreactors include a sample system connected to the sample pipe which enables cell culture to be drawn into a sample bottle that can then be replaced aseptically. This system simply uses a pipette to draw cell culture from the sample pipe.

8.5.2.1. Locate the clamped tubing connected to the sample pipe on the bioreactor head plate. Connect a pipette to the sample pipe tubing.

8.5.2.2. Release the clamp, withdraw 5 mL of cell culture, and re-clamp. (Sample pipe volume = 4mm ID X 25 cm = 3.2 cm³ = 3.2 mL)

Use this sample for cell counts and product assays per the process SOP.

8.6. Harvesting the cell culture

A process SOP should specify when the cell culture is to be harvested.

8.6.1. Stop the controllers

8.6.1.1. Login as Operator per section 8.1.1.3 if not already logged in.

8.6.1.2. Stop all control loops:

Home > Menu > Start/Stop all controllers > Stop all controllers

8.6.1.3. Close the CO₂ tank.

8.6.1.4. Turn off the air compressor.

8.6.2. Disconnect the bioreactor

The bioreactor must be disconnected from the controller so that the bioreactor can be decanted.

8.6.1.1. Locate the tubing that connects the alkaline bottle to the bioreactor. Clamp the tubing near the bioreactor. Disconnect the tubing from the alkaline bottle and remove the tubing from the controller pump.

8.6.1.2. Lift the stirrer motor from the bioreactor head plate and set the motor aside.

8.6.1.3. Disconnect the gas filter on the bioreactor sparger inlet from the tubing to the aeration outlet of the controller.

8.6.1.4. Unwrap the heating blanket from around the bioreactor vessel and set the blanket aside being sure it is lying flat.

8.6.1.5. Disconnect the pH sensor cable from the pH sensor. Cover the pH sensor connector with the pH sensor screw cap.

8.6.1.6. Repeat step 8.6.2.5 for the DO sensor.

8.7. Cleaning the bioreactor

8.7.1. Clean and store the sensors

8.7.1.1. Remove pH and DO sensors from the bioreactor head plate.

8.7.1.2. Rinse the pH and DO sensors thoroughly with MilliQ water, being careful to remove all broth-residue. Gently pat dry with a clean lint-free laboratory wipe. Spray with 70% IPA and gently pat dry with a clean lint-free laboratory wipe

8.7.1.3. Rinse with MilliQ water and pat dry with a clean lint-free laboratory wipe.

8.7.1.4. Fill the protective cap of the pH sensor 1/2 full with 3M potassium chloride (KCl) solution. Cover the tip of the pH sensor with its protective cap. Verify that the pH electrode is completely immersed in KCl solution.

8.7.1.5. The DO sensor can be stored in an electrolyte solution upright for short term. For

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long term it is stored dry.

8.7.1.6. Cover the pH sensor connector with the pH sensor screw cap. Repeat for the DO sensor.

8.7.2. Decant the cell culture

8.7.2.1. Place the disconnected bioreactor in a biosafety cabinet if specified by the process SOP.

8.7.2.2. Loosen the six mill nuts that fasten the head plate on the bioreactor vessel and remove the head plate.

8.7.2.3. Decant the cell culture into a suitable container per the process SOP.

8.7.2.4. Re-mount the head plate on top of the vessel and fasten with the six mill nuts finger-tight.

8.7.3. Pre-clean the bioreactor in place

8.7.3.1. Fill the bioreactor with a working volume of 0.1M NaOH solution (2.4 liters for a 3-liter bioreactor).

8.7.3.2. Connect the stirrer motor per section 8.3.7.

8.7.3.3. Activate the stirrer at 250 RPM for 30 minutes. Visual check for dissolution of foam, debris and other contamination in the bioreactor.

8.7.3.4. Stop the stirrer. Lift the stirrer motor from the bioreactor head plate and set the motor aside. Drain the bioreactor

8.7.4. Disassemble the bioreactor and clean all parts

8.7.4.1. Remove all tubing and gas filters from the bioreactor head plate assembly and discard in biohazardous waste.

8.7.4.2. Remove the septum from the head plate.

8.7.4.3. Remove the air outlet condenser from the head plate and disassemble the condenser for cleaning.

8.7.4.4. Remove the head plate from the bioreactor vessel.

8.7.4.5. Clean all parts carefully and thoroughly using a small soft bristle brush (e.g. tooth brush) and a dilute laboratory glassware cleaner. Rinse with MilliQ water and then repeat with a 10% bleach solution. Rinse thoroughly with MilliQ water and spray with 70% IPA and place on paper towels on a lab bench to dry. Let dry all the parts

8.5.1. Clean the porous sparger tip (optional)

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

8.5.1.1. 1. Remove the sparger tip from the air inlet pipe.

8.5.1.2. 2. Soak the sparger overnight in a solution of 10 mg/mL pepsin / 0.01M HCl.

8.5.1.3. 3. Use ultrasonic cleaning with water and/or ethanol.

8.5.1.4. 4. Replace the sparger tip onto the air inlet pipe.

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9. Attachments



Figure 1. Applikon ez-Control Bioreactor Controller and Bioreactor

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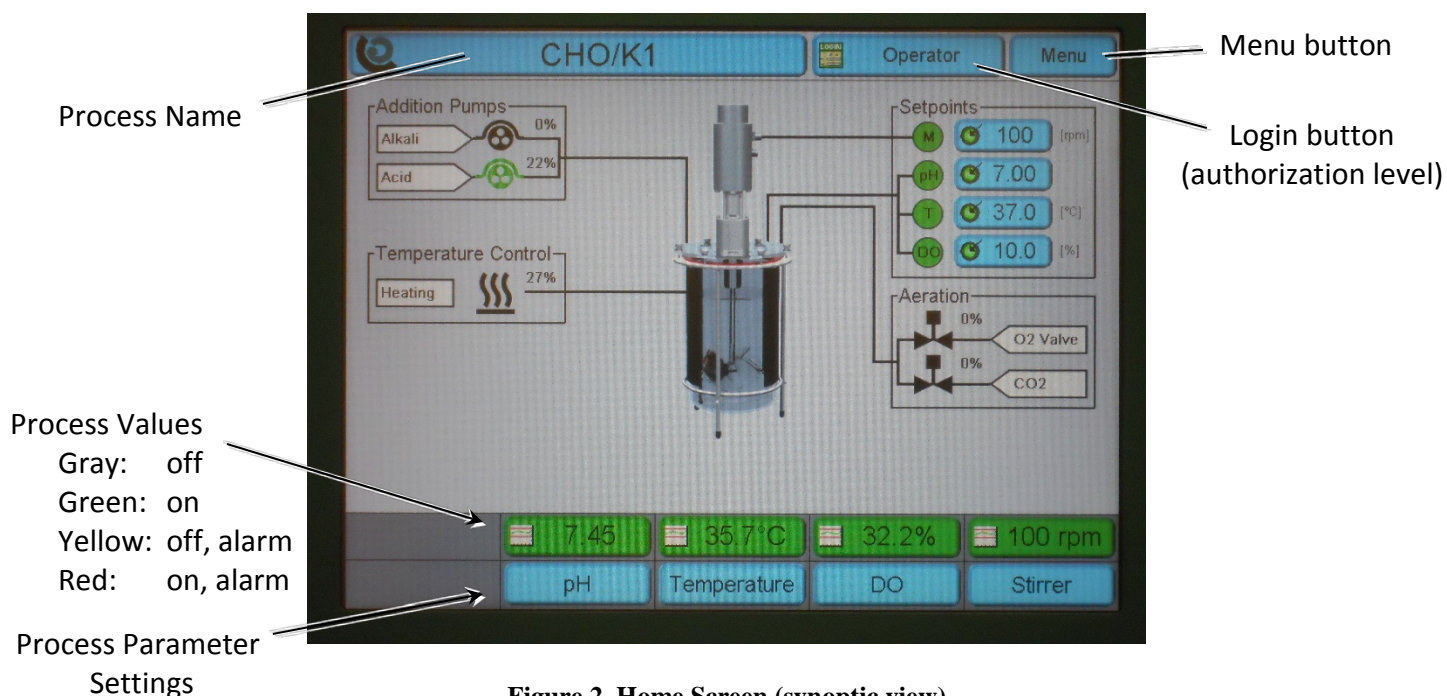


Figure 2. Home Screen (synoptic view)

Example 1. Navigating the Controller Screens to Login

Instructions	Explanation
Home > Login (top middle) > Operator > Login Enter the Operator password: "0000"	<p>On the Home screen, touch the Login button (top middle). The display changes to the Access Control screen.</p> <p>On the Access Control screen, touch the button Operator (left side). The display changes to the Operator screen.</p> <p>On the Operator screen, touch the button Login. The display changes to a keyboard and prompts for the Operator password.</p> <p>Enter the Operator password: "0000" Then touch the button Enter. The display changes back to the Home screen.</p>

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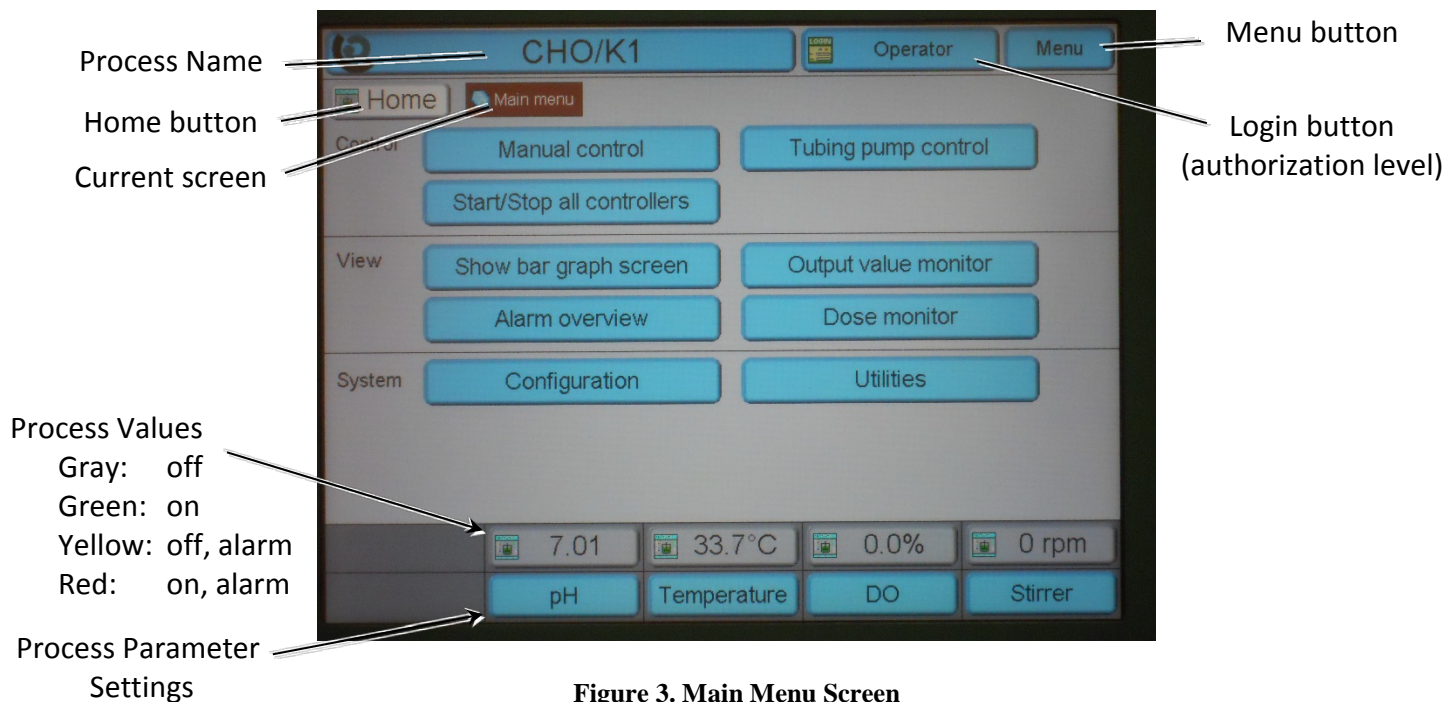


Figure 3. Main Menu Screen

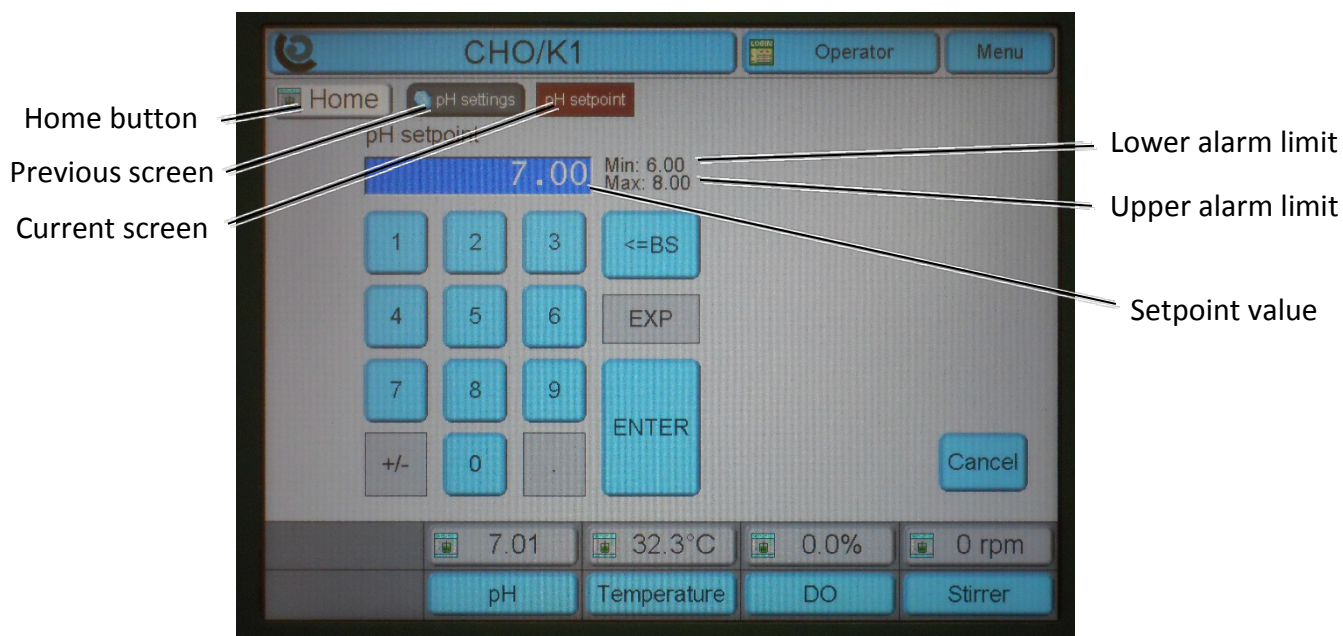


Figure 4. pH Setpoint Screen

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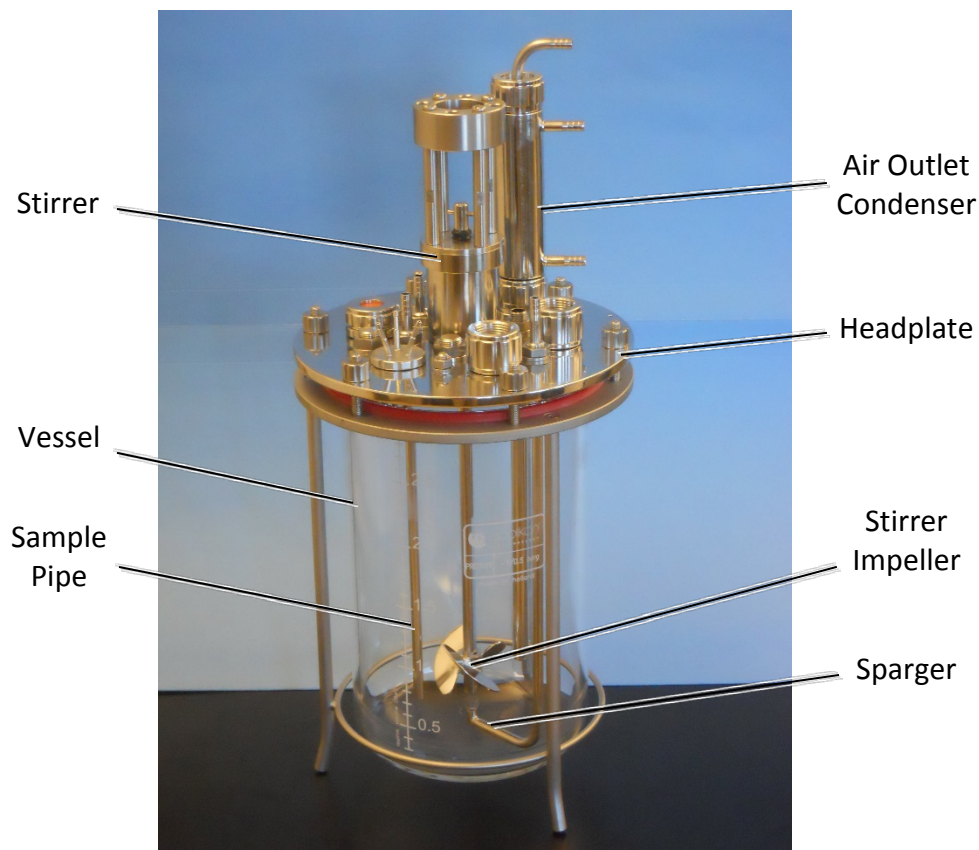


Figure 5. Applikon 3-Liter Glass Autoclavable Bioreactor

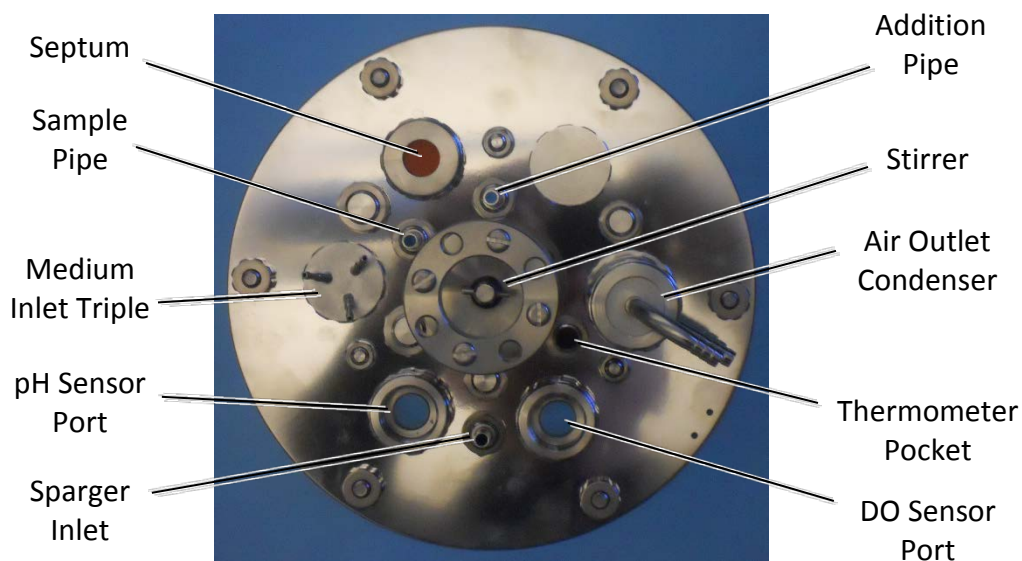


Figure 6. Bioreactor Head Plate (top view, unassembled)

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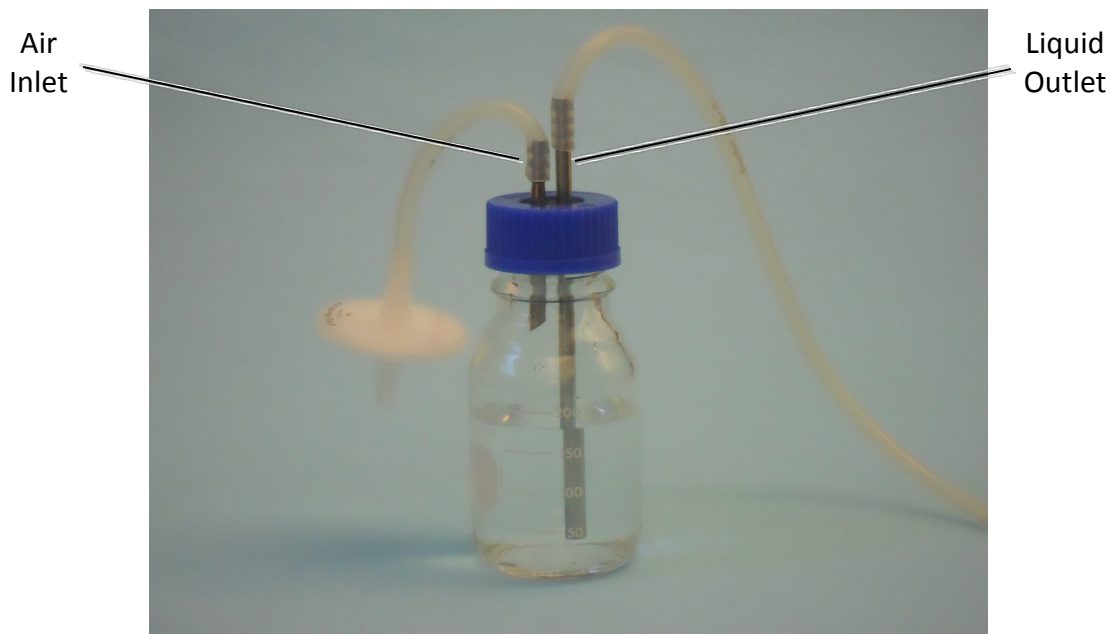


Figure 7. Liquid Addition Bottle

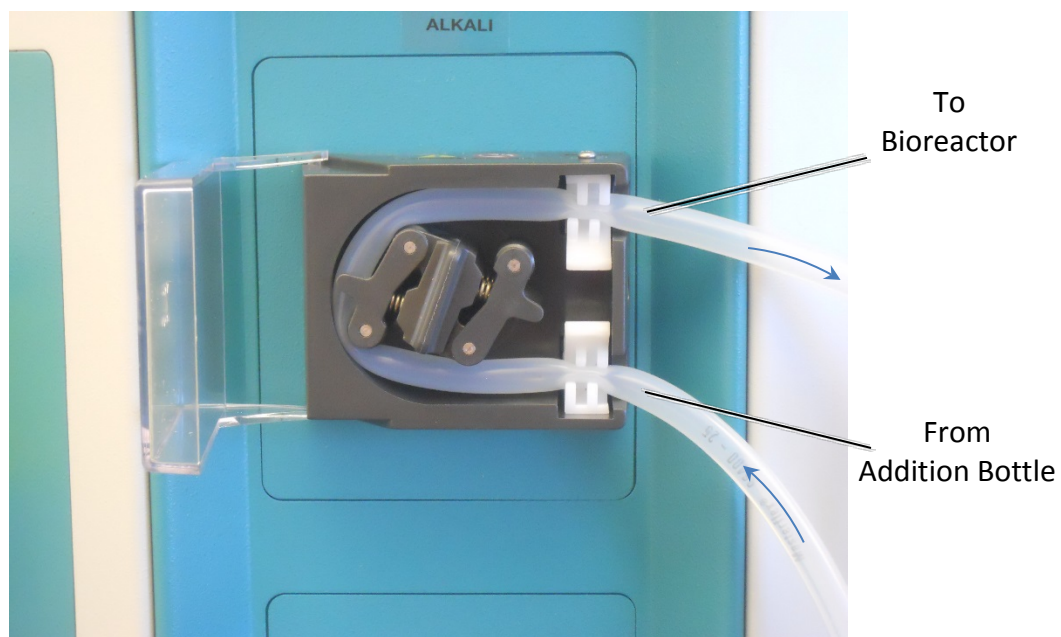


Figure 8. Peristaltic Pump (Alkaline Pump)

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10. History

<i>Revision Number</i>	<i>Effective Date</i>	<i>Preparer</i>	<i>Description of Change</i>
0	20DEC13	John Buford	Initial release
1	16SEP14	Jason McMillan	Various fixes and adjustments
2	9JULY17	Hetal Doshi	Various fixes and adjustments