



RC 300 AND RC350 RESPIRATION CELLS

INSTRUCTIONS FOR USE

Introduction

The RC300 and RC350 comprise a water-jacketed respiration chamber with an adjacent 'parking chamber'. The respiration chamber is made from precision bore glass and is marked with a white spot. The parking chamber is unmarked and is used to hold the electrode at the correct temperature when not in use during an experiment. A small magnetic spinbar should be introduced into the respiration chamber and the whole unit placed on a magnetic stirrer.

For rapid metabolic events, (i.e. those in which the oxygen in the solution would be depleted in under 10 minutes), it may be preferable to use the more permeable FEP membranes with their faster response times. (Order Part No: SI025 and the special electrode jacket, Part No: SI035.)

Electrode holder assembly

The electrode holder is made from black acetal. The electrode is positioned in the holder by pressure exerted by the cap on three O rings at the cable-entry end of the electrode. This seals the nose of the electrode against the precision shaped plastic tip of the holder, so that only the membrane-covered cathode protrudes.

Unscrew and remove the cap of the electrode holder.

Insert the electrode into the electrode holder. Feed the cap of the electrode holder over the cable and screw it up 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.

Connect the electrode to the 781 or 782 meter, or to the interface of the 928.

Calibration of chamber volume

The respiration chamber volume which is used in experiments can be varied from c 0.3 to 1.0 ml in the RC300 and from 1 ml to 3 ml in the RC350, by means of the collar on the stem of the holder. Rotate this collar to the left until the first screw thread is exposed. Drop the magnetic spinbar into the chamber. Pipette into the chamber the volume of the liquid which will be used in the experiment. Insert the probe holder into the chamber, with the milled groove towards you, until the collar rests on the top surface of the cell. Angle the top of the chamber away from you and slowly rotate the knurled collar to the right whilst exerting slight downward pressure on the probe holder. This will advance the holder into the chamber and will gradually gather the air bubble. The air passes up the milled groove. Allow the meniscus of the liquid in the groove to rise the 5mm to the level of the recessed area. It may be helpful to do this under a strong light, in order to illuminate the meniscus.

The probe holder will now be calibrated to the required chamber volume, and care should be taken to ensure that the collar is not knocked, jarred or rotated after this. Remove the electrode and holder and insert it into the parking chamber.

Making respiration measurements

Connect the water jacket to a pumped source of constant temperature water set at the desired temperature for the experiment. The temperature should be maintained $\pm 0.05^{\circ}\text{C}$. Bring the respiring preparation to this temperature and keep it aerated by bubbling a fine stream of air through it.

Switch on the oxygen meter, or the 928 interface and computer, and calibrate the electrode, within its holder, at the temperature of the experiment, as follows:

Zero calibration: Pour about 1cm depth of 4% sodium sulphite solution into a 100ml beaker and dip the electrode holder into this. Wait until the minimal reading is obtained (c. 3 - 4 minutes with polypropylene membranes; c. 1 - 2 minutes with FEP membranes) With the 781 meter, use the 'set-zero' knob to give a zero display, and set the chart recorder to zero. With the 949 or 928 system follow the calibration instructions in the manual.

100% saturation calibration: Carefully rinse all traces of sulphite solution from the electrode holder with distilled water. Slide the electrode holder into the parking chamber and leave for 10 minutes. Pipette the calibrated volume of water into the respiration chamber and drop in the magnetic spinbar. Place the cell on a magnetic stirrer and switch on. Leave for 10 minutes to ensure full aeration of the liquid.

Switch off the stirrer, and angling the top of the cell away from you, slide in the electrode holder with the slo towards you as before. Switch the stirrer on again. Wait until a stable reading is obtained and then set the 781 meter to 100% saturation or to the concentration of oxygen expected in the preparation at time zero (see section 4.3 of the Model 782 oxygen meter manual), using the 'Fine' control knob. Set the full scale on the chart recorder. If using the 949 or 928 system, follow the instructions in the manual.

Withdraw the electrode holder and transfer it to the parking chamber. Suck out the contents of the chamber (do not lose the magnetic spinbar during this). Dry the inside with a piece of absorbent tissue. Reinsert the spinbar. Add the desired volume of respiring preparation to the chamber and then insert the electrode holder as before, ensuring that no air bubbles remain in the chamber. Start the respiration rate recording.

Metabolic studies on mitochondria, cell suspensions etc.

Small quantities of inhibitor or substrate may be added to the chamber during a respiration run by injecting it through a fine stainless steel needle (supplied) connected to a syringe pipette (Hamilton or similar) and passed down the groove. When doing this, ensure that there is no air bubble at the end of the needle when it enters the chamber. A volume of liquid corresponding to the volume injected, will be displaced upwards into the recessed area behind the tip of the electrode holder.

General points

It is important that air bubbles do not remain attached to the tip 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 electrode 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.

When not in use, the 1302 oxygen electrode can be kept within the electrode holder in the parking chamber, until the membrane next requires to be changed. Keep a drop of distilled water at the base of the chamber, to create a saturated atmosphere so that the electrolyte does not dry out.

Avoid introducing any fine grit into the chamber since this will scratch and score the probe holder.

Downward Drift

If the electrode is inserted into aerated distilled water in the chamber and left to run for 1 - 2 hours, it is often found that there is a downward drift of the oxygen values. Surprisingly, this is invariably due to bacterial contamination of the distilled water. This can be remedied by keeping the chamber filled with hypochlorite solution (e.g. dilute solution of bleach) overnight and then by using freshly boiled, then cooled and re-aerated distilled water to check the stability of the electrode.

Remember to wash the hypochlorite out with several rinses of distilled water before starting an experimental run.

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