



EK1

Ussing Electrode Kit

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INSTRUCTION MANUAL

102301



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World Precision Instruments

Contents

Introduction 1
Electrode Description..... 1
Set-Up2
Agar Gel Preparation.....3
Gel loading instructions.....3
Operating Instructions.....7
Storage and Maintenance7
Reference7
Warranty9

Warranty

WPI (World Precision Instruments, Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of 30 days from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

- Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within 10 days after receipt of shipment. Claims for lost shipments must be made within 30 days of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim settles. In some instances, photographic documentation may be required. Some items are time sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container.
- WPI cannot be held responsible for items damaged in shipment en route to us. Please enclose merchandise in its original shipping container to avoid damage from handling. We recommend that you insure merchandise when shipping. The customer is responsible for paying shipping expenses including adequate insurance on all items returned.
- Do not return any goods to WPI without obtaining prior approval and instructions (RMA#) from our returns department. Goods returned unauthorized or by collect freight may be refused. The RMA# must be clearly displayed on the outside of the box, or the package will not be accepted. Please contact the RMA department for a request form.
- Goods returned for repair must be reasonably clean and free of hazardous materials.
- A handling fee is charged for goods returned for exchange or credit. This fee may add up to 25% of the sale price depending on the condition of the item. Goods ordered in error are also subject to the handling fee.
- Equipment which was built as a special order cannot be returned.
- Always refer to the RMA# when contacting WPI to obtain a status of your returned item.
- For any other issues regarding a claim or return, please contact the RMA department.

Warning: This equipment is not designed or intended for use on humans.

Introduction

The EK1 Electrode Kit contains two **EKC** (current) electrodes, two **EKV** (voltage) electrodes and four male luer-tipped cartridges. Each electrode is threaded and screwed securely into the larger end of an empty cartridge. When ready to use, the cartridge is filled with an electrolyte gel mixture and the luer tip of each cartridge is then plugged securely into a luer fitting in the Ussing Chamber, thus effecting a direct-connect electrode. The cable from each electrode terminates in a 2 mm pin which may be plugged into voltage/current clamps, such as WPI's **DVC-1000** or **EVC-4000**.

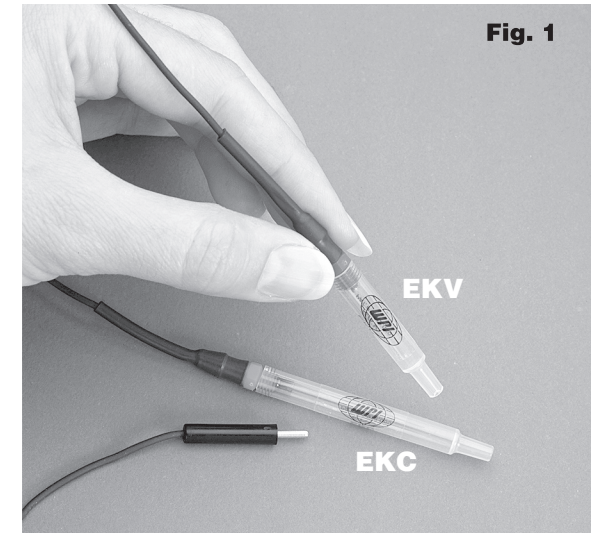


Fig. 1

Electrode Description

The EKC electrode has a red colored body, a large Ag/AgCl pellet and a longer cartridge than the EKV electrode, which has a blue colored body. Each electrode is supplied dry inside a light-protective plastic storage bag.

Before using the electrode, the cartridge needs to be filled with a gel containing a mixture of electrolyte and agar to form an agar salt bridge. This bridge serves a dual purpose: it slows the migration of the Ag ion from the electrode into the Ussing chamber when current is flowing and it minimizes the risk of cross-contamination between experiments. Should the Ussing chamber contain toxic or radioactive chemicals that could diffuse into the electrodes, the user can simply discard the gel, thereby eliminating any potential contamination of the electrode.

Set-Up

Parts List

The package should contain:

Part # Description

EKC	Ussing Current Electrode (red) plus cartridge inside a light-protective bag (2)
EKV	Ussing Voltage Electrode (blue) plus cartridge inside a light-protective bag (2)
EK1-IM	This Instruction Manual

Unpacking: Upon receipt of this product, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. Please read the section entitled “Claims and Returns” on the Warranty page of this manual.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA number required) and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in shock absorbing material. Please read the section entitled “Claims and Returns” on the Warranty page of this manual.

Materials Required for Set-Up

- Electrolyte-gel, *e.g.*, agar and KCl
- KCl saline solution
- 1 mL syringe and 16 gauge 1½-in. needle *or* plunger from a Becton Dickinson (B-D) disposable 1 mL syringe
- Disposable gloves
- Glass beaker
- Glass tray or baking dish
- Hot plate

Operating Instructions

Place the assembled electrodes into the proper ports on the Ussing chamber as described in the Ussing System Manual. Connect the 2-mm pin of each electrode to the appropriate jack.

During usage, the two red EKC current-passing electrodes should be swapped at least daily to avoid excessive buildup of AgCl at the anode and depletion of AgCl at the cathode.

Storage and Maintenance

Filled Cartridge/electrode: To prevent the gel from drying out in the loaded cartridge, soak the open end (bottom) of the electrode cartridge in electrolyte solution similar to the one used to prepare the gel. Alternatively, proceed immediately to load the filled electrode cartridges onto the Ussing Chamber.

Short-term storage of electrodes: The electrodes tips should be immersed in an electrolyte solution, ideally the same solution as used to make the agar gel. In addition, it is recommended that the 2-mm pins of each pair of electrodes (EKV to EKV and EKC to EKC) be connected to each other during wet storage in order to equilibrate the potential offset. This is can be accomplished for example, by wrapping aluminum foil around the pair of pins.

Long-term storage of electrodes: Remove the cartridge. Discard the agar gel. Rinse the electrode with distilled water, wipe or air dry and store dry in the light protective bags.

If the voltage readings start to drift, the EKV voltage sensing electrodes may be very gently sanded using a very fine sand paper (300 or higher grit).

Reference

H.H. Ussing and K. Zehran, *Acta Physiol. Scand.* **23** 110 (1951) 1/92

Fig. 5



the plunger from a 1ml B-D syringe. Remove the cartridge and the electrode from the heated water and dry off the outside of both. Insert the plunger into the cartridge and push it to the bottom. Place the open end of the cartridge into the gel container. Draw the gel into the cartridge until the black tip of the plunger is halfway out of the cartridge (Fig 5). Remove the cartridge from the gel and place a gloved finger over the open end. Holding the lower open end of the cartridge firmly so that the gel won't leak out, remove the plunger and place the electrode into the cartridge top. Holding the cartridge vertically, check to see that there are no air bubbles in the gel. If air bubbles are present, the cartridge must be emptied and refilled until no air bubbles are observed. Now, screw the electrode into the cartridge until it is snug or won't go any further.

It is important that the gel not be allowed to dry out in the loaded cartridge. If the filled electrode is not to be used immediately, soak the open end (bottom) of the electrode cartridge in electrolyte solution similar to the one used to prepare the gel. This will prevent the gel from drying out.

Agar Gel Preparation

The agar gel can be made by dissolving 2-4 % agar (by weight) in a KCl solution at 80° C. The concentration of KCl depends on the experimental requirements. 3 M KCl will give the lowest resistance for passing large currents. However, highly concentrated KCl will slowly diffuse into the Ussing chamber during use: the total diffusion rate for all four electrodes is about 0.00004 mole per hour at room temperature. If the experiment is to be performed over a relatively long time, the concentration of KCl in the circulating chamber bath may increase significantly thereby inducing error in the resistance and current/voltage measurements. For this reason, we recommend using the same saline solution (~150 mM of KCl and NaCl) as used in the chamber bath to make the agar gel. Although this will increase the resistance of the current path from 5 K Ω to 11 K Ω , it will not adversely affect the measurement, unless very low noise or a very large current is required. When salts other than KCl are used, some chloride ions must be present to ensure stability of the Ag/AgCl electrodes.

Gel loading instructions

When the gel preparation has been completed, the electrode is unscrewed from the cartridge and the cartridge is filled with the agar electrolyte gel mixture. The electrode is then screwed back into the cartridge and the luer tip of the cartridge is plugged securely into a luer fitting in the Ussing Chamber.

There are a number of ways to fill the electrode cartridges with the agar gel. Two of the most commonly used methods are described here. The first method uses a 1 mL syringe with a 16 gauge, 1½-in. needle. The second method utilizes the plunger from a 1 mL Becton Dickinson (B-D) disposable syringe that fits the barrel of the WPI cartridge. Disposable gloves should be worn with both procedures

1. Remove the Cartridge from Electrode Body

The cartridge comes assembled to the electrode body. Remove the cartridge from the electrode by unscrewing the cartridge and pulling the electrode *straight out*.

NOTE: The pellet of the EKC electrode is fragile. It can be easily damaged during removal of the electrode from the cartridge. Care must be taken not to pull the cartridge off the electrode at an angle, as this will put pressure on the electrode, causing it to snap.

2. Melting the agar – Indirect heating

To prevent the agar mixture from burning during the melting process, it is recommended that the agar electrolyte mixture is put into a small glass beaker and the

beaker then placed in a larger glass dish filled with water. The large dish can now be placed on the hot plate. This method of indirect heating is sufficient to melt the agar without causing it to burn. See Fig. 2.

3. Maintaining the temperature of the heated agar

Agar will melt at about 80°C and quickly becomes thick below this temperature. In order to facilitate loading the cartridges with this mixture, it is recommended that all the materials needed to fill the cartridges be kept in the warmed water of the large glass container (see Fig. 2). This includes the cartridges to be filled, the syringe, needle and/or plunger, along with the tips of the electrodes.

Fig. 2



Method I: Syringe Loading

With gloves on, remove the cartridge and the electrode from the heated water and dry off the outside of both. Mount the 16G needle on the 1 mL syringe. Place the syringe needle into the gel and draw gel in and out of the syringe several times to warm the syringe. Fill the syringe with gel. Holding the syringe with the needle down, slide the smaller opening of the cartridge onto the needle until it touches the colored luer fitting. Loosely plug the electrode into the wider end of cartridge. Do not push the

Fig. 3



electrode all the way inside. An air gap is necessary to permit the gel to fill the cartridge up to the electrode without creating an air bubble. (See Fig. 3).

Fill the cartridge until the end of the needle is immersed in gel. Allow a little of the gel to leak out from around the electrode to ensure that no air is trapped in the cartridge. Now, screw the electrode into the cartridge until it is snug or will go no farther (Fig. 4). To remove the needle, make sure that the syringe is firmly on the cartridge and slowly inject more gel as you move the needle out of the cartridge. Holding the cartridge vertically, check to see that there are no air bubbles in the gel. If air bubbles are present, the cartridge must

be emptied and refilled until no air bubbles are observed.

It is important that the gel not be allowed to dry out in the loaded cartridge. If the filled electrode is not to be used immediately, soak the open end (bottom) of the electrode cartridge in electrolyte solution similar to the one used to prepare the gel. This will prevent the gel from drying out.

4. Method II: Plunger Loading

With gloves on, remove

Fig. 4

