RC 650 6 - CELL RESPIROMETER
INSTRUCTIONS FOR USE

Introduction

The RC650 has been designed to enable up to 6 simultaneous closed cell respiration measurements to be made in volumes of between 1 and 3 mls, in conjunction with the Strathkelvin Instruments model 928 6-channel repirometer system. It is essential that you are familiar with the operation of the 928 system and we recommend that you read the Instruction Manual before commencing a respiration run.

The RC650 comprises:

A sealed water bath which is connected to your pumped source of constant temperature water.
6 'active' cells for use in respiration measurements.
6 'parking' cells to keep the electrodes at the correct temperature when not in use.
6 electrode holders which can be adjusted to set the volume in the 'active' respiration cells to between 1.0 and 3.0 mls.
A 6-place 12 volt magnetic stirrer unit.

Bath Unit

The 6 'parking' cells are located at the rear of the bath. The 6 'active' cells are at the front of the bath and are accurately centred over the coils of the magnetic stirring unit beneath. Each cell is sealed by a neoprene 'o' ring.

Setting up the bath

The bath unit comprises a grey base unit which houses the magnetic stirrer, an acrylic bath unit which locates onto two pins on the top of the base, and a vertical upstand with 6 holes for the electrode cables. Screw the two water connectors into the bath unit and tighten to compress the 'O' rings. Fix the vertical upstand to the back of the bath, using the bolts provided. Connect the plug from the transformer unit to the socket at the rear of the bath and plug the transformer unit into the mains. Connect the bath inlet and outlet tubes to your pumped source of constant temperature water, maintained at the temperature of the experiment and regulated to ± 0.05ºC. Pump water into the

bath. In order to completely fill it, tilt the whole bath unit to allow the air bubble to be drawn into the outlet tube.

Electrode holder assembly

The electrode holders are 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 the cap of the first electrode holder and insert the electrode. Feed the cap of the electrode holder over the cable and screw it up tight. Place the electrode holder in the first 'parking' cell and pass the cable through the first cable guide hole above. Connect the Lemo connector on the end of the cable to Channel 1 of the 928 Interface. Repeat for the other electrodes.

NB. Make sure the electrode holders are vertical when inserting and removing them from the cells. Do not tilt the holders as this might break the glass cells.

Calibration of chamber volume

Insert the first electrode holder into the first 'active' cell.

The volume of the cell can be varied from 1 ml to 3 ml, 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 cell. Pipette into the cell the volume of the liquid which will be used in the experiment. Insert the probe holder into the cell, with the milled groove towards you, until the collar rests on the top surface of the cell. Slowly rotate the knurled collar to the right whilst exerting slight downward pressure on the electrode holder. This will advance the holder into the cell and will gradually gather the air bubble into 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 electrode 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. Repeat with the other electrode holders in their respective cells.

Making respiration measurements

Electrode Calibration

With the water bath set to the desired temperature, place each of the electrode holders in its respective 'parking' cell.

Switch on the 928 interface and select the 928 program on the computer. Follow the Set up procedures described in the 928 Instruction Manual.

To calibrate zero, add the calibrated volume of approximately 4% sodium sulphite solution to each of the 'active' cells. When instructed by the computer, insert each electrode holder into its corresponding 'active' cell. It is not necessary to stir during zero calibration.
At the completion of this part of the calibration, remove each holder, rinse the tip with distilled water from a squeeze bottle and replace in the 'parking' cell. Suck out the contents of each 'active' cell and rinse **several times** with distilled water.

Finally, dry the cells.

To calibrate 100% saturation, first ensure that the spinbar is located in each 'active' cell. Pipette in the calibrated volume of water and switch on the magnetic stirrer. Stir for five minutes to ensure full aeration of the liquid.

When instructed by the computer, switch off the stirrer and transfer each electrode holder to its respective 'active' cell.

Switch on the stirrer again. When a stable reading is obtained, carry out the calibration steps as detailed on the computer screen. Switch off the magnetic stirrer and then return the electrode holders to their 'parking' cells.

Suck out the calibrating solution from the cells, remove the spinbars and dry the interior of the cells with absorbent paper tissue.

Zero and 100% calibration is now complete.

Respiration Measurement

Add the spinbars to the 'active' cells and then pipette the calibrated volume of respiring preparation to each cell. Ideally this should have been aerated and maintained at the experimental temperature immediately beforehand.

Switch on the magnetic stirrer and stir for about a minute to equilibrate. Switch off the magnetic stirrer and transfer each electrode holder to its 'active' cell. Switch on the magnetic stirrer again.

Start the recording on the computer as per the 928 Instruction Manual.

At the end of the run, switch off the stirrer, remove the electrode holders, rinse with distilled water and replace in their 'parking' cells. Suck out the contents of the 'active' cells, rinse several times with distilled water and dry with absorbent paper tissue. Take care not to lose the spinbar!

Metabolic studies on mitochondria, cell suspensions etc

Respiring preparations in metabolic studies are often conducted at 37ºC and have a fast time course. For these studies, it is preferable to use 12.5µ F E P membranes which have a very fast speed of response - typically 95% in 6 - 8 seconds. These membranes have to be mounted on special electrode jackets (part number SI035) which have a very fine capillary hole opposite the anode. This hole prevents distension of the membrane which would otherwise be caused by pressure building up as the electrode jacket is screwed on.

Small quantities of inhibitor or substrate may be added to the chamber during a respiration run by injecting it down the groove, using the 9cm spinal needle connected to a Hamilton or similar precision syringe. 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 holder in a dilute detergent solution. 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, until the membrane next requires to be changed. Keep the electrode holder in the ‘parking’ cell which should have a drop of water added to provide a saturated atmosphere so retarding the rate of water loss from the electrolyte through the membrane.

Removal of solutions from the 'Active' cells can be speeded up by using a pasteur pipette connected via a water trap to a vacuum pump or to water tap suction unit.

An plastic extraction tool is provided with the RC650 to allow easy removal of the cells for cleaning.

Downward Drift

If the electrode is inserted into aerated distilled water in the 'Active' cell and left to run for 1 - 2 hours, it is often found that there is a downward drift of the meter display. Surprisingly, this is invariably due to bacterial contamination of the distilled water. This can be remedied by keeping the cell filled with hypochlorite solution (e.g. dilute solution of bleach) overnight. The stability of the electrode can then be checked using freshly boiled, then cooled and re-aerated distilled water. Remember to wash the hypochlorite out with several rinses of distilled water before starting an experimental run.

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