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1.0 Purpose

This procedure describes the operation procedures for the Eppendorf BioFlo 320 benchtop bioprocess system.

2.0 Scope

This SOP applies to all Biopharmaceutical Development Program (BDP) personnel operating the Eppendorf BioFlo 320 benchtop bioprocess system.

3.0 Authority and Responsibility

- 3.1 The Director, Technical Operations, BDP has the authority to define this procedure.
- 3.2 Production personnel are responsible for training on this procedure and for documenting training to Biopharmaceutical Quality Assurance (BQA).
- 3.3 Production personnel are responsible for the implementation of this procedure.
- 3.4 BQA is responsible for quality oversight of this procedure.

4.0 Materials and Equipment

- 4.1 Eppendorf BioFlo 320 benchtop bioprocess system
- 4.2 70% Isopropyl Alcohol (IPA), BDP PN 30129
- 4.3 pH 4 buffer, BDP PN 30107.
- 4.4 pH 7 buffer, BDP PN 30108.
- 4.5 pH 10 buffer, BDP PN 30109

- 4.6 WFI
- 4.7 Appropriately sized **single use** vessel
- 4.8 Appropriately sized heating blanket
- 4.9 Appropriately sized oxygen probe
- 4.10 Appropriately sized pH probe

5.0 Procedure

5.1 Utility Startup

- 5.1.1 Confirm that the magnetic drive motor is plugged into the cabinet before powering the unit on.
- 5.1.2 Electrical
 - 5.1.2.1 Power on the control station.
 - 5.1.2.2 Login to the operator interface panel on the control station
- 5.1.3 Oxygen Gas
 - 5.1.3.1 Confirm that the oxygen gas is properly connected to the control station
 - 5.1.3.2 Open the oxygen gas valve on the utility panel.
 - 5.1.3.3 Verify that the O₂ pressure gauge reads 6 PSIG max.
- 5.1.4 Pharmaceutical Air
 - 5.1.4.1 Confirm the pharmaceutical air is properly connected to the control station.
 - 5.1.4.2 Open the pharmaceutical air valve on the utility panel.
 - 5.1.4.3 Verify that the pharm air pressure gauge reads 6 PSIG max.
- 5.1.5 Nitrogen Gas
 - 5.1.5.1 Confirm that the nitrogen gas is properly connected to the control station
 - 5.1.5.2 Open the nitrogen gas valve on the utility panel.
 - 5.1.5.3 Verify that the Nitrogen pressure gauge reads 6 PSIG max.
- 5.1.6 Carbon Dioxide Gas
 - 5.1.6.1 Confirm that the carbon dioxide gas is properly connected to the control station
 - 5.1.6.2 Open the carbon dioxide gas valve on the utility panel.
 - 5.1.6.3 Verify that the CO₂ pressure gauge reads 6 PSIG max.

5.2 Vessel Preparation

- 5.2.1 Inspect vessel packaging for damage
- 5.2.2 Carefully tear open packaging. Do not use any sharp objects. Keep package label information for probe calibration. (Information is also located on the vessel wall but is easier to read on the package label.)
- 5.2.3 If connections will not be made using a tubing welder, make all necessary connections as directed by the BPR, aseptically in a BSC (per **SOP 19102 Routine Use and Disinfection of Biological Safety Cabinets, Incubators, Shakers and Centrifuges**) prior to connecting the vessel to the control station.
 - 5.2.3.1 If the BPR dictates using a syringe for antifoam addition, it is critical to make this connection in the BSC prior to connecting the vessel to the control station.
- 5.2.4 Put the heater blanket on the vessel to protect the pH sensor from light.
- 5.2.5 pH Probe Installation
 - 5.2.5.1 For Optical pH:
 - 5.2.5.1.1 Inspect the optical pH sensor for visual damage.
 - 5.2.5.1.2 Connect the pH sensor to the control cabinet, confirm that a purple light is pulsing from the tip of the sensor (about twice per minute). Disconnect the probe from the cabinet until required for use.
 - 5.2.5.2.3 Insert the sensor into the headplate port labeled "pH" by gently turning while pressing it into the port.
 - 5.1.5.2 For analog pH
 - 5.1.5.2.1 The probe must be calibrated per step 5.4.3 before installation.
 - 5.1.5.2.2 With the vessel inside of a BSC, remove the cap from the PG13.5 port labeled "sensor" by unscrewing it.
 - 5.1.5.2.3 Carefully thread the sensor into the port.
- 5.2.6 Dissolved Oxygen (DO) Probe Installation
 - 5.2.6.1 Inspect the DO sensor for possible damage.
 - 5.2.6.2 Unscrew the protective cap from the electrode and check the electrolyte level, fill if necessary (per **SOP 12118 Maintenance and Storage of Dissolved Oxygen and pH Probes**).
 - 5.2.6.3 Gently insert the sensor into the headplate port labeled "DO". The probe should cause the membrane to protrude from the sleeve about a 1/4". Do not apply excessive force to the sensor as this may damage the membrane.

- 5.2.6.4 Connect or verify connection to the DO cable to the probe and control cabinet.
 - NOTE:** Both the analog and the optical DO probe are inserted into the non-contact sleeve.
- 5.2.7 RTD sensor installation
 - 5.2.7.1 Insert the RTD temperature sensor into the headplate port labeled "Temperature" making sure that the sensor reaches to the bottom of the well.
 - NOTE:** Do not use low resistivity thermal grease when installing the sensor as this may cause a temperature offset.
 - 5.2.7.2 Attach the RTD cable to the temperature connector on the control cabinet
- 5.2.8 Motor assembly installation
 - 5.2.8.1 Use the locating notch to orient the motor on top of the coupling
 - 5.2.8.2 Turn the motor until the groove in the motor is aligned with the upper sparge barb.
 - 5.2.8.3 Gently push the motor downward onto the sparge barb.
- 5.2.9 Sparge connection
 - 5.2.9.1 Using flexible tubing attach the sparge outlet on the control cabinet to the sparge filter on the vessel headplate.
- 5.2.10 Overlay connection
 - 5.2.10.1 If the overlay is required, using flexible tubing, attach the overlay outlet on the control cabinet to the overlay filter on the vessel headplate.
- 5.2.11 Exhaust heater
 - 5.2.11.1 Wrap the exhaust tube heater around the exhaust tube and brace it with the support rod
 - 5.2.11.2 Attach the exhaust tube heater cord to the control cabinet.
- 5.2.12 Scale (optional)
 - 5.2.12.1 If you are using the scale, ensure it is plugged into the control cabinet.
 - 5.2.12.2 Place the vessel (with all the sensors attached and the heat blanket on) onto the scale.
 - 5.2.12.3 Go to the "unit view: calibration" screen of the OIT.
 - 5.2.12.4 Select volume and then press "set zero"
 - 5.2.12.5 Enter 0

5.2.13 Vessel Selection

- 5.2.13.1 From the unit view screen, select the set-up screen.
- 5.2.13.2 In the vessel row, select the type of vessel being used (autoclavable or BioBLU SU)
- 5.2.13.3 Select the size of vessel being used (e.g. 50c)
- 5.2.13.4 Confirm the sensors listed are the sensors being used for the process. It may be necessary to plug the sensors into the cords coming from the control station for the sensor type to register accurately.

5.3 Vessel Batching

- 5.3.1 Batch the vessel per the BPR or Supervisor's instructions
- 5.3.2 If you are batching in a BSC:
 - 5.3.2.1 Disconnect all the probes and the heater blankets from the control station and move the vessel next to the BSC.
 - 5.3.2.2 Spray the appropriate lines with an approved disinfectant before placing them in the BSC.
 - 5.3.2.3 Remove the cap from the line to aseptically connect the desired components to the vessel.
 - 5.3.2.4 Once all necessary connections and or additions are made, return the vessel to the control station.
- 5.3.3 If you are batching outside of the BSC, follow the steps in section 5.3.2 to add sterile connectors, or set up a tube welder capable of welding C-Flex following the appropriate SOP for the tube welder.
- 5.3.4 Verify the vessel and exhaust heater blankets are plugged in to the control station and securely in place.
- 5.3.5 Verify all required probes installed securely in the vessel head plate and plugged into the control station.

5.4 pH Probe standardization

- 5.4.1 Ensure vessel is at operating temperature and stable before starting calibration of an optical pH probe
- 5.4.2 For an optical pH probe:
 - 5.4.2.1 Plug in the optical pH cable.
 - 5.4.2.2 Go to the calibration screen on the control tower and select pH-Opt in the loops field.
 - 5.4.2.3 Touch the edit box in the sensor data field.
 - 5.4.2.4 Enter the data for the sensor from the vessel packaging.

- 5.4.2.5 Touch the check mark on the keypad to accept typed values.
- 5.4.3 For an analog pH probe:
 - 5.4.3.1 Connect the probe to the control station using the appropriate cable.
 - 5.4.3.2 Go to the calibration screen and select pH.
 - 5.4.3.3 Rinse the probe with WFI and immerse in pH 7 buffer.
 - 5.4.3.4 Touch “set zero” and enter 7. Touch “ok”.
 - 5.4.3.5 Rinse the probe in WFI and immerse in second buffer (eg. pH 4 or 10)
 - 5.4.3.6 Allow the reading to stabilize and the touch “set span” edit box
 - 5.4.3.7 Enter the value of the pH buffer and touch “ok”
 - 5.4.3.8 When the current view reading stabilizes touch “set span”
- 5.5 pH Probe Re-standardization
 - 5.5.1 If required per the batch production record or other appropriate guidance the pH can be restandardized from the calibration screen.
 - 5.5.2 Select pH or pH-Opt from the loops field (whichever is being used) and then touch the restandardize button.
 - 5.5.3 Enter the offline value using the keypad that opens. Press ENTER to accept the value entered.
- 5.6 DO probe standardization
 - 5.6.1 Set the flow of the sparge and the overlay to 100% N₂
 - 5.6.2 Allow the nitrogen to flow into the vessel until the DO reading stabilizes. This may take a half an hour or more.
 - 5.6.3 In the calibration screen, select the DO sensor in the loops field.
 - 5.6.4 Press “Set Zero”
 - 5.6.5 Change the flow for the sparge and overlay to 100% air at the setpoint to be used for the production.
 - 5.6.6 Allow the air to flow into the vessel until the DO reading stabilizes again.
 - 5.6.7 In the calibration screen, select the DO sensor in the loops field.
 - 5.6.8 Press “Set Span”
- 5.7 Assign and calibrate built in pumps
 - 5.7.1 The unit has four built in pumps that can be assigned to various functions as needed.



- 5.7.2 The pumps can be used as fixed speed at 25 rpm or variable speed from 5 rpm – 25 rpm
- 5.7.3 Pumps 1 – 4 can be used with 0.8mm, 1.6mm, 3.2mm, and 4.8mm ID tubing.
- 5.7.4 Pump 4 can also be used with 6.4mm ID tubing.
- 5.7.5 Validated pump flow rates are as follows:

<i>Pump Number</i>	<i>Tubing size (mm)</i>	<i>Minimum Flow Rate (mL/min)</i>	<i>Maximum Flow Rate (mL/min)</i>
1 – 3	0.8	0.2	1
	1.6	0.7	3.5
	3.2	2.35	11.75
	4.8	4.25	21.25
4	0.8	1.2	6
	1.6	5	25
	3.2	17	85
	4.8	38	190
	6.4	60	300

- 5.7.6 Calibrate the pumps when required per the BPR or area supervisor.
 - 5.7.6.1 Calibration values can be copied for pumps 1 – 3 as long as the same type and size of tubing is used in each pump
- 5.7.7 To start calibration, install the type and size of tubing that will be used into the pump head.
 - 5.7.7.1 Place one end of tubing into a container with an appropriate volume of water. Place the other end in an empty graduated measuring device or empty tear weighted container.
 - 5.7.7.2 In the “pumps” screen, select the desired pump by pressing the name of the pump under “loop”.
 - 5.7.7.3 Ensure the direction selected under “rotation” is the direction in which the pump will be used and that the filled container and empty container are on the correct ends of the installed tubing.
 - 5.7.7.4 Prime the tubing to fill it with water.

- 5.7.7.5 Touch “start” under calibrate
- 5.7.7.6 The pump will run for 3 minutes.
- 5.7.7.7 After the pump has shut off, use the pop-up keypad to enter the actual volume of liquid pumped into the empty container.
- 5.7.8 Enter the desired setpoint
- 5.7.9 Select the desired type (fs = fixed speed, vs = variable speed)
 - 5.7.9.1 For fixed speed, both a desired setpoint in mL/min and a period are required.
 - 5.7.9.2 For variable speed only a desired setpoint in mL/min is required.
- 5.7.10 In the assignment section, select the appropriate assignment.
- 5.7.11 The pumps can be set to the following:
 - 5.7.11.1 None
 - 5.7.11.2 Harvest – based on the scale
 - 5.7.11.3 Add – based on the scale
 - 5.7.11.4 Acid – the pump will run based on pH cascade
 - 5.7.11.5 Base – the pump will run based on pH cascade
- 5.7.12 Lines can be primed using the “prime” mode.
- 5.8 Setting Vessel Parameters
 - 5.8.1 At the operator interface terminal on the control station, verify each of the needed control loops/alarms are controlling and set to their appropriate mode per the BPR.
 - 5.8.2 Select the gas mix specified in the BPR.
 - 5.8.2.1 The 4-gas mix uses N₂, CO₂, O₂, and air.
 - 5.8.2.1.4 The operator must set a flow rate that will be a constant
 - 5.8.2.1.2 The composition of the gas going into the vessel is determined by pH loop demand and DO loop demand
 - 5.8.2.1.3 Acidic demand from the pH loop will increase the CO₂ content of the gas mix.
 - 5.8.2.1.4 Low DO will increase the O₂ content.
 - 5.8.2.1.5 The output % for this mode is -100% to +100%
 - 5.8.2.2 The 3-gas mix uses CO₂, O₂ and air.

- 5.8.2.2.1 The operator must set a minimum and a maximum flow rate
- 5.8.2.2.2 The composition of the gas going into the vessel is determined by pH loop demand and DO loop demand
- 5.8.2.2.3 Acidic demand from the pH loop will increase the CO₂ content of the gas mix.
- 5.8.2.2.4 Low DO will increase the O₂ content.
- 5.8.2.2.5 The output % for this mode is 0% to +100%
- 5.8.2.3 The O₂ enrich mix uses O₂ and air.
 - 5.8.2.3.1 The operator must set a minimum and a maximum flow rate
 - 5.8.2.3.2 The composition of the gas going into the vessel is determined by DO loop demand
 - 5.8.2.3.3 Low DO will increase the O₂ content.
 - 5.8.2.3.4 The output % for this mode is 0% to +100%
- 5.8.2.4 The N₂ enrich mix uses N₂ and air.
 - 5.8.2.4.1 The operator must set a minimum and a maximum flow rate
 - 5.8.2.4.2 With 0% output for DO, the gas supply is completely nitrogen. As DO output increases, the air content increases.
 - 5.8.2.4.3 The output % for this mode is 0% to +100%
- 5.8.2.5 The sparge and overlay have totalizers that can be used to calculate the quantity of each gas used.
- 5.8.3 Set the cascades as directed by the BPR
 - 5.8.3.1 A cascade from DO to sparge and/or overlay should not be set when one of the pre-defined mixes are being used. The software will automatically control the gas composition based on DO as described in section 5.8.2.
 - 5.8.3.2 A cascade from pH to sparge or an assigned pump should not be set when using 3-gas mode, 4-gas mode or have pumps assigned as "acid" and/or "base". The software will automatically control the pH in these conditions.
 - 5.8.3.3 In the cascade screen select the "cascade from" button. This allows you to select the loop that controls the other outputs.

- 5.8.3.4 Select the “+” button under “cascade to” in order to add a new target loop.
 - 5.8.3.4.1 For example, if low DO should increase agitation, select “DO” as the “cascade from” and “agitation” is the “cascade to”.
- 5.8.3.5 In the column under the specified “cascade to” input the setpoints in the row that corresponds to the desired output % per the BPR.
- 5.8.3.6 User defined output % can be added to the table by selecting the next empty box in the “Out %” column.
- 5.8.3.7 Add additional loops in the “cascade to” fields as directed in the BPR.
- 5.8.3.8 Touch the circle below each loop in the “cascade to” to activate the cascade(s).
- 5.8.4 Set up timed profile(s) as directed by the BPR
 - 5.8.4.1 Timed profiles drive setpoints for loops based on the elapsed fermentation time.
 - 5.8.4.2 If required per the BPR, touch the “profile” tab at the bottom of the “cascade” screen to enter a timed profile.
 - 5.8.4.3 Touch the “cascade to” box to select a loop for which a timed profile is desired.
 - 5.8.4.4 Enter the EFT, the new setpoint, and the action.
 - 5.8.4.4.1 The action determines whether the setpoint will step directly to the next setpoint or ramp to the next setpoint.
 - 5.8.4.4.2 Add a new row for each new setpoint desired throughout the run.
 - 5.8.4.5 Touch the enable button to enable the profile for that loop.
 - 5.8.4.6 Repeat for other loops as required.
 - 5.8.4.7 In order for timed profiles to activate, the batch timer must be started when the vessel is inoculated
- 5.9 Vessel Inoculation
 - 5.9.1 Pull a sterile BI sample before inoculating the vessel using the sampling procedure outlined in step 5.11. Refer to the BPR for procedural timing and additional details.
 - 5.9.2 Verify the growth parameters, control cascades, and pump assignments and rate and period values are set as described in the BPR.

- 5.9.3 Re-standardize the pH probe to match the offline BI value by following the Steps in 5.5.
- 5.9.4 Add the inoculum to the vessel using the addition procedure outlined in step 5.10.
- 5.9.5 Press the start button of the batch timer in the timed profile window.
- 5.10 Aseptic Additions
 - 5.10.1 Attach the addition/inoculation/feed assembly to an addition port inside a portable hood, by tube welding, or by using the appropriate assembly attached in step 5.2.3.
 - 5.10.2 Open the clamp on the addition leg of the vessel to allow the flow of material to enter the vessel.
 - 5.10.3 Pump the material into the vessel using a peristaltic pump. Close the clamp on the vessel tubing when addition is complete.
- 5.11 Vessel Sampling
 - 5.11.1 Spray the luer connector on the sampling leg and the luer on a new syringe with IPA.
 - 5.11.2 Attach the syringe via the luer connection.
 - 5.11.3 Unclamp the tubing on the sampling leg.
 - 5.11.4 If the vessel has been previously sampled, draw out any liquid that had been in the line previously and attach a new clean syringe following the steps in 5.11.1 and 5.11.2. If the vessel has not been previously sampled, proceed to 5.11.5.
 - 5.11.5 Draw the amount of sample required per the BPR or supervisor's direction into the syringe.
 - 5.11.6 Disconnect the syringe and recap the sampling leg or connect a new syringe using step 5.11.1.
- 5.12 Vessel Harvest
 - 5.12.1 Connect flexible tubing (or use the assembly attached in step 5.2.3 if sterile harvest is required) to the harvest line of the vessel and direct the other end to the harvesting equipment or receiving vessel.
 - 5.12.2 Set vessel control loops as specified in the BPR for harvest. Make a comment in the BioCommand software indicating the start of the harvest.
 - 5.12.3 Once the harvest procedure is ready to begin, unclamp the tubing on the harvest line.
 - 5.12.4 Using a peristaltic pump, pump the contents of the vessel out per the BPR or supervisor's instructions.
- 5.13 Vessel Shutdown

- 5.13.1 End the batch by pressing the stop button for the EFT timer on the operator interface terminal (if the timer was started).
- 5.13.2 If required by the BPR or directed by the area supervisor, export the run data from the control station.
 - 5.13.2.1 Insert a drive into the USB port of the control station.
 - 5.13.2.2 Open the trend screen and select export
 - 5.13.2.3 Select USB external memory as the export location.
 - 5.13.2.4 Touch the file name edit button to name the file with the lot number and/or other pertinent identifying information.
 - 5.13.2.5 Touch "OK" followed by "Save". The USB device can now be removed.
- 5.13.3 The data can also be exported from the Biocommand software if required.
- 5.13.4 Turn off all vessel utilities that were turned on or opened in Steps 5.1.2 through 5.1.6.
- 5.13.5 Remove the heat blankets, probes and the RTD from the vessel.
- 5.13.6 Disconnect all tubing from between the vessel and the control station.
- 5.14 Autoclave the single use vessel per **SOP 19500 Operation and Maintenance of BMT Steam Sterilizers** discard the vessel.

6.0 Documentation

- 6.1 Document the performance of this procedure on the BPR and in the equipment logbook.

7.0 References and Related Documents

- 7.1 **BDP SOP 12118** *Maintenance and Storage of Dissolved Oxygen and pH Probes*
- 7.2 **BDP SOP 19102** *Routine Use and Disinfection of Biological Safety Cabinets, Incubators, Shakers and Centrifuges*
- 7.3 **BDP SOP 19500** *Operation and Maintenance of the BMT Steam Sterilizers*

8.0 Attachments

- 8.1 **Attachment 1** Disposable Vessel Example
- 8.2 **Attachment 2** Head Plate Diagram

Attachment 1: Disposable Vessel Example

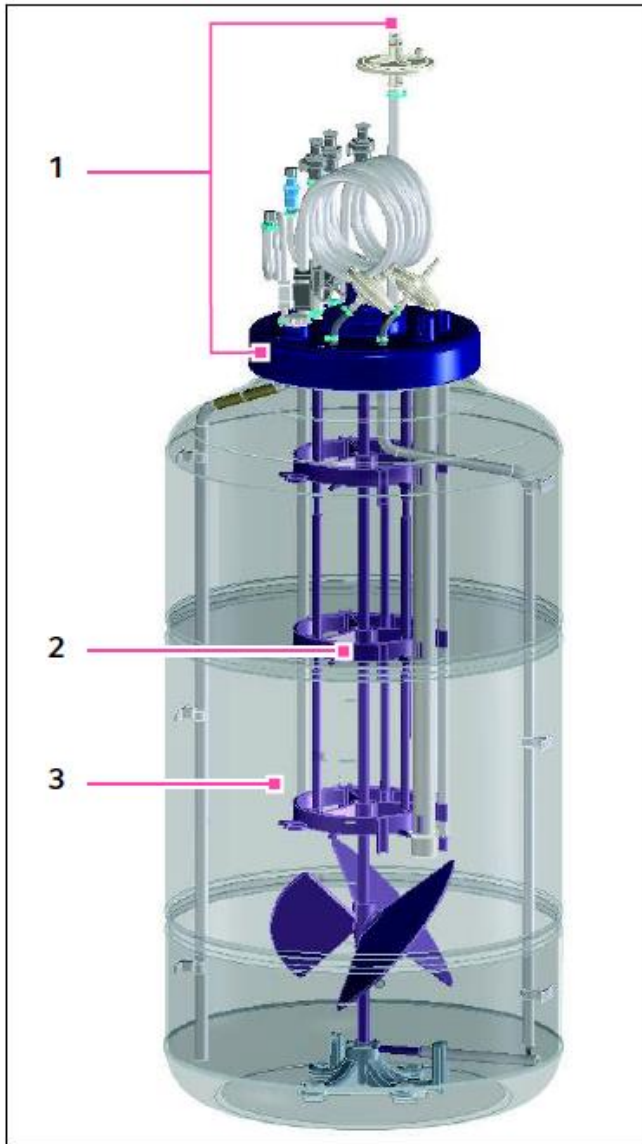


Fig. 4-41: BioBLU 50c

1 Headplate and tubing assemblies

2 Vessel

3 Internal components

Attachment 2: Head Plate Diagram

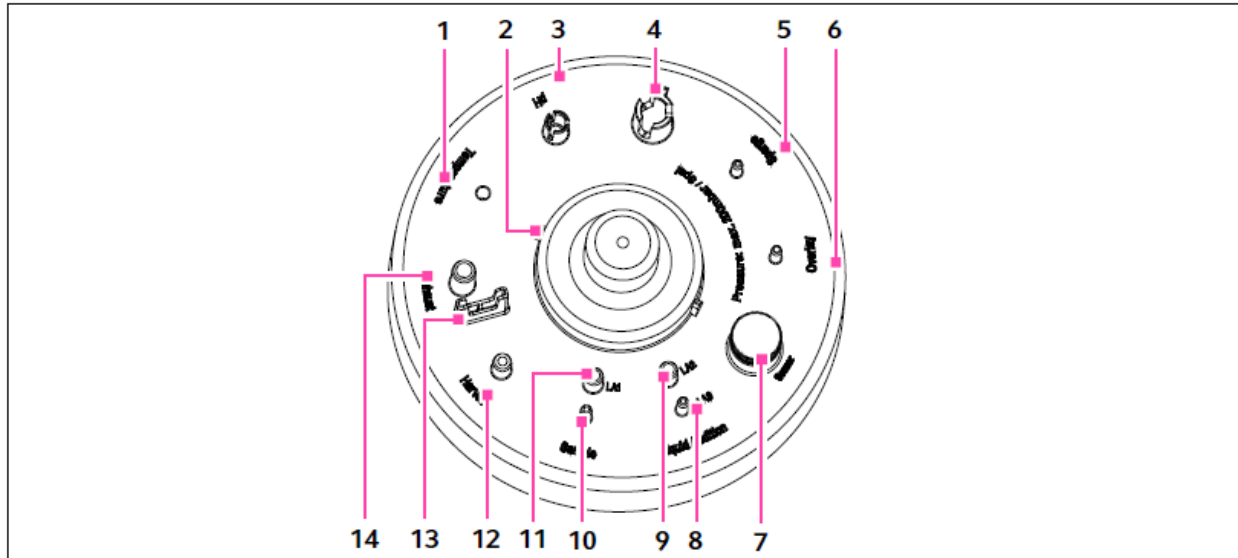


Fig. 4-44: Headplate Arrangement (shown without tubes and connectors)

- | | |
|---|--|
| 1 Temperature port for RTD and thermowell | 7 Threaded PG 13.5 sensor port
Plugged as standard |
| 2 Motor coupling
Note ridges for proper alignment | 8, 9, 11 Liquid Addition ports 1, 2 and 3 |
| 3 pH port | 10 Sample port |
| 4 DO port | 12 Harvest port |
| 5 Sparge port | 13 Exhaust heater support |
| 6 Overlay port | 14 Exhaust port |