

**The Care and Use of
RC Series Recording Chambers**

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RC Series Recording Chamber

Description

The RC Series perfusion chambers are designed for physiological experiments performed with the aid of inverted or dissecting microscopes. They are particularly useful for single-channel and whole-cell patch clamping and other electrophysiological applications including recording from oocytes, isolated perfused tubule, and cell impalement. They have also been used as a perfusion chamber in fluorescence microscopy.

The chambers come in a variety of sizes with either rounded or slotted shape, and can be fitted to standard microscope stages. The rounded tissue compartment is useful when two or more electrodes must be introduced (e.g., oocyte clamping) or when the tissue preparation requires a relatively large area (e.g., cultured cells grown on various supports). The slotted tissue compartment is preferred when a minimal bath volume and reasonably stable bath level and laminar flow are desired. A standard No. 2 thickness coverslip forms the bottom of all chambers for added durability while still providing high-quality optics.

The perfusion input is a Teflon manifold. A single input manifold is supplied with the chamber. Multi-input minimal dead space manifolds accommodating from 2 to 8 input lines may be employed (see Accessories and Supplies). The manifolds fit into a tapered hole in the chamber and are easily interchanged. They accept standard PE-160 polyethylene tubing (1/16" OD). An adjustable capillary tube attached to the chamber serves as the outflow pipette, obviating the need for a separate manipulator. Input perfusate can be gravity-fed with flow rates in excess of 10 ml/min. and removed via suction, or alternatively, input and output can be pump-controlled.

Electrical grounding of the bath is achieved either by placing a chlorided silver wire into a well containing pipette solution (with an agar salt bridge to the outflow reservoir) or directly into the outflow reservoir (for symmetrical solutions).

Typically cells grown on (or attached to) a coverslip are placed in the tissue compartment, so the microscope optics must have the ability to focus through the combined thickness of the coverslip containing the cells and the No. 2 coverslip forming the floor of the chamber. Capillary forces are usually sufficient to keep the cell coverslip in place, but the rounded chambers are also equipped with a narrow space between the glass and the polycarbonate so that the cell coverslip can be "slid and held" under this ledge.

Standard Accessories

Each chamber is supplied with:

1. One single input manifold (see Accessories and Supplies for optional manifolds)
2. Five (5) No. 2 glass coverslips 24 x 60mm
3. Two (2) glass capillary tubes (suction)
4. One (1) stainless steel capillary tube (suction)
5. Allen wrench to adjust suction tube tightness
6. Two screws for mounting chamber to a base
7. Half-moon washer (RC-11,13 & 16 only).

Preparation

The floor of the chamber is a 24 x 60 mm #2 cover glass, which is applied to the bottom of the chamber using one of the following:

1. High Vacuum Grease (Dow Corning).
2. Silicone RTV adhesive (Dow Corning or GE).
3. Preferred method - Sylgard™ 184 Silicone Elastomer (Dow Corning Corp., Midland, MI, Tel. (517)-496-6000).

The cover glass is placed in the milled channel. High vacuum grease is the simplest to apply especially if the coverslip needs to be replaced often but does not work well if the solutions are heated or if the chamber is to be autoclaved. Silicone RTV has been used with success but, depending on the formulation, leaching of chemicals in the adhesive could be a problem. The Sylgard™ 184 is the most permanent of the adhesives but the application is more involved.

Attaching The Cover Glass

High Vacuum Grease

- a. Apply a thin layer of grease to the areas in contact with the glass and press a cover glass into position, gently moving it back and forth to spread the grease evenly. Remove the cover slip and discard.
- b. Remove any excess grease, especially any in the working area and the small duct connecting the reservoir.
- c. Carefully place a new cover slip in place and press firmly without any lateral movement to avoid getting grease in the working area.

Silicone RTV Adhesive -

- d. 1) Follow the same procedure to secure the glass as outlined above taking care to avoid getting adhesive in the small duct.

- e. 2) Remove any excess adhesive after cured with a scapel or exacto knife.

Sylgard™ 184 Elastomer

- f. 1) Apply a thin layer of Sylgard™ Prime Coat (also available from Dow Corning) with a fine brush to the clean surface where adhesion is desired. Allow the prime coat to air dry for 1-2 hours.
- g. 2) Mix the Sylgard™ 184 base and curing agent in a 10:1 (by weight) ratio. Mix with glass stirring implements in a smooth action to minimize air bubble formation.
- h. 3) Apply a thin layer of the mixture with a fine brush to the primed surface and carefully attach the cover glass. Use two strips of transparent tape to hold the glass in place.

Curing

Place the chamber "bottom up" in an oven at 65° C (149° F) for four hours to cure the Sylgard™. If no oven is available, curing can occur over 24 hours at ambient (25° C), but full mechanical strength will not be achieved for 7 days (although the majority of its physical strength is present after 24 hours).

Cooling

After cooling, remove the tape and the chamber is ready for use.

Using the Chamber

Mounting

RC Series chambers are used with inverted or upright microscopes. Mounting to the stage is done with a mounting adapter such as those available from Warner Instrument Corp. to fit the Nikon Diaphot or Olympus IMT and IMT-2. Bases for other microscope models are also available. Provide a sketch with dimensions to our sales department for quotation.

If you choose to make your own, refer to the "RC Recording Chamber Base Cut Out Detail" drawing included with these instructions.

Perfusing

Solution inflow is through the teflon input manifold. Manifolds accept 1/16 inch OD polyethylene tubing. Select the manifold (standard single line or optional multi-line) for the number of solutions to be used and insert it into the chamber. Control of the solutions is done externally by manual or program controlled valves

Outflow

One metal and two glass outflow tubes are provided with the chamber. For most applications, the metal tube is the best choice. However, some users have reported improved recordings, particularly with small signals (lower noise levels) when using the glass tubes. We therefore supply both and suggest you experiment to determine which works best in your application.

Plastic tubing with a 1/16 inch ID will slide onto the outflow tube. The fluid level in the chamber is adjusted by moving the outflow tubing up or down. Once the desired level is attained, gently tighten the cap screw to prevent the tubing from moving.

Maintenance

The chamber is quite durable and can be easily cleaned by rinsing with water. Other solvents may damage the chamber and should be avoided. The most fragile parts of the chamber are the floor (a No. 2 glass coverslip) and the glass (if used) outflow tube.

Replacing the Chamber Floor

Either by accident or normal wear and tear, the cover glass will crack or become leaky. Removal must be done with care to avoid damage to the surface area the glass attaches to. Pry the old glass off using a razor blade or Exacto knife being careful to avoid digging or scratching the chamber surface. If the glass was attached with either the silicone RTV or Sylgard™, the adhesive residue should peel off easily by mechanical action such as rubbing. This may be assisted by the application of isopropyl alcohol, but only when absolutely necessary since solvents may damage the polycarbonate. Clean the exposed surface with a very fine abrasive paper and apply the new glass as described above.

Additional coverslips are available by ordering catalog number CS-24/60.

Replacing the Outflow Tube (Glass Type)

The glass outflow tubes are fragile and can be expected to break occasionally. If you find that the glass tubes work better in your application, replacements can be fabricated as follows:

1. Start with a 100mm length borosilicate glass with 1.5mm OD and 1.1-1.2mm ID (our part no.
2. Simply break the tube in two roughly equal pieces by snapping it in the middle as if you were breaking a pencil (i.e., DO NOT use a glass cutting tool). Often this results in the broken ends appearing as shown in Fig. 1 (if not, just try again).

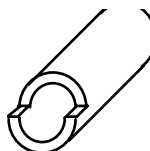


Fig. 1

3. Using a small flame, briefly fire the broken end to "polish" it.
4. Holding the broken tip over the flame to soften the glass, use a 20 gauge needle to gently pull down on the tip to form the shape shown in Fig. 2. A small glass bead at the bottom is desirable to make the tip less hydrophilic, and/or the tip can be dipped in Sylgard™ 184. The Sylgard™ can then be cured using the same high-temperature coil used for Sylgarding patch pipettes.

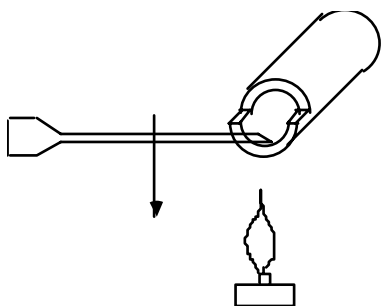


Fig. 2A



Fig. 2B

5. Using the small flame to soften the glass at the appropriate site, put a slight bend in the glass tube so that it resembles those originally supplied (see Fig. 3). Be careful to keep the bend diametrically opposed to the Sylgarded glass bead.

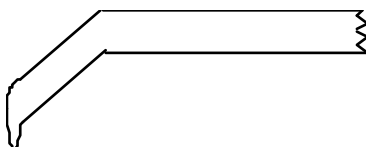


Fig. 3

6. To reduce the chance of subsequent breakage, cut the excess glass tubing such that when positioned on the chamber the tube is as short as possible. Attach a 50mm piece of Tygon TM tubing (Formulation R-3603, O.D. 1/8 in., I.D. 1/16 in.) to the cut end. Place a small (~10 mm) piece of metal tubing in the end of the Tygon TM tubing.
7. Position the end of outflow tube in the middle of the outflow reservoir, adjust to an appropriate angle, and tighten the Allen screw.
8. Attach suction tubing to the free end of the metal rod so that subsequent detachment will not place direct force on the glass tube.

Sample Application

Consider an experiment involving patch-clamping cultured cells grown on small glass coverslips. The goal is to record single-channel activity from cell-attached and excised inside-out membrane patches. The inside-out patches are to be exposed to three experimental solutions in addition to the control Ringer, for a total of four input solutions.

Preliminaries

1. Attach a 3-way stopcock valve to four syringes and adapt PE-160 tubing to the output of each stopcock (e.g., via cut 18 gauge needles).
2. Place the other end of the PE-160 tubing into each hole of the 4-input manifold.
3. With the stopcocks closed, fill each syringe with a solution (the control is in syringe #1).
4. Prime the lines by opening each stopcock until the solution drips out of the manifold. Turn off all lines and insert the manifold into the input hole of the chamber.

5. Place the chamber on the stage of the inverted microscope and turn on syringe #1 to fill the chamber with control solution. Connect the suction line and adjust the bath level as desired.
6. Place a chlorided silver ground wire into the small reservoir through the hole provided and fill this reservoir with the solution to be used in the patch pipette. If an agar bridge is not to be used, insert the wire directly into the outflow bath reservoir.
7. Place an agar bridge (made up of pipette solution) from the small (ground) reservoir to the outflow (bath) reservoir (see sketch).

Experiment

1. Place the coverslip containing the cells in the middle of the tissue compartment filled with the control solution. Gently tap down to eliminate air bubbles and maximize capillary forces between the cell coverslip and the bottom of the chamber. Run some control solution to insure that the cell coverslip is "fixed" in place.
2. Using the stage controls, select the cell to be patched.
3. Guide the patch pipette to this cell and form a cell-attached patch.
4. Record single-channel activity from the cell-attached patch in control Ringer bath.
5. Excise the patch to form an inside-out patch with the former cytoplasmic side exposed to the bath and record activity in control Ringer.
6. Open syringe #2 to exchange the bath. Close syringe #2 and record activity in solution #2.
7. Open syringe #1 to wash with control solution. Close syringe #1 and record activity.
8. Open syringe #3 to exchange to another experimental bath. Close syringe #3 and record.
9. Open syringe #1 to washout with control solution.
10. Continue in this fashion as dictated by your experimental protocol.

Accessories and Supplies

Stage Adapter Bases

Cat. No.	For Chamber Model	To Fit Microscope
CB-1N	RC-8, RC-10, RC-5/18 & RC-5/25	Nikon Diaphot
CB-1O	RC-8, RC-10, RC-5/18 & RC-5/25	Olympus IMT
CB-1O/2	RC-8, RC-10, RC-5/18 & RC-5/25	Olympus IMT-2
CB-2N	RC-11, RC-13 & RC-16	Nikon Diaphot
CB-2O	RC-11, RC-13 & RC-16	Olympus IMT
CB-2O/2	RC-11, RC-13 & RC-16	Olympus IMT-2

MP Series Near Zero Dead Space Manifolds

These manifolds differ from those supplied with the chambers. The inputs converge to a single output with near zero internal dead space. A small length of PE160 tubing can be inserted into the output so that the solution is directed close to the specimen in the bath. MP Series manifolds plug fit all RC Series chambers.

Cat. No.	Description
MP-2	2 to 1
MP-3	3 to 1
MP-4	4 to 1
MP-5	5 to 1
MP-6	6 to 1
MP-7	7 to 1
MP-8	8 to 1
CM6-1	6 to 1, supplied with a set of pins to plug unused inputs

Supplies

Cat. No.	Description
PE160/10	Polyethylene Tubing, 1.57mm OD x 1.14mm ID x 3meters (10') long
CS-24/60	#2 Coverslip, 24 x 60mm, approximately 40 per pkg.