Notes on the Care and Use of Bilayer Chambers and Cuvettes

Description

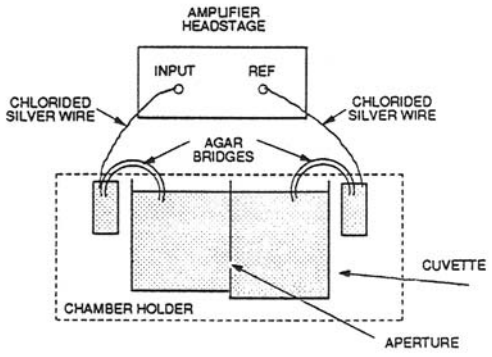
The bilayer cup/chamber system consists of a holder (chamber) and cuvette (cup) which incorporates the bilayer aperture. The chamber is made from black Delrin and cups are made from either translucent polystyrene or white Delrin. When mounted in the chamber, a set-screw securely holds the cuvette in place. Two agar bridge wells, with centrifuge tubes, are provided to facilitate electrical coupling with the amplifier. In addition, each cup and chamber is supplied with a magnetic stirbar which is designed to fit into the small well at the base of the cup or chamber. This design facilitates stirring of solutions with a minimum of mechanical noise associated with the stirbar colliding with the cup or chamber walls. Replacement stirbars can be ordered from Warner Instruments.

Using the chamber

Bilayer membranes are formed across an aperture in a septum which separates two chambers. The most common configuration is that of the cup and holder. The interior of the cup represents one chamber while the interior of the holder is the other chamber. The cup wall is the septum. Electrical connections are made via agar salt bridges into each chamber. The whole assembly must be shielded from electrical and vibrational interference to obtain low noise recording of bilayer currents.

Assembly

The general assembly of a cup/chamber is shown in the figure to the right. Since many membrane channels are sensitive to trace levels of silver, headstage leads should not be connected directly to the bath solutions. Instead, headstage leads are routed to wells containing a salt solution which are in turn connected to the solution baths via agar bridges. Salt bridge wells should ideally contain the same solution as used in the formation of the salt bridge, typically 1 M KCl. In addition, these wells should be adjacent to the baths so that the agar bridges used to complete the circuit from well to bath are as short as possible.



A typical setup.

Membrane formation

Bilayer membranes will spontaneously form after spreading a thin film of lipids over the aperture in the cup. In general, the aperture is first pre-coated with lipids before assembling the cup/chamber and forming the membrane. Lipid solutions are usually comprised of a cocktail of lipids suspended at 25-50 mg/ml in decane. The lipid solution should always be clear without visible turbidity. Contamination with water will make the lipids unusable and turn the decane solution milky.

The aperture must be prepared to accept lipids prior to membrane formation. This is achieved by 'coating' the hole with the lipid cocktail before adding solutions to the cup or chamber. Several techniques are employed to coat the hole prior to membrane formation. While

the choice of technique used will depend on your application, the materials at hand, and your ingenuity and training, two methods are presented below.

One method used to coat the hole is to insert several lipid covered hairs from a Red Sable paintbrush through the aperture. The brush is then revolved in a small circle until the hole is uniformly coated with lipid. (Use a size 00 or 000 Red Sable artists dotting brush which has been trimmed to present 3-5 hairs of the same length. The brush is cleaned and dipped into the lipid cocktail before coating the hole.)

An alternative method uses a small (1-2 mm) ball formed on the end of a glass rod or Pasteur pipette using a Bunson burner. An advantage of this technique is that it is straightforward to keep the glass rod, and hence the resulting membrane, free from contamination. The rod is used to pre-coat the hole by applying lipids to the outside rim of the hole. Finally, the rod is used to form a membrane by dipping the clean rod into the lipid solution and then 'touching' the hole with the coated rod tip. If properly performed, a bilayer membrane will be easily formed.

Once the hole has been pre-coated, but before the membrane is formed, the cup is inserted into the chamber and both sides filled with appropriate solutions. Take care to not over-tighten the cuvette set-screw as this can put undue stress on the cup and shorten its lifetime. A rubber plug has been inserted into the end of the screw hole to reduce the occurrence of cracking due to over-tightening of the set-screw. Additional rubber (1") is supplied to make replacements as needed.

MAINTENANCE

Cleaning

The stability of plastic materials must be taken into account when cleaning cups and chambers. For example, chloroform and decane can etch or dissolve polystyrene while Delrin is significantly more tolerant to these chemicals. Moreover, the stability of polystyrene is seriously compromised at pH greater than 9 or less than 4.

Cleaning of cups and chambers can be performed using a dilute detergent solution. Many investigators use Joy dish detergent since they can be assured of complete removal of the soap once the lemon scent has been rinsed away. Alternatively, Warner instruments has developed a TSP wash protocol which gives very good results. Contact our Technical Support staff or download the protocol in PDF format at http://www.warneronline.com.

Bridge formation

Agar bridges can be formed from 1-2% agar in a high salt solution (usually 3 M KCl). The agar is usually inserted into PE or glass tubing make the bridge, and can be stored for up to 1 month. As above, you can download an agar bridge protocol from our website.

Repair

The glass window on the chamber is glued into place with RTV silicone rubber adhesive (General Electric) and secured by screws. While unlikely, if the glass seal develops a small leak, it will become apparent as an electrical drift caused by a short circuit to ground during recording. The occurrence of this problem can be avoided by monitoring for signs of moisture beneath the chamber after use. If necessary, the leak can be repaired by carefully removing the glass slide with a scalpel and gently peeling off the residual silicone cement. After the glass and chamber surface have been thoroughly cleaned, the glass slide can be re-glued with RTV adhesive, the chamber reassembled and left to dry for 24 hours.

REORDER INFORMATION

Description	Part Number
BCH-13A bilayer chamber	
13 mm polystyrene chamber with 150 um aperture	CP13A-150
13 mm polystyrene chamber with 200 um aperture	CP13A-200
13 mm polystyrene chamber with 250 um aperture	CP13A-250
13 mm Delrin chamber with 150 um aperture	CD13A-150
13 mm Delrin chamber with 200 um aperture	CD13A-200
13 mm Delrin chamber with 250 um aperture	CD13A-250
BCH-22A bilayer chamber	
22 mm polystyrene chamber with 150 um aperture	CP22A-150
22 mm polystyrene chamber with 200 um aperture	CP22A-200
22 mm polystyrene chamber with 250 um aperture	CP22A-250
22 mm Delrin chamber with 150 um aperture	CD22A-150
22 mm Delrin chamber with 200 um aperture	CD22A-200
22 mm Delrin chamber with 250 um aperture	CD22A-250
PTFE coated stirbars	
2x5 mm stirbars for BCH-13 cup and chamber (5 pack)	MAG-13
2x7 mm stirbars for BCH-22 cup and chamber (5 pack)	MAG-22