



## RC400 RESPIRATION CHAMBER

### INSTRUCTIONS FOR USE

#### Introduction

The RC400 respiration chamber is an acrylic chamber with a detachable perforated floor on which the animal is placed. The space below the floor is occupied by a spinbar driven by a magnetic stirrer. Although microcathode electrodes like the 1302 do not require the water to be stirred, a gentle stirring is nevertheless necessary to produce a homogeneous distribution of oxygen in the chamber. The lid of the chamber screws on to the bottom half, making a seal against an 'O' ring. The 1302 electrode is fitted into the EH100 electrode holder supplied, which is then inserted through an 'O' ring in the centre of the lid.

#### Fitting the electrode into the EH100 holder

Unscrew the cap on the electrode holder. Insert the electrode into the holder. Pass the cap over the cable and screw it on tight.

**When doing this it is essential that the electrode holder is held vertically, so that the topmost O ring is centred symmetrically around the cable. If you do not do this, the top O ring can be distorted up through the opening in the cap. This in turn will mean that insufficient pressure is exerted on the electrode, which may cause leakage when the tip of the holder is immersed in liquid.**

#### Preparation of the chamber for a respiration run

Calibrate the electrode within its holder as detailed in the 782 or 929 Instruction Manual. Keep it at experimental temperature while setting up the chamber as detailed below.

Remove the perforated base of the chamber and insert the magnetic spinbar. Add aerated water at the correct temperature up to about the level of the 'O' ring. Insert the perforated base using forceps and swirl the chamber to remove adhering air bubbles. Check carefully for the presence of trapped air bubbles. A fine paint brush is quite useful for dislodging these.

Add the experimental animal to the chamber. Screw the lid on, until it seals against the 'O' ring. Insert the electrode holder, with the electrode in place, through the hole in the centre of the lid so that the tip just projects into the chamber.

Place the chamber in a constant temperature waterbath and position it over a magnetic stirrer. The water level of the waterbath should be below the top surface of the chamber at this stage.

Then **either**

push the electrode down until its tip is just below the surface of the water in the chamber. Switch on the magnetic stirrer to bring the water back to full aeration again before topping up with aerated water through the conical holes in the lid and sealing with suitable bungs.

**or**

fill the chamber through the holes, and then insert a water circulation tube through each hole and pump temperature-controlled aerated water through the chamber during the settling-in period.

Before sealing the chamber at the start of a respiration run, it is essential to make a quick visual inspection to ensure that there are no air bubbles present in the chamber. If there are, guide them up to and expel them through one of the holes in the lid. A moistened pipe cleaner is quite useful for coaxing bubbles which may tend to stick to the acrylic surfaces.

### **Water Volume**

In order to calculate respiration rate in a closed chamber, it is necessary to know the water volume. This will vary with the volume of the animal in the chamber. The easiest way of determining water volume is to wait until the end of the experiment, and pour off the water into a beaker and then to a measuring cylinder.

### **Notes**

The electrode should be calibrated in its holder.

Take care to protect the exposed tip of the electrode and preferably keep the tip covered in water when not in use for short periods.

It is important that air bubbles do not remain attached to the top of the electrode holder when it is inserted into the chamber. This could happen if the holder develops a thin film of grease. If this should happen, clean the end of the holder in a dilute detergent solution, taking care not to touch the membrane of the protruding electrode tip. Then rinse with distilled water and dry carefully with paper tissue.

### **Caution**

Ensure that water, particularly seawater, does not leak down the cable into the electrode causing irreparable damage to the electrode. Symptoms of problems of this sort are a very high short circuit current so that it becomes impossible to zero the meter when the electrode is being calibrated in a zero oxygen solution.