

P2020-S EasyMount Electrode Set

Instructions

Electrode Description:

The electrode set consists of two pairs of electrodes - one pair for passing current, the other for sensing voltage. Each electrode consists of a metal electrode in a threaded nylon housing with a 1 mm gold pin connector at the opposite end on a short length of wire. The voltage-sensing electrode (black housing) is a small pellet formed by compressing AgCl about an Ag wire. AgCl is used for sensing the voltage because it provides a relatively stable electrode potential and is reversible. The current electrode (white housing) is Ag wire that has been plated with chloride ions to lower its resistance. Ag wire is chosen here because it is more rugged than the AgCl pellet, offers lower resistance to current flow than other metals, and can readily be reconditioned. Each type of electrode is inserted into a pipette tip and makes connection to the saline in the chamber via a salt-filled agar bridge. Both are nontoxic to the tissue when isolated from the tissue by the agar bridge. Preparation of the agar and filling the electrode tips are described below.

Electrode Tip Preparation:

The electrodes tips are precut to length so that they just reach the center of the chamber when fully inserted into the electrode ports. If necessary they can be trimmed further using a sharp razor blade or scalpel. Make cuts on a bias to increase the exposed area and deter trapping of air bubbles in the tip.

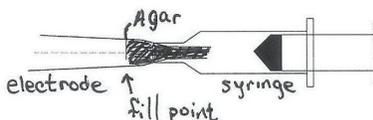
The first step in electrode preparation is to fill the electrode tip with KCl-filled agar solution followed by back-filling the electrode with 3M KCl. The agar functions to keep the concentrated KCl from flowing into the chamber except by simple diffusion. KCl is chosen as the filling solution because K^+ and Cl^- have nearly equal free solution mobility and, therefore, will not cause a significant diffusion potential even though the concentration gradient from the pipette to the chamber is very large. Methods for agar preparation and filling are described below.

3M KCl-Agar Preparation:

Make a 3%-4% solution of agar in 3M KCl (3-4 g agar/100 ml of 3M KCl). Stir the solution gently while heating until it begins to thicken. Be careful not to stir in air bubbles as these will inevitably cause problems later. Several methods of heating may be used including direct heating on a hotplate, microwaving the mixture, and using a double boiler. We prefer the latter as it provides the most uniform heating and least bubble production.

A simple double boiler can be constructed using two carefully selected glass beakers – an inner beaker and an outer beaker. The inner beaker is used to hold the agar solution and must be large enough to get your fingers and pipettor into the opening (e.g., 50 ml). The inner beaker is suspended in the outer beaker by such means as to provide easy access to the inner beaker. The outer beaker is filled with water to a level well below the top of the inner beaker to prevent contamination of the KCl-agar. The inner beaker should be placed on a layer of glass boiling beads/small marbles or suspended from the top of the outer beaker using a wire mesh with an appropriately cut hole or using a crucible triangle.

- Stir the solution gently to prevent forming air bubbles.
- When the solution thickens draw the agar into a 1-3 ml syringe (no needle). Insert the tip of an electrode into the syringe until the taper of the electrode makes a seal with the barrel of the syringe. Inject agar into the electrode until it fills the barrel of the electrode to approximately 5mm past the entry into the barrel of the pipette. *Do not fill the entire electrode with agar.* Place the electrode into a beaker of 3M KCl. Continue filling additional tips while the agar is still hot in the syringe.



- After filling the electrode tips with agar, the remaining volume of the pipette must be backfilled with 3M KCl. To do this use a long, small gauge needle or PE tubing attached to a syringe filled with 3M

KCl. Place the tip of the delivery tube inside the pipette and near the surface of the agar. Slowly withdraw the tube while injecting KCl to completely backfill the pipette. Place the filled pipette in KCl and seal the container until use. If placed in the refrigerator and in KCl, these tips can be stored for many months.

- To assemble the electrode, first apply a thin film of silicone vacuum grease (stopcock grease) to the black rubber seal on the threaded shaft of the electrode. Remove a backfilled tip from the container and insert the threaded end of the electrode into the pipette and turn clockwise until rubber seal is snug against the pipette mouth. Caution: Do not over tighten the screw as this will deform the seal and result in a leak. This seal must be air tight in order for the electrodes to be relatively maintenance free.

Installing the Electrodes in the Chamber

- Insert two voltage-sensing electrodes (black) into the two electrodes ports closest to the tissue.
- Press the electrodes in firmly until they fully seat in the port. The electrode tip makes a leak-free seal by forcing the soft tip through a hole that is slightly smaller in diameter. You will feel resistance when inserting the electrode. As you push through this resistance, you should "feel" the electrode seat itself in the hole.
- Note that the pipette tip has been cut on a bias like a hypodermic needle. The open side should face the tissue.
- The two current passing electrodes (white) insert similarly in the two electrode ports furthest from the tissue.

Storage of the Electrodes

- The electrodes may be removed at the end of the experiment and can be stored for later use by placing them in a container with the tips immersed in KCl. Note: The gold pins should be first cleaned with DI water and wiped dry. Contact of the gold pins with the KCl will cause them to corrode and eventually fail. A used pipette storage container works well as a container.
- Alternatively, the electrodes can be stored in the chambers themselves for short periods (1 -3 days). For storage in the chamber, wash and rinse the chambers well after use with DI water. Place a layer of Parafilm over the surface of a tissue slider or a blank Snapwell into a slider and reassemble the chamber. Fill the chambers with about 1 ml of saline so that the electrode tips are immersed. Lay a microscope slide across the top of the chamber to prevent evaporation. Just prior to the next experiment, rinse the chamber several times with DI water followed by physiological saline to remove contaminants. Fill the chamber halves with physiological saline to keep the electrode tips wet.
(Note: Do *not* use ethanol or other aromatic solvents on the acrylic chambers. These solvents may cause fines cracks (crazing) to form throughout the material. Clean the chambers only with mild soaps and water. Excessive sonication can cause internal heating sufficient to melt the acrylic and distort the machined channels).
- For longer storage times, we recommend the electrodes be disassembled, rinsed with distilled water and stored dry.

Reconditioning Electrode Surfaces

- Current electrodes – Sand the wire using 600 grit wet/dry sandpaper to expose a bright Ag finish. Immerse the wire in fresh Clorox bleach (sodium hypochlorite, ClHO) for 30 sec or more until the surface becomes light gray in color.
- Voltage electrodes – carefully sand the bottom of the AgCl pellet to freshen the surface. Note: the pellet is made by compressing AgCl powder and as such is very easily broken. Use only very light pressure when sanding and do not apply pressure to the edge of the pellet.

Physiologic Instruments, Inc.
12335-6 World Trade Drive
San Diego, CA 92128
Tel: 858 451-8845
Fax: 858 451-6012
Email: PhysInst@aol.com or
info@physiologicinstruments.com