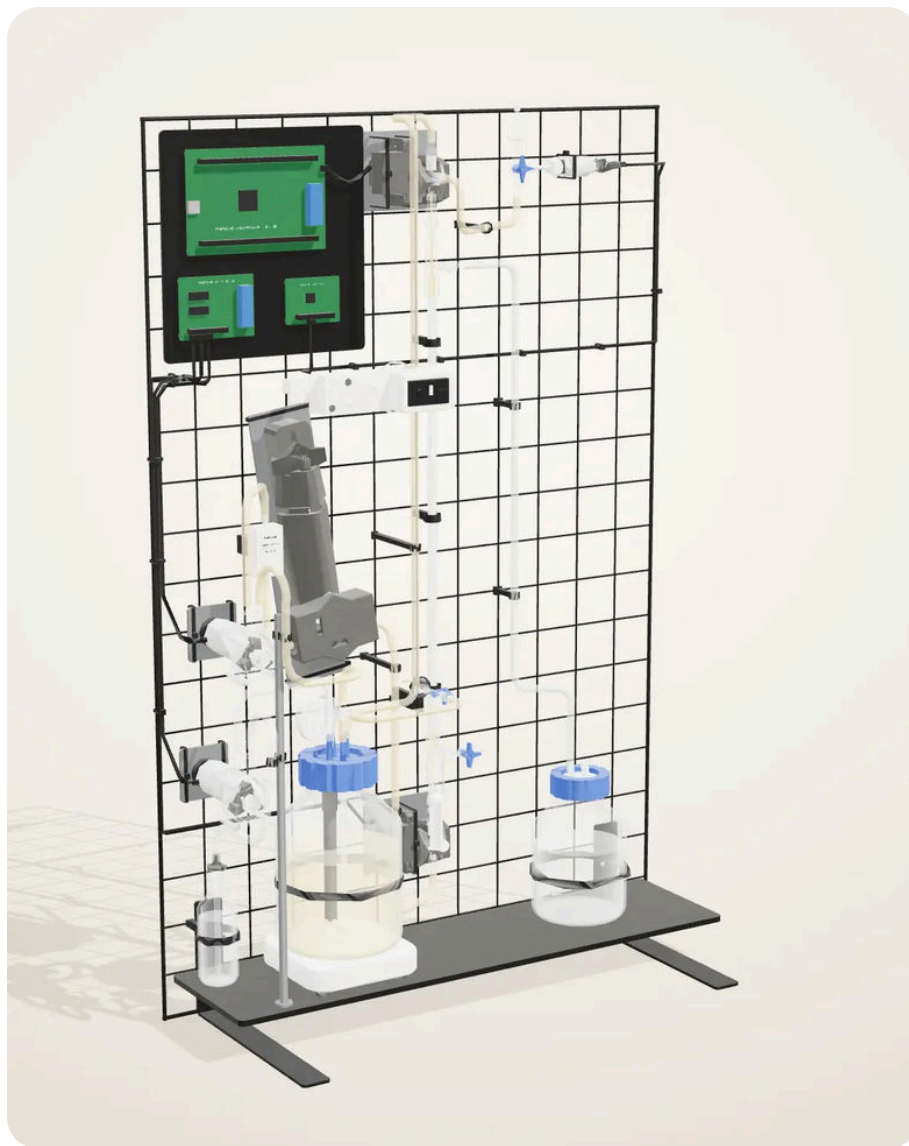


Perfusion Bioreactor

Assembly guide

A continuous-flow bioreactor. *B. subtilis* 168 stays on one side of a hollow-fiber membrane, and only the protectant it secretes crosses to the other: the product leaves the system, the organism does not.



⚠ SAFETY

- ◆ Wear gloves for all plumbing, cleaning and culture work.
- ◆ 3.5 % hydrogen peroxide irritates skin and eyes. Wear gloves and eye protection.
- ◆ KOH is corrosive. Wear gloves and eye protection, and label the KOH bottle.
- ◆ *B. subtilis* 168: handle it under your lab's biosafety rules (BSL-1). Inactivate the culture and everything it touched before disposal, as your institution requires.
- ◆ Keep the power off while you connect tubing, and keep electronics away from liquids.
- ◆ If a pump or servo makes an odd noise, smell or light, cut the power at once.
- ◆ The soldering iron runs at 350 °C. Keep it in its stand, solder in a ventilated room, and wear eye protection.

Tools and supplies

- Metric and imperial screwdrivers
- 3D printer, for the valve parts
- Sharp scissors or a tubing cutter
- Ruler
- Dropper, for the leak test
- Soldering iron, lead-free solder, wire stripper, multimeter, hot-glue gun
- Gloves and eye protection

Files**Print files**

Download the STL files from the Design Files section of our wiki's Bioreactor page.

Parts checklist from the wiki's materials list (Bioreactor page, 9.1) and the draft

QTY	PART	DETAILS
CULTURE AND MEMBRANE		
☐	1 Pro photometer	built with its own assembly guide, with its flow cuvette
☐	1 Hollow-fiber membrane	Cobetter Biophsep MiniLab: mPES, 0.2 µm, 200 cm ² , 11 fibers
☐	1 Culture bottle (lumen side, 1 L)	SCHOTT bottle
☐	1 Protectant bottle (shell side, 1 L)	SCHOTT bottle
☐	1 KOH bottle (~100 mL)	SCHOTT bottle
☐	1 Magnetic stirrer and stir bar	USB, variable speed
☐	1 Green LED strip, 3 m	for the green light that switches production on
PUMPS, VALVES AND SENSORS		
☐	1 Culture pump	ZP4000-N79H peristaltic, 1 L/min
☐	1 Liquid pump	1.5 L/min
☐	1 KOH pump	5 mL/min
☐	10 Luer valves	three-way stopcock, Fr6-10
☐	5 Filters	hydrophobic PTFE disc vent filter, 0.45 µm: 4 ports and the culture bottle
☐	4 Pressure transducers	BP8G-AGB: 0–1 MPa gauge, 0–5 V out

	QTY	PART	DETAILS
☐	1	Flow meter	ZJ-TM02
☐	1	pH sensor	DFRobot Gravity analog pH V2: board and glass probe
☐	5	Servo motors	STS3215, 12 V: 1 for the pinch valve, 4 for the automated Luer valves
☐	1	Servo bus adapter	bus serial servo adapter
☐	2	M4 nuts	for the pinch valve
☐	2	M4 button-head screws	for the pinch valve
THE SENSOR ADAPTER CABLE			
☐	1	Perfboard	3 × 8 holes, 2.54 mm
☐	2	3-pin connectors	one female socket, one male header, 2.54 mm
☐	2	Resistors	15 kΩ and 22 kΩ
☐	1	Ceramic capacitor, 0.1 μF	marked 104
☐		Wire and hot glue	red and black hook-up wire
TUBING, PRINTS AND SUPPLIES			
☐		Tubing	silicone, 4 × 6 mm and 5 × 7 mm, 50A
☐		Filament	black PLA and TPU 95A, for the valve parts
☐		Gloves	
☐	1 L	Hydrogen peroxide, 3.5 %	for sterilizing
☐	~5 L	Distilled water	for the water run and the rinses
☐		Clean culture medium	for the photometer's blank

STAGE 1 Build the Pro photometer

YOU NEED

-  The Pro Photometer Assembly Guide
-  The Pro photometer's parts

- 1** Build the Pro photometer with its own assembly guide, up to and including its Test 4.
Do not blank it yet: you blank it in Stage 7, after sterilizing and rinsing.
- 2** Keep its flow cuvette in place: it goes into the culture line in Stage 5.

CHECK

1. The photometer passes its Tests 1 to 4.

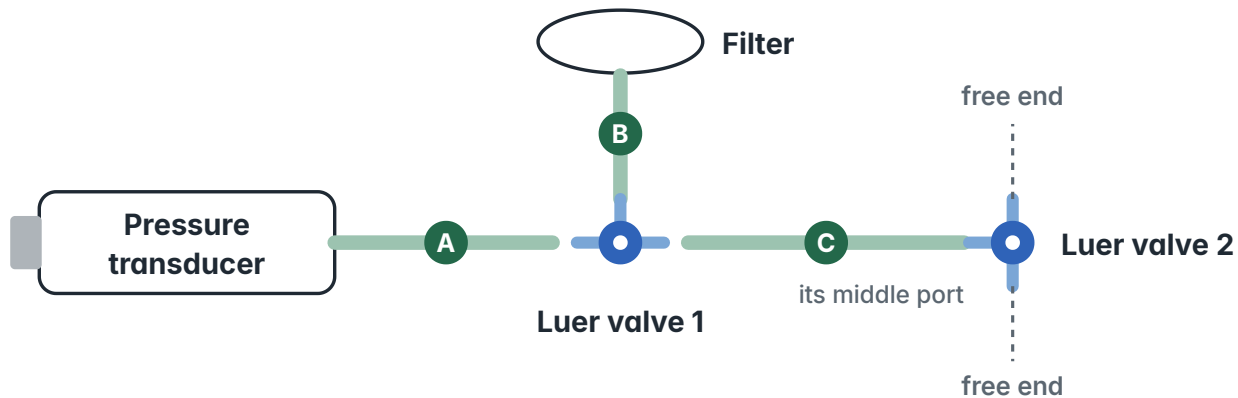
STAGE 2

Make the four port assemblies

Make 4: one for each membrane port, In-Lumen, In-Shell, Out-Lumen and Out-Shell. All four are built the same way.

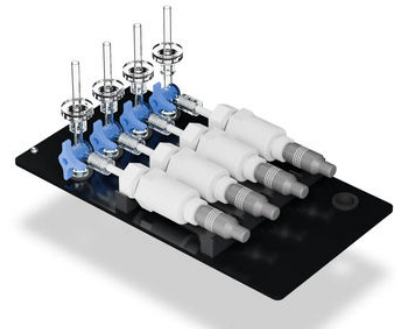
YOU NEED

- 4 filters
- 8 Luer valves
- 4 pressure transducers
- Tubing
- Sharp scissors
- For the check: a dropper and water



One port assembly. A, B and C are short pieces of tubing.

- 1 Cut three short pieces of tubing: A, B and C.
- 2 Push A onto the left port of the first Luer valve, B onto its middle port and C onto its right port.
- 3 Push the free end of B onto the filter.
- 4 Push the free end of A onto the pressure transducer.
- 5 Push the free end of C onto the middle port of the second Luer valve.
- 6 Label the assembly with its port: In-Lumen, In-Shell, Out-Lumen or Out-Shell.
- 7 Make the other three the same way.



The four pressure ports, in the bioreactor's 3D model.

✓ CHECK · LEAK TEST, EACH ASSEMBLY

1. Turn the second valve's handle so its middle arm points at the right port. This closes the left path.
2. Hold the assembly with that right port facing up.
3. Drip 3–5 drops of water into the right port with the dropper.
4. Slowly turn the first valve's handle through its positions.
5. Watch every joint: no water may escape.

If it fails: Check the tubing: refit a loose piece, replace a damaged one. If it still leaks, replace the valve.

STAGE 3

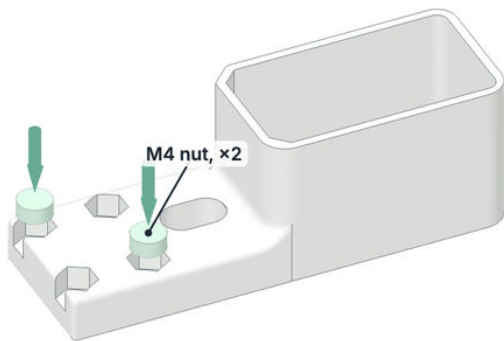
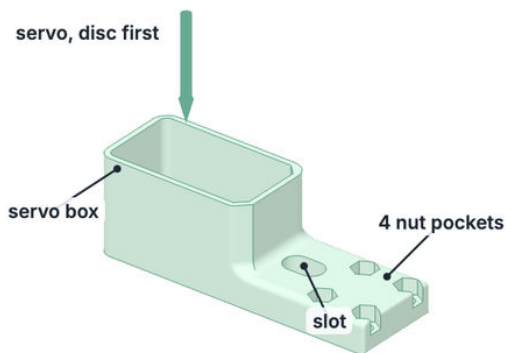
Fit the valve servos

Each of the five servos sits in a printed box: one in the pinch valve's body, four in the Luer-valve servo mounts. Green in the pictures marks what each step adds.

THE PINCH VALVE · ONE

YOU NEED

- 1 STS3215 12 V servo
- 4 servo screws
- 2 × 4 mm button-head screws
- 2 M4 nuts
- The printed pinch-valve body
- Screwdriver



- 1 Print the pinch-valve body (releaf-pinch-valve-body.stl). A box for the servo, and an arm with four nut pockets.
- 2 Fit the servo's metal disc onto its rotor.
- 3 Screw a servo screw into the hole at the centre of the disc.
- 4 Lower the servo into the box, disc end first, until it stops on the two ledges inside the far end.
Its back and cable socket stay above the box, as in the photo below.
- 5 Press an M4 nut into each of the two diagonally opposite hex pockets shown in the picture.
Each nut sits flat, below the arm's face. The 2 button-head screws go into these nuts.

CHECK

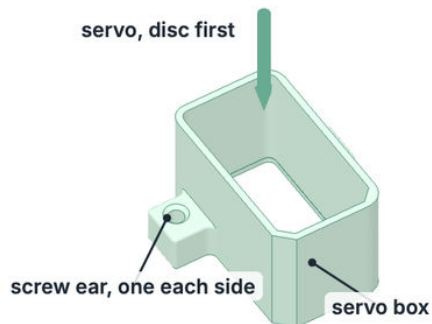
1. The servo sits square in its box and does not rock.
2. Both nuts sit flat in their pockets.



The team's pinch valve, assembled. The servo body is on top, with the valve's lower parts under its arm.

YOU NEED

- 4 STS3215 12 V servos, each with its metal disc and centre screw
- 4 printed Luer-valve servo mounts
- Screwdriver



- 6 Print 4 Luer-valve servo mounts (releaf-luer-valve-servo-mount.stl).
The same box as the pinch valve's, with a screw ear on each side. Each ear has a 4.2 mm hole.
- 7 Fit each servo's metal disc onto its rotor, and screw a servo screw into the centre hole.
- 8 Lower each servo into its mount, disc end first, until it stops on the two ledges inside the far end.

CHECK

1. Each servo sits square in its mount and does not rock.

YOU NEED

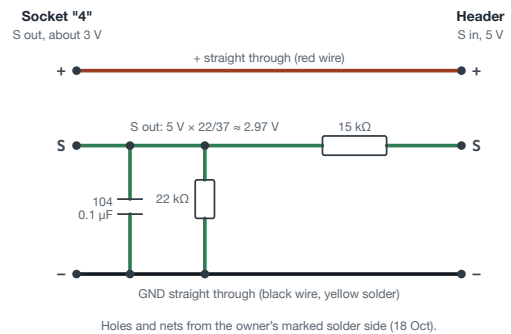
- A small perfboard, 3 × 8 holes at 2.54 mm
- One 3-pin female socket and one 3-pin male header, 2.54 mm
- Resistors: 15 kΩ and 22 kΩ
- One 0.1 μF ceramic capacitor (marked 104)
- Red and black hook-up wire
- Hot glue
- Soldering iron, solder, multimeter, a 5 V supply

Most of our sensors give a 5 V signal, but the Arduino UNO Q's inputs take 3.3 V. The adapter sits on the sensor's cable and steps the signal down: + and – pass straight through, and S goes through 15 kΩ, with 22 kΩ and a 0.1 μF capacitor from the output to –. 5 V in gives $5 \times 22 / 37 \approx 2.97$ V out.

- 1 Solder the socket at one end of the board and the header at the other, and mark both ends –, +, S in the same order.

On the team's adapter the 5 V signal comes in on the header, and the socket's lead carries the divided signal out.

- 2 Join + to + and – to – from end to end with red and black wire.
- 3 Fit the 15 kΩ between the header's S (in) and the socket's S (out).
- 4 Fit the 22 kΩ and the 0.1 μF capacitor between the socket's S (out) and –.
- 5 Check it (below), then seal the board in hot glue.



The adapter's circuit, as the team built it.

On the team's 3 × 8 board: the 15 kΩ lies in the S row from column 6 to 3, the 22 kΩ in the + row from 3 to 6, and the capacitor's leads go into the – row at 3 and 6. Column 3 is soldered together as S out; column 6 of the + and – rows and the header's – are soldered together as ground. Any layout that makes the same connections works.

CHECK · THE ADAPTER

Set a multimeter to continuity, then to Ω, then to volts with a 5 V supply.

1. **Pass-through:** + beeps end to end, and – beeps end to end; + and – do not beep to each other.
2. **Series:** S reads about 15 kΩ from the header to the socket.
3. **To ground:** the socket's S reads about 22 kΩ to – (it settles after a moment, as the capacitor charges).
4. **Divider:** with 5 V on the header's S (– to ground), the socket's S reads about **2.97 V**.

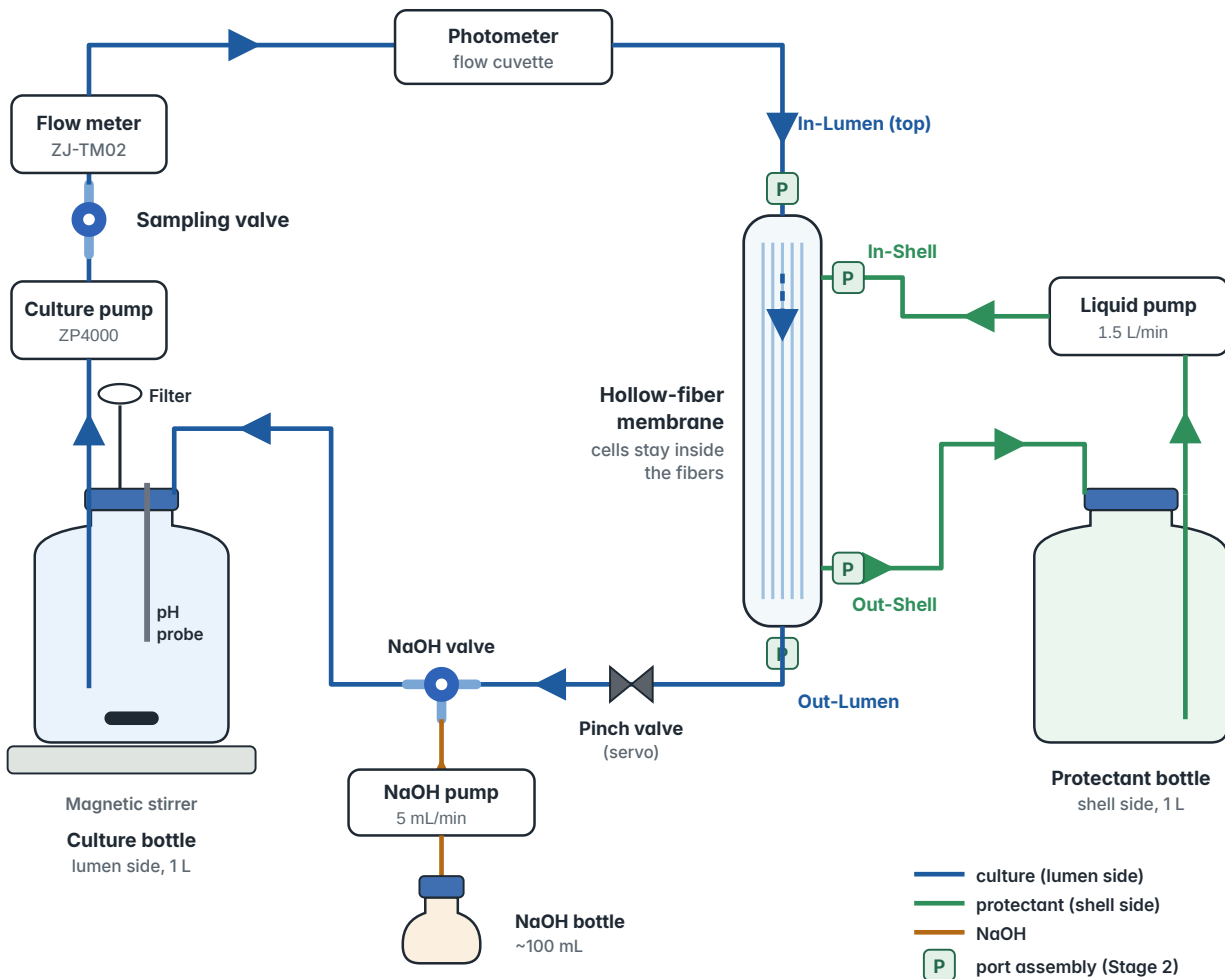
If it fails: Above 3.3 V: the two resistors are swapped. 0 V: S is open or shorted to –. Reflow the joint and check again.

STAGE 5

Connect the loop

YOU NEED

- The membrane and the 4 port assemblies
- The Pro photometer
- Flow meter
- Culture pump, liquid pump and KOH pump
- Culture, protectant and KOH bottles
- 1 filter
- 2 Luer valves: sampling and KOH
- The pinch valve
- Stir bar and magnetic stirrer
- pH probe and its board
- Tubing, scissors, ruler
- Gloves



The loop as these steps connect it: a map of what joins what, not of where each part sits.

THE MEMBRANE

- 1 Put on gloves.
- 2 Push each port assembly onto its port on the membrane: In-Lumen, In-Shell, Out-Lumen and Out-Shell.

THE CULTURE LINE: BOTTLE TO MEMBRANE

- 3 Put the stir bar in the culture bottle.

- 4 Under the cap, fit a ~75 mm (3 in) tube to one cap port. It must reach near the bottom, stay clear of the centre, where the stir bar spins, and not press against the wall.
Fit it with the cap off. Check it again with the cap on.
- 5 Join that cap port, on top, to the culture pump's inlet with a 150 mm (6 in) tube.
- 6 Join the culture pump's outlet to the flow meter's inlet with a 100 mm (4 in) tube.
The flow meter's arrow points away from the pump.
- 7 Cut this tube in two and join the halves with the sampling Luer valve.
- 8 Join the flow meter's outlet to one end of the photometer's flow cuvette with a 50 mm (2 in) tube.
- 9 Join the cuvette's other end to the In-Lumen port assembly, at the top of the membrane, with a 75 mm (3 in) tube.
Tug each tube to check the fit, gently: the cuvette is glass and breaks.

THE CULTURE BOTTLE'S CAP

- 10 Fit a filter to a second cap port with a 50 mm (2 in) tube from the filter's Out.
No tube inside: this port only lets air in and out, and keeps the bottle sterile.
- 11 Put the pH probe into the slot in the cap. Lead its wire to the pH board, and plug the sensor into the Arduino.
- 12 Stand the culture bottle on the magnetic stirrer.

THE RETURN LINE AND THE KOH LINE

- 13 Join a third cap port, on top, to end port C of a Luer valve, the KOH valve, with a ~150 mm (6 in) tube.
- 14 Join the KOH valve's middle port B to the KOH pump's outlet with a ~40 mm (1.5 in) tube.
- 15 Join the KOH pump's inlet to the KOH bottle's cap port with a ~40 mm (1.5 in) tube.
- 16 Under the KOH bottle's cap, fit a ~40 mm (1.5 in) tube to the same port. Screw the cap on tight.
- 17 Join the KOH valve's end port A to the Out-Lumen port assembly with a ~250 mm (10 in) tube.
- 18 Fit the pinch valve on this tube, about 50 mm (2 in) from the Out-Lumen connection.

THE SHELL SIDE

- 19 Under the protectant bottle's cap, fit a ~75 mm (3 in) tube to one cap port. It must reach near the bottom and not press against the wall.
Fit it with the cap off. Check it again with the cap on.
- 20 Join that cap port, on top, to the liquid pump's inlet with a ~100 mm (4 in) tube.
- 21 Join the liquid pump's outlet to the In-Shell port assembly.
- 22 Join a second cap port of the protectant bottle to the Out-Shell port assembly with a ~200 mm (8 in) tube.

✓ CHECK

1. Every tube is pushed fully on and tugged.
2. All the caps are tight.
3. The dip tubes are clear of the stir bar and the bottle walls.

STAGE 6

Water run

YOU NEED

- Distilled water

- 1 Fill the culture bottle with distilled water.
- 2 Run the bioreactor.
- 3 Check every joint for leaks.

✓ CHECK

1. No leaks anywhere.
2. The photometer reads correctly.
3. The pinch valve causes no breaks in the loop.
4. The pH sensor reads accurately.

If it fails: Refit a loose tube or replace a damaged one. Replace a valve that still leaks.

STAGE 7

Sterilize and blank

YOU NEED

- 1 L of 3.5 % hydrogen peroxide
- About 5 L of distilled water
- Starch-iodide test paper
- Clean culture medium
- Gloves and eye protection

- 1 Run 1 L of 3.5 % hydrogen peroxide through the bioreactor for 15 minutes.
- 2 Drain the peroxide.
- 3 Run 1 L of distilled water through the bioreactor for 30 minutes, then drain it.
- 4 Test the rinse with starch-iodide paper. Repeat step 3 until no peroxide is left and the rinse water's pH is neutral.
Five rinses of 1 L are usually enough.
- 5 Fill the photometer's flow cuvette with clean medium.
- 6 Blank the photometer: press **Calibrate blank** in its app.
Always blank after sterilizing and rinsing, never before.

✓ CHECK

1. No peroxide is left in the rinse water.
2. The rinse water's pH is neutral.
3. The photometer is blanked with medium.