

FIRST TIME USE

1

Your mini chamber system for intermittent respirometry comes with up to four chambers positioned in a chamber holder, miniature DC pumps mounted on the outside of the water bath, tubing, and fittings (1). Four of the pumps are used for flushing the chambers, and four pumps are used for recirculating (mixing) water inside the chambers during the closed measuring phase.

2

Attach the open T-pieces with the barb hose fitting (2a) to each overflow and a stopper (2b) to each drain hole on the water bath. Fill the water bath with experiment water until the water level reaches the overflow. Connect a DC power adapter (3) to each of the eight pumps, and insert the power adapters for the four flush pumps into the four sockets on one of the two PowerX4 power strips. Use the second PowerX4 to power the four recirculating pumps.

3

Set the DC power adapter to the 12 V setting (3). Connect each PowerX4 to a grounded wall outlet. This will start the pumps after a delay of 1 second. Input voltages between 5-12 VDC will change the flow.

The pump must be fully purged of air before pump operation. Any air in the system will cause inconsistent pump performance and a risk of overheating. To prime the pump with water, the pump intake must be below the level of the water surface at all times.

Let the pump run for 10 seconds to empty it for any air trapped inside the pump housing (3.1). Then stop the pump and repeat this step to make sure that the pump is fully purged of air. Air is less likely to get trapped inside the pump, if the pump is placed with the ports pointing straight upwards. Also, an increase in water temperature can force dissolved gasses out of the solution forming numerous small gas bubbles on all solid surfaces. Leave everything at a constant temperature for a while to reduce the risk of this phenomenon.

4

Mount oxygen sensor spot(s) centered on the inside of the chamber(s) by following the instructions in the online FAQ (4). Keep chambers/spots in a dark place between trials to avoid bleaching of the oxygen sensitive dye. Sensor spot performance check (amplitude value): Good performance =  $10000 \leq 50000$ . Bad performance  $< 10000$ .

5

Insert chamber(s) in holder using adapter rings, if necessary. Use the tubings and fittings matching your specific chamber ID to connect pumps as shown in the picture (5):

1. Connect flush circuit (blue). Leave the open end of the flush tubing slightly above water level.
2. Connect recirculating circuit (red). Use supplied steel tube, and attach recirculation tubing with a smaller piece of tubing (green) inside, if needed.
  - Chamber ID 28 = ID 4.79 mm tubing
  - Chamber ID 18.5 = ID 3.14 mm tubing
  - Chamber ID <18.5 = ID 1.59 mm tubing
  - Use tube reduction fittings, if necessary.
3. Keep tubing submerged and as short as possible while avoiding collapse of the tube.

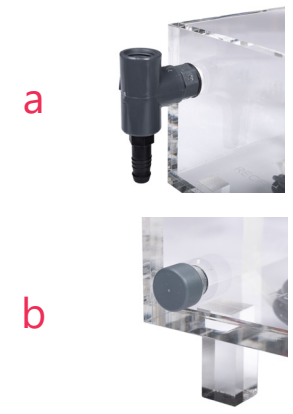
6

Set up your Witrox instrument by following its quick guide or online FAQ. Insert the bare tip of the fiber optic cable(s) into cable holder(s) through the 45 degree hole. Make sure that the tip is directly above and as close to the internal sensor spot as possible. Then fix the fiber cable with the red finger knob and fix the chamber with the nylon screw (6). Do **NOT** overtighten!

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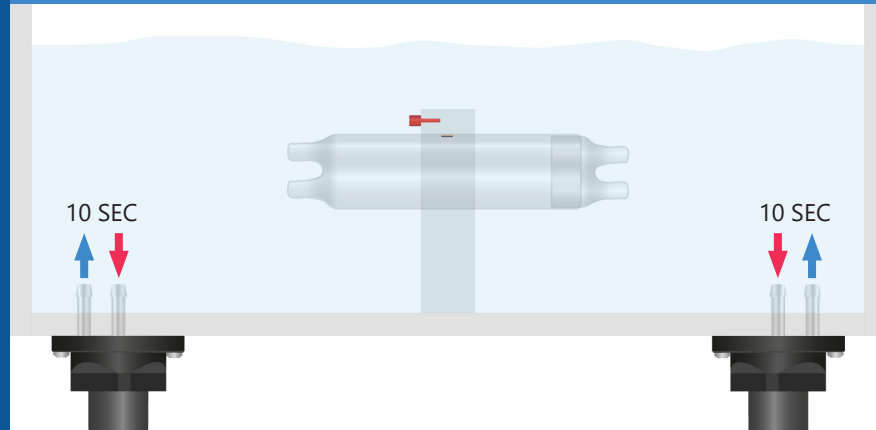
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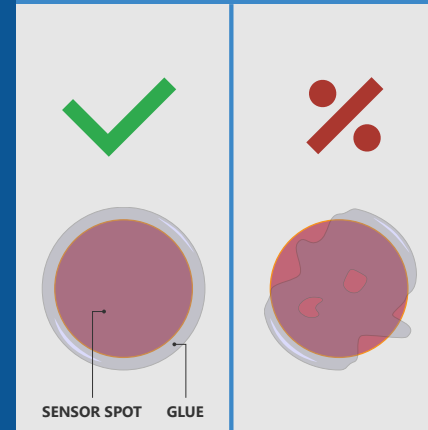
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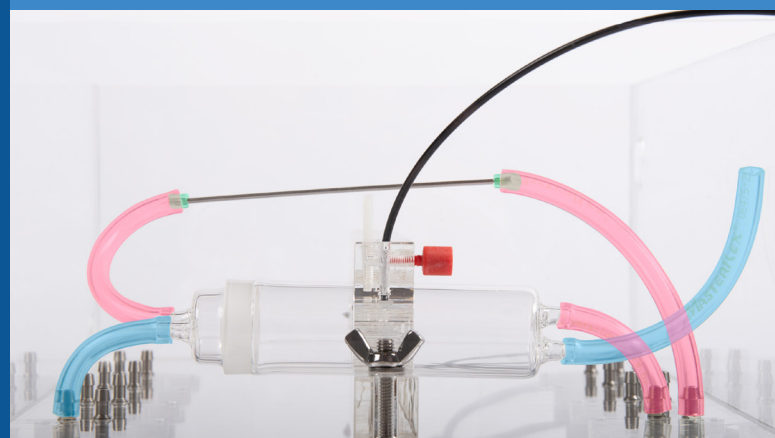
3.1



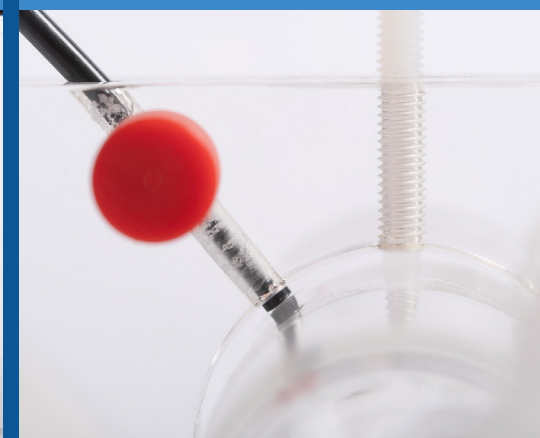
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6



FOR EACH TRIAL

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Start each trial by purging the pumps of air (see step 3). Acclimate the system by flushing chamber(s) for 30 min. at constant temperature (7). Make sure that water exits open tube end throughout the acclimation period. Remove any air bubbles from the chamber(s) before adding the organism(s).

**MAKE SURE** to attach/detach the glass stopper using a twisting motion (rather than push/pull). This is easier if the ground glass joints are wet. Use non-toxic silicone grease, if necessary.

8

Add organism to chamber. For most animal species, a soft tubing allows easy access to chamber while limiting addition of air bubbles. Check that fish face flush-end of the chamber, so that their gills are being ventilated (8).

**MAKE SURE** that the flush pump is on and that water exits the open tube. Otherwise, your organisms may suffocate during setup!

CALIBRATION, SERVICE & MAINTENANCE

9

Follow instructions in **Quick guide for Witrox instrument** to calibrate oxygen sensors.

10

Pumps should be stored clean, empty and dry between use. Detach any tubing, empty the bath/vessels/chambers and refill with tap water adding mild detergent or bleach, if needed. Let pumps run with solution at 12 VDC for a few minutes (10). Empty pumps and bath/vessels/chamber and leave pumps upside down to drain pumps completely.

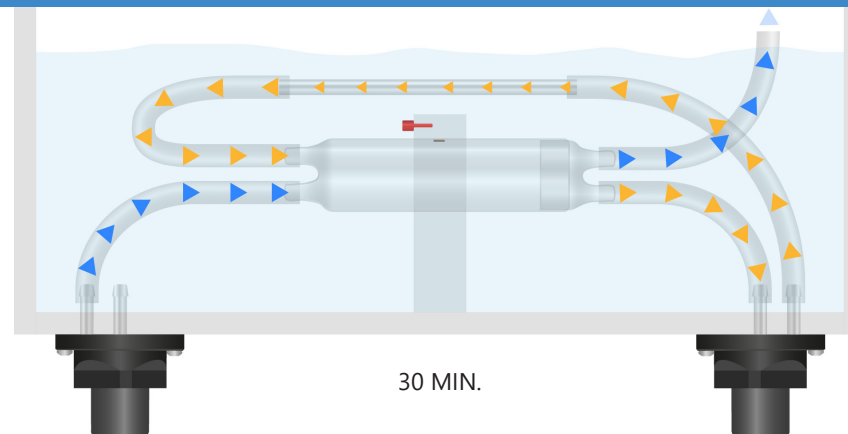
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Any chambers with sensor spots or individual sensor spots should be stored in a dark place between trials to avoid exposing the fluorescent dye to UV light. UV light will bleach the sensor and cause signal drift.

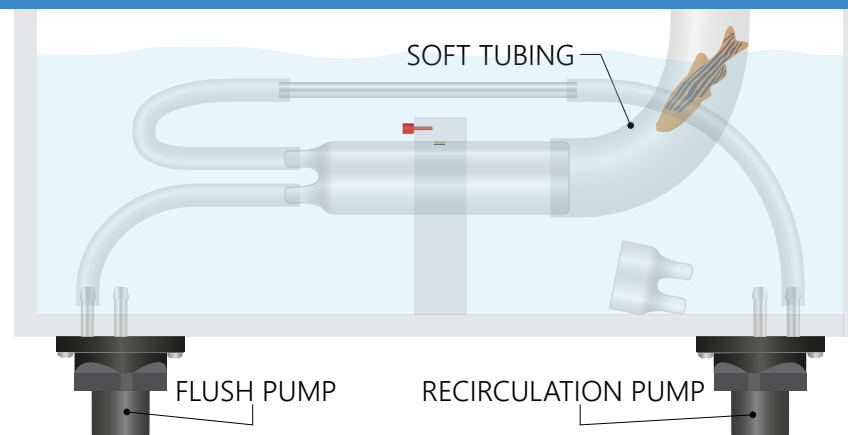
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Do **NOT** use alcohol on acrylic parts (water bath, chamber holder and adapter rings) as it can cause cracks (12). Use instead bleach or detergents for cleaning.

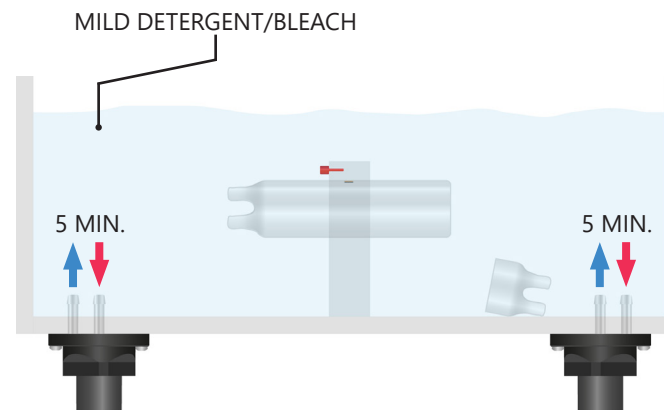
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**DO NOT USE ALCOHOL FOR CLEANING**