

Setting up the Qplus Bioreactor

Day 1

Quick checklist a day before fermentation

Before autoclave:

- For 20 minutes Calibrate pH probe and insert into Bioreactor
- Replace electrolyte in pO₂ probe and insert into Bioreactor
- Connect relevant solutions (acid, base, anti-foam)
- Remove Motor
- Disconnect from controller:
 - Sparger Jacket Condenser
 - Acid Base and Anti-foam Solutions from pumps
 - pH and pO₂ probes (at the head on the bioreactor)
 - Temperature probe (at the controller)
- Clamp and close relevant connections:
 - Solutions to Bioreactor
 - Condenser to air filter
 - Sampler to Bioreactor
 - Any open, unused ports
- Cover:
 - Air filters (w/ foil)
 - Temperature probe plug (w/ foil)
 - pH and pO₂ with relevant caps
 - Rotor (w/ foil)

Autoclave:

- Unscrew one septum at top
- Place autoclave tape
- Autoclave for 20 minutes at Liquid20 cycle
- Screw back septum when taking out of autoclave

After autoclave:

- Reconnect water jacket and set desired temperature
- Reconnect sparger. Spurge air. Check outlet air filter for airflow.
- Replace motor and start spinning impeller.
- Reconnect condenser, probes, and solutions to pumps
- Start pO₂ calibration
- Prepare inoculum

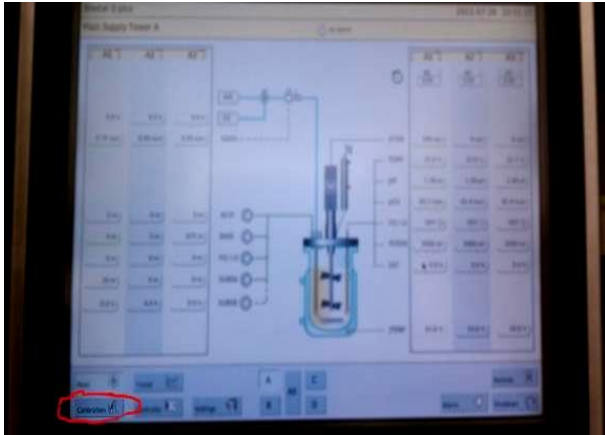


- 1 - Impeller;
- 2 - pH-electrode;
- 3 - Exhaust cooler;
- 4 - Inoculation port;
- 5 - pO₂-electrode;
- 6 - Harvest pipe;
- 7 - Aeration tube;
- 8 - 4-way-sampler;
- 9 - Level probe;
- 10 - Reserve;
- 11 - Antifoam probe;
- 12 - Temperature sensor

Setting up the probes

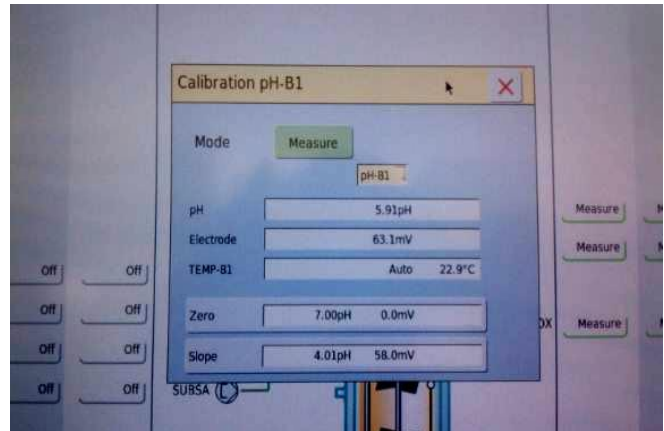
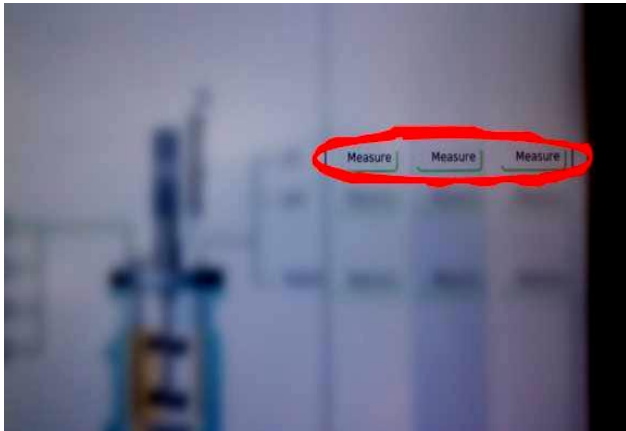
Calibrate the pH probe.

Remove the pH probe from the case or reactor. Wash with water into beaker. Place into buffer solution of pH 7.

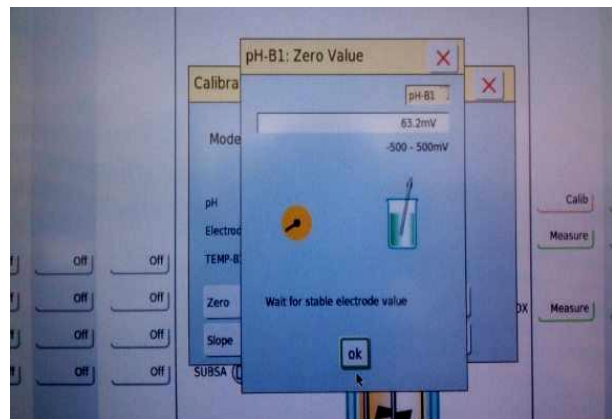
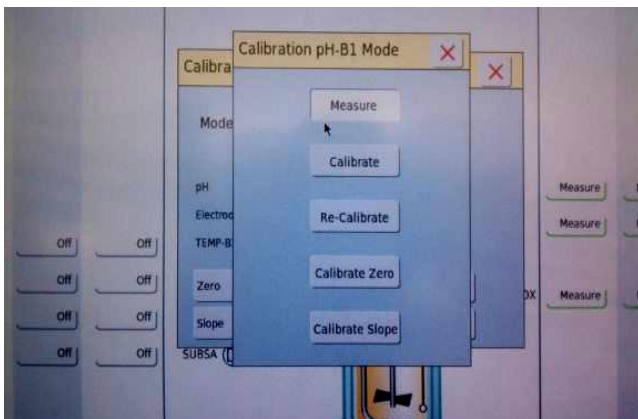


On the controller, go to “Calibration” screen (button in the bottom left corner):

Click the group of reactors that you’re interested in (A, B, or C). Click “Measure” next to pH for the relevant reactor: Choose “Single Point Calibration”. This should bring up a new menu. Where you click on the “Measure” button: This will allow you to select a Calibration. Choose “Calibrate” to first do the zero point calibration



Wait for the pH to stabilize and then hit “OK” (it should get close to 0 eV). Then you’ll do another point (presumably pH of 4). Wash the pH probe with water and place into appropriate buffer. When the eV value stabilizes, press “OK”.





pH probe with cap



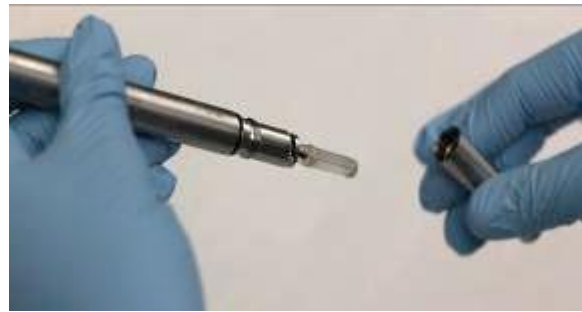
Buffer solutions

Change electrolyte solution in pO₂ probe

Remove the pO₂ probe from the bioreactor (if necessary).



1. Unscrew the bottom.



2. Pour solution out.



3. Fill ½ to ¾ with new electrolyte solution.



4. While holding the probe slanted, screw the probe back into the cap. Some of the solution should spill out.



← Electrolyte solution

Setting up the solution connections

Connect the base solution

Connect the long pipeline of the base solution to one of the entrance ports of the bioreactor. Close this line with a clamp to prevent base solution from being sucked in during a pressure gradient created by autoclaving. Cover the base air filter with aluminum foil to prevent it from getting wet during the autoclave process.



The entrance ports



Clamp to close tubing



Air filters

Base, Acid and Antifoam solutions

Connect the acid and anti-foam solutions

Follow the same instructions as connecting the base solution.

Disconnect from the controller

Remove motor

Unscrew the thumb screw and remove motor from top.



The thumb screw

The motor on rotor



The motor itself



The rotor without motor

Disconnect the solution lines from the pumps

Disconnect the base, acid, anti-foam, and any substrate lines from the respective pumps.

Disconnect the jacket plugs

Disconnect at the easy plug. Do NOT plug these into each other. Glass will crack under pressure of the autoclave.

Disconnect the condenser plugs

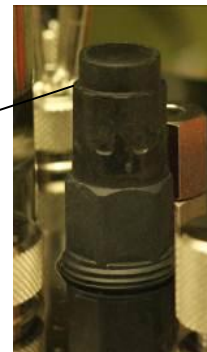
Disconnect the inlet first, and then disconnect the outlet plug directly from the controller.

Disconnect sparger and probes

When disconnecting the sparger, leave the air filter on. pH and pO₂ probes can be disconnected at the top. Place appropriate cover on probes. Temperature probe must be disconnected from the controller, and the end must be covered with foil.



pO₂ probe



pH probe

Cover on the probe heads.

Clamp and Protect

The following connections should be clamped:

- Base, Acid, and Anti-foam to bioreactor
- Sampler to bioreactor

The following ports should have tubing and be clamped:

- Any free ports that's not being used

Protect the following with aluminum foil:

- All air filters (prevents air filters from getting wet)
- The rotor
- The temperature probe plug

Preparing to autoclave

Open top and pour in media

Open the top of the reactor by unscrewing three knobs. If resting the top down, do on the side with condenser. Do not rest on any of the probes. Pour existing water/media out and add needed media.

Pictures:

- Bioreactor on side
- Bioreactor with new media

Leave plug open

Open one of the plugs on the bioreactor. Set down into port so that it doesn't block it, but it also doesn't leave completely open. This will allow pressure to be relieved during the autoclave cycle. You'll put this back on when taking the bioreactor out of the autoclave.

The upper connector of jacket must not be closed or clamped. The thermostat liquid in the jacket expands during heating up in the autoclave. Excess liquid must be able to flow out.

For an optimal heat transition the jacket must completely be filled with thermostat medium.

Place piece of autoclave tape on reactor

Autoclave

Autoclave Liquid20 cycle for 20 minutes. Be sure to autoclave with bioreactor in autoclavable bin.

When done autoclave, screw closed the open plug as taking bioreactor out of autoclave. This will prevent contamination.

Re-connect Bioreactor

Connect the water jacket plugs back to the controller

Connect the appropriate plugs and the temperature probe. Then set a temperature. This will start cooling the bioreactor immediately.

Connect the sparger

Connect the sparger and then start flowing air. This will help the heat transfer.

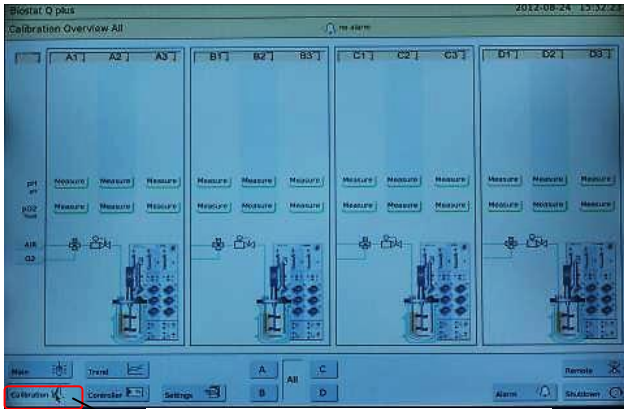
Connect the motor

Connect the motor and start spinning the impeller to help heat transfer.

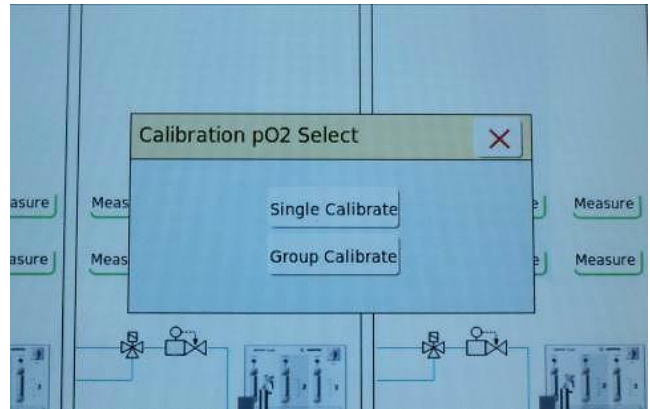
Connect the pH probe

Start pO₂ Calibration

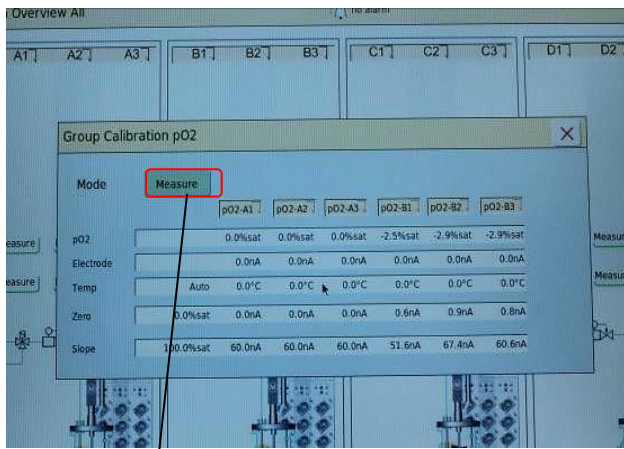
With the temperature, spinner, and sparger set to your appropriate reactor conditions, start calibration of the pO₂ probe.



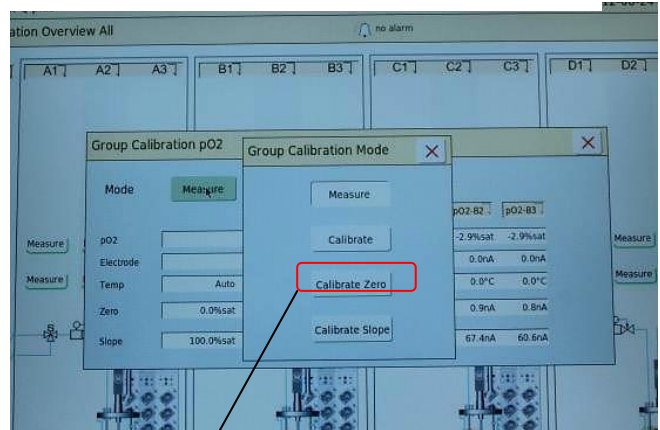
1. Go to the calibration menu



2. Choose Single or Group calibration

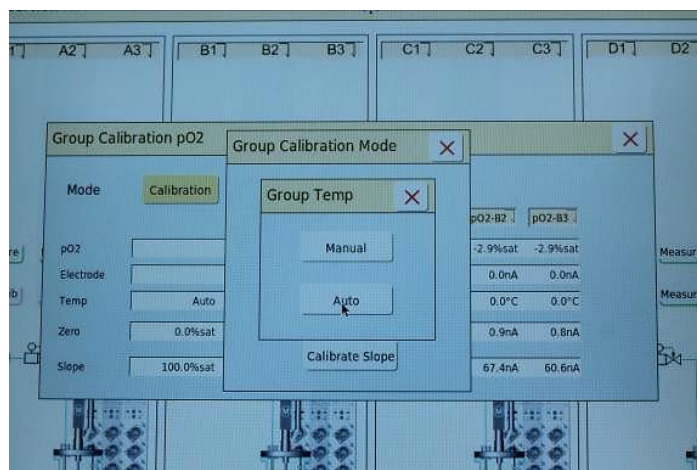


3. Press Measure button



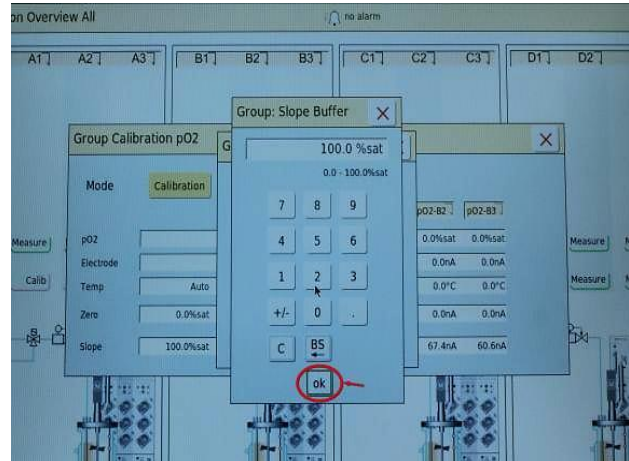
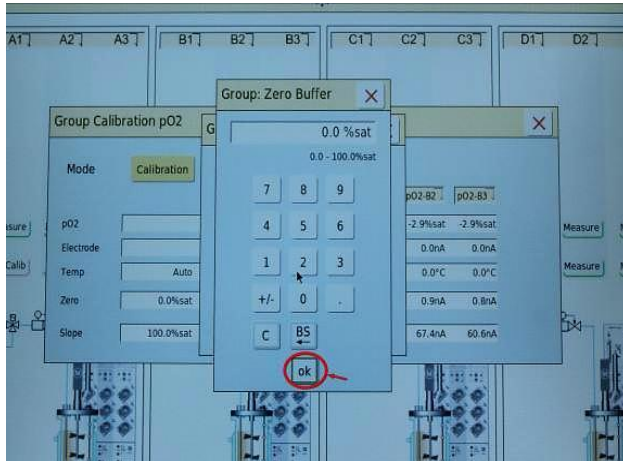
4. Choose the Calibrate Zero

First do the zero point, where the probe isn't connected to the controller.



Then connect the probe to the controller and start the 100% (slope) calibration point. For slope adjustment enable supply of air (gas containing oxygen) and adjust the stirring speed. The medium should be optimally gassed (max. flow rate intended for your process) and mixed. At a stable display of the measured value you can calibrate this as “100% pO2”.

This will take 2 to 4 hours and can be done overnight.



Prepare overnight cultures:

- Use an inoculation loop to inoculate night culture media with bacteria from a glycerol stock.

Calculate volume of night culture media (usually it is LB with appropriate antibiotics) as 20 ml for 1 L of TB (or other) fermentation media (2%). Use 50 ml tubes.

- Repeat for all cultures
- Leave tops loose so air can get in. In order not to lose tops fix them with a tape.
- Grow overnight at 37°C on a rotary shaker (220 rpm).
- To refreeze glycerol stock by soaking in liquid nitrogen and then place in freezer at -80°C.

Day 2

Quick checklist

- Connect all sensors and solutions
- Turn on the fermenter and set all necessary parameters
- Inoculate overnight culture into the Bioreactor
- Induce the culture
- Collect the harvest

Final preparations for fermentation

Connections:

- Connect the base, acid and anti-foam lines to the respective pumps

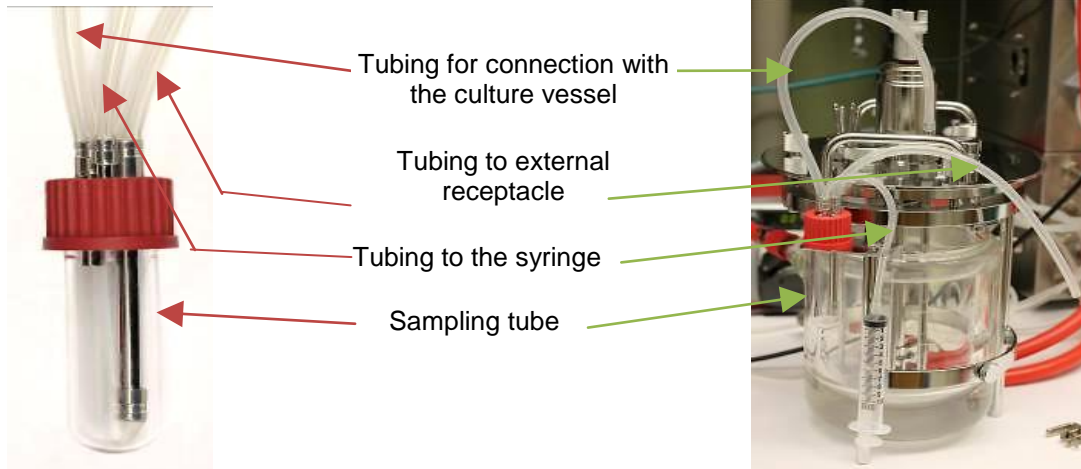
From the buffer →
To the entrance port →



Tubing going through pump

Make sure that the tubes are connected properly!

- Connect the temperature sensor
- Connect the foam sensor
- Connect (or check the connection of) motor, exhaust cooler, sparger, pH and pO₂ probes
- Connect the Manual Sampling Kit



- Clamp all tubings for a while.

Setting the measurement and control system:

- Set the temperature to 37⁰C. Takes about 10 minutes to stabilize.
- After temperature has stabilized, turn the pH controller on. Set to 7.1 (or adjusted value)
- Set the stir rate to 600 rpm
- On the Main control screen, set the air flow rate to 8 SLPM
- Turn on the foam sensor. You may adjust sensitivity of sensor.

Now bioreactor is ready for inoculation

Inoculate overnight culture into the fermenter:

- Measure the OD₆₀₀ of overnight cultures using 1:20 dilution in blank media. Calculate inoculum volume below:

$$V_{\text{starter}} * OD_{\text{starter}} = V_{\text{ferm}} * OD_{\text{init}}$$

The OD_{init} is calculated from the desired OD at harvest and the number of doublings i (6-8 is acceptable):

$$OD_{\text{init}} * 2^i = OD_{\text{harvest}}$$

Don't forget to take a sample of blank media from the culture vessel for further OD measure while cultivation.

- Sterilize the septum injection port by removing the plug and filling it with 70% ethanol. Using a sterile syringe and Gauge 20 needle, inject the desired amount of overnight culture through the septum into the fermenter. To maintain sterility, load the syringe in the biosafety cabinet or next to a flame (the butane torch works well).
- Upon inoculation, synchronize the batch by right clicking on the batch in the Batch Management window list and choosing Synchronize.
- Take the OD of a small sample immediately after inoculation. This reading should match the calculated OD_{init}

- Grow until absorbency is greater than 0.8 (usually 3.5 – 4 hours). Prepare IPTG solution.

Induction:

- Add IPTG* solution to the culture vessels using a funnel through one of the free ports on the top.

***Calculate the amount of IPTG as 0.14 g of IPTG powder for 1L of culture. Dissolve in water, 2 ml per 1L culture.**

- Reduce temperature to 25⁰C.
- Double check all connections and parameters. Leave overnight.

Day 3

Prepare lysis buffer and place in refrigerator at +4⁰C.

Change fermenter settings below:

- Set the air flow rate at 4 SLPM
- Set the stir rate to 100 rpm
- Turn off the pH control.
- Set the temperature to 4⁰C.

Harvest the culture

- Remove the sleeve on the harvest port. Attach the cooling coil to the port and drain culture into sterile
- 2 L bottles
- Transfer culture to 1 L centrifuge bottles. Balance the bottles.
- Spin bottles in the Beckman Avanti J-E Centrifuge using rotor JLA-9.1 at 8000 g 10 min, +4⁰C.
- Remove supernatant by pouring. Dispense the remaining culture in centrifuge bottles on top of the first round of pellets. Re-centrifuge.

Remaining steps should be done on ice to keep as cold as possible.

- Resuspend pellet in lysis buffer, usually 100 mL for 1 L of culture is enough.
- Transfer suspended culture to 50mL centrifuge tube, about 40-45 mL each
- Freeze at -20⁰C.