



Ussing System

For investigation of epithelial transport

INSTRUCTION MANUAL

Serial No. _____

112717

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ABOUT THIS MANUAL

The following symbols are used in this guide:



This symbol indicates a CAUTION. Cautions warn against actions that can cause damage to equipment. Please read these carefully.



This symbol indicates a WARNING. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and TIPS contain helpful information.

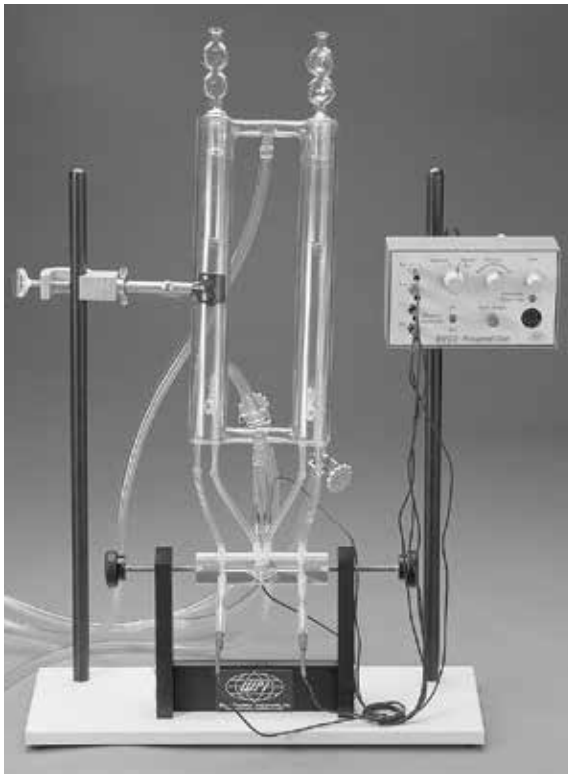


Fig. 1—The Ussing Chamber is setup.

INTRODUCTION

WPI's Ussing System offers researchers a quick, effective means of making low-resistance electrical connections to the Ussing chamber without need of long agar bridges or Calomel half-cells. Ag/AgCl half-cells screw into short tubes which plug firmly into place in the chamber's luer ports. These direct-connect electrodes

eliminate the inconvenience and expense of Calomel half-cells in open liquids. The System includes one Ussing Chamber (eight sizes available), Support Stand, Electrode Kit, Glass Circulation Reservoir (two sizes available), and a Tubing start-up kit (25 feet of 0.375-in. tubing, 10 feet of 0.156-in. tubing, plus four male luer fittings, two compressor clamps, one Y-connector, and one clip). Components are also available separately.

WPI's classical Ussing Chambers are well established perfusion chambers that are easy to operate, easy to control temperature, and easy to clean after use. Hundreds of them are used daily by scientists in the field. Ussing Chambers are machined from solid acrylic with eight entry ports for fluid lines, electrodes, or agar bridges. For easy, leak-free attachment of tubing and electrodes, all eight ports are luer type. The four ports for voltage and current electrodes are recessed to prevent formation of air bubbles in the chamber. The fluid compartments in each side of the chamber are separated by the epithelial membrane being studied. Sharp stainless steel pins on one side of the chamber hold the membrane in position and mate with holes in the opposite chamber interface. (In the CHM4, tissue is held by an O-ring instead of pins.) The CHM5 chamber adapts the Costar Snapwell, a cell culture insert for monolayer cell culture, into WPI's "classical" epithelial voltage clamp system. Until now, classical Ussing Chambers have not been widely used for monolayer cell culture inserts because most inserts have a very deep profile, limiting good fluid perfusion at the surface of the membrane - and limiting voltage electrodes from measuring the potential close to the surface of the membrane. CHM5 solves these problems: Perfusion fluid is introduced into the chamber at an angle so that it will flow directly to the surface of the membrane. The voltage electrode is also inserted into the chamber at an angle so as to reduce the distance between the surface of the membrane and the electrode. Drains may be added to Ussing chambers to allow quick and complete evacuation of radioactive or toxic substances.

Cartridge Electrodes—The Electrode Kit contains four voltage/current electrodes, plus four luer-tipped cartridges. Electrodes are threaded and screw securely into the end of each cartridge. The luer tip then plugs securely into the luer openings of the chamber. The cable from each electrode terminates with a 2mm pin which may be plugged into voltage/current clamps such as WPI's SYS-DVC1000 or SYS-EVC4000. The miniature electrode-gel cartridge is a small plastic tube with a male luer tip identical to those at the tip of hypodermic syringes. The tube may be filled with different gel materials; agar is commonly used but other gel materials may also be satisfactory.

Circulation Reservoirs—Hand-blown borosilicate glass, with jacketed chambers for temperature control. Available in two sizes - #5210 holds 20 mL per side, and #5362 holds 10mL per side (useful when expensive chemicals are involved). Reservoir condenser caps prevent air bubbles and turbulence in fluid reservoirs.

Water Bath—The 503843 Julabo Constant Temperature Water Bath has been tested at WPI and found to be ideal for WPI's Ussing system. It can provide simultaneous warming for up to 16 systems. Menu-driven control module allows easy temperature setting, correction and display, with temperature accuracy of $\pm 0.02^{\circ}\text{C}$.

Unpacking

Upon receipt of this instrument, 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 damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 9 of this manual. Please contact WPI Customer Service if any parts are missing at 941.371.1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100mm (four inches) of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 9 of this manual.

OPERATING INSTRUCTIONS

Mounting a Tissue

1. Separate the two halves of the Ussing chamber. Steel pins on one side of the chamber mate with corresponding holes on the opposite side. In the CHM1, CHM2, CHM3, CHM6, and CHM7 the pins are used to mount the tissue sample as well as to align the two halves of the chamber. In the CHM4, the tissue is held in place by an O-ring and the pins are used solely for chamber alignment. The CHM5 uses O-rings to hold a tissue insert.
2. Mount the tissue by stretching and pressing it onto the pins (CHM 1, 2, 3, 6, 7) or on the O-ring (CHM4). For best results, apply a small amount of vacuum grease (silicone) to ensure a good seal. Align the red dots and press chamber halves together.
3. With the red dots facing toward you and four of the luer ports facing upward, place the chamber into the stand. With the screw tips in the indentations in each end of the chamber, tighten the knobs to hold the chamber securely. For high resistance epithelia such as urinary bladder and frog skin, care must be taken when tightening the knobs. Too little pressure can leave the preparation unsealed (allowing edge leakage), and too much pressure may injure the tissue under the edge of the chamber (causing edge damage which can also produce a leak pathway between the fluid compartments). A small amount of sealant such as silicone grease on the inside rim of the Ussing chamber helps produce a better seal without excessive pressure. Alternatively, cut four small rings of Parafilm® to act as a soft gasket, placing two rings on each side of the chamber before joining them.

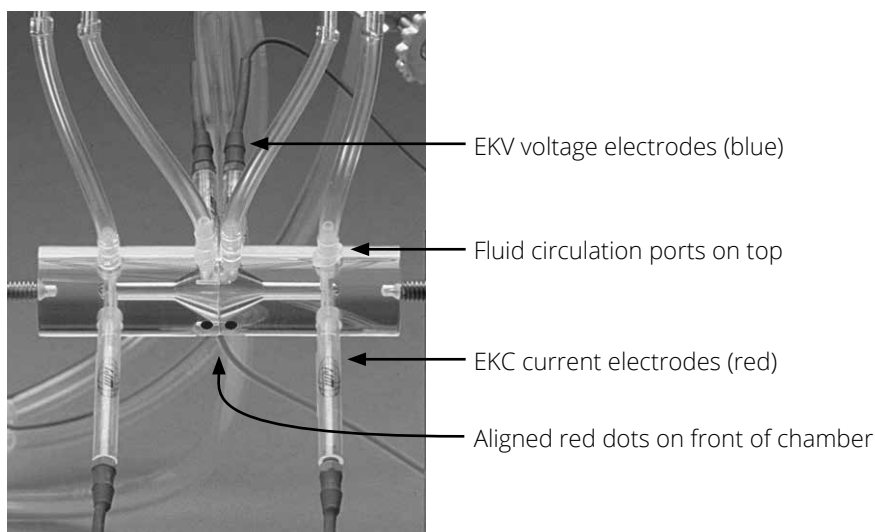


Fig. 2—Port configuration of all chambers.

4. The Ussing chamber has eight luer ports. The four ports on top of the chamber are connected to the solution reservoir, as shown in Fig. 2, using the 9/32-inch OD Tygon tubing supplied. Use as short a piece of tubing as possible to avoid problems with air bubbles.



CAUTION: Be careful when attaching the tubing to avoid breaking the glass connectors on the circulation reservoir.

Opposed 90 degrees to the four connectors on the top of the chamber and located near the red alignment dots are two ports for the blue **EKV** voltage electrodes; on the opposite side and near each end of the chamber are two ports for the red **EKC** current electrodes.

Volume of the Chamber

	CHM1 (M)	CHM2 (S)	CHM3 (L)	CHM4 (XS)	CHM5 (Snap)	CHM6 (Rect., S)	CHM7 (Rect., L)	CHM8 (XS)
Reservoir Opening	12 mm	9 mm	13.5 mm	4 mm	12 mm	5x14.5 mm	7x30 mm	4 mm
Half-Chamber Volume	1.0 mL	0.75 mL	1.2 mL	0.5 mL	1.7 mL	0.8 mL	5.5 mL	0.5 mL
Pin Circle Diameter	17 mm	12 mm	18.5 mm	6 mm*	N/A	7x16.5 mm	9x32 mm	5.5 mm
Exposed Tissue Diam.	12 mm	9 mm	13.5 mm	4 mm	12 mm	5x14.5 mm	7x30 mm	4 mm
Surface Area	113 mm ²	63.5 mm ²	143 mm ²	12.56 mm ²	113 mm ²	67.12 mm ²	199.46 mm ²	12.56 mm ²

Assembled chambers are 101.6 mm (4 in.) long. *O-ring diam.

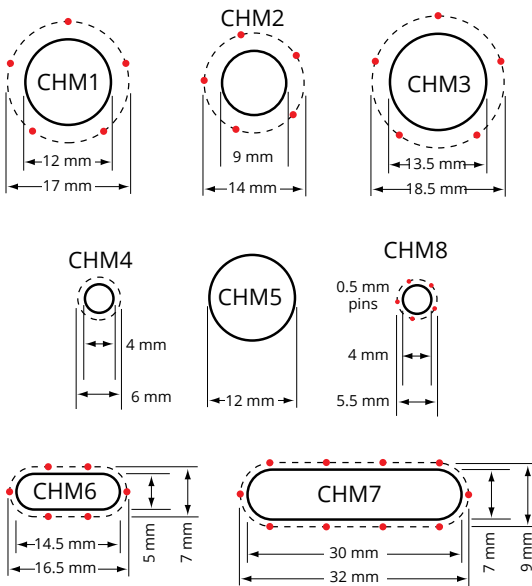


Fig. 3—Diameters and dimensions of the chambers

Circulation Reservoir

1. Use the T connections between the solution columns to connect a thermally regulated water flow. Connect the in flow to the bottom T and the return flow to the top T using the supplied 3/8-inch OD Tygon tubing.
2. Use the large connectors on each inside solution column to connect an air lift system. Normally, a 95% O₂ and 5% CO₂ air mixture is used as the lifting air, which also supplies the necessary gases to keep the tissue oxygenated.
3. Connect two pieces of 3/8-inch OD Tygon tubing to the plastic Y fitting provided. Place the two clamps provided are between the solution column and the plastic Y and fully tighten the. The Y is connected to an air source of approximately 5 to 10 psi. The clamps are then slowly opened so a gentle air flow is the same to each solution column.
4. The chamber may also be used for perfusion without electrical recording. In that case, the electrode ports can be blocked with luer plugs.

Electrodes

1. Fill the electrode cartridge tubes with an agar gel. The gel may be made by dissolving 2-4 % (by weight) agar in KCl solution at 80°C. The concentration of KCl depends on the experimental requirements. 3M KCl gives the lowest resistance

when passing large currents. However, the highly concentrated KCl slowly diffuses into the chamber (the total diffusion rate for all four electrodes is about 0.00004 moles per hour at room temperature). If the experimental time is relatively long the concentration of KCl in the saline solution may increase significantly, inducing error in the resistance and I-V measurements. For this reason, we recommend using the same saline solution (~150mM of KCl and NaCl) as used in the chamber bath to make the agar gel. Although this increases the resistance of the current path from 5K Ω to 11K Ω , it will not adversely affect the measurement, unless very low noise is needed or very large currents are required. When salts other than KCl are used, some chloride ions must be present to ensure stability of the Ag/AgCl electrodes.

2. Press the red EKC current-passing electrodes into the long cartridge and the blue EKV voltage-sensing electrodes into the short cartridge before the agar gel hardens. Place these assembled electrodes into the proper ports on the Ussing chamber as shown in Figure 4. Connect the 2-mm pin of each electrode to the proper jack on the **EVC3** preamplifier box if **EVC4000 Voltage/Current Clamp** is used, or to the electrode adaptor (WPI# 3993) if an **EVOM2** is used.

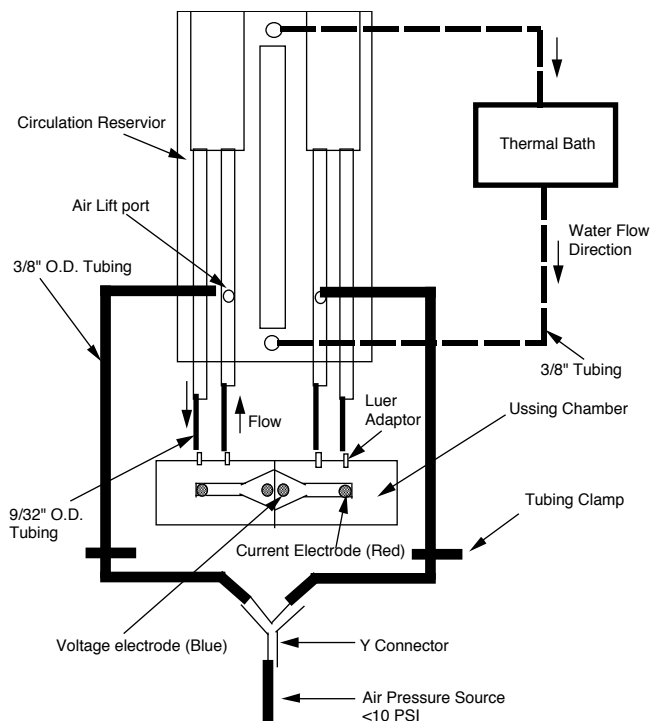


Fig. 4—The circulation flow diagram.

Optional Drains

The Ussing chamber with optional drains has drain ports at the bottom, one on each side of the chamber. It also comes with a kit containing one piece of tubing (for you to cut), two male luer stoppers (to plug the drains when they are not needed), two slide clips (for pinching the tubing shut when the drains are being used) and two male luer fittings (for attaching tubing to drain holes).

Use a twisting motion to remove or insert the male luer fittings (both the plugs and tubing connectors) in the drain openings .

Reference: H. H. Ussing and K. Zehran, *Acta Physiol. Scand.* 23 110 (1951)

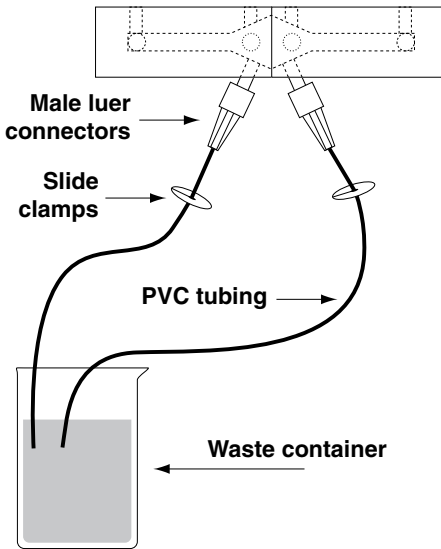


Fig. 5—Setup of the Ussing Chamber with optional drains.

MAINTENANCE

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. If the voltage readings start to drift, the blue EKV voltage sensing electrodes may be very gently sanded using a very fine emery cloth (800 or higher grit). If the two voltage-sensing electrodes exhibit a large potential difference (>5 mV), they should be shorted together at their 2-mm pins and left in salt solution (*i.e.*, 1 M KCl) overnight.

Do not wash the Ussing chamber in organic solvents such as acetone or ethanol. Wash only with mild detergent, such as Enzol (WPI# **7363**), and water. Store the chamber dry. Any rust on the pins may be removed by gentle abrasion.

The Ussing chamber is made of acrylic and cannot be autoclaved nor used with alcohol. The chamber can be sterilized chemically with Cidex Plus (WPI# **7364**). When working with live preparations, be sure to remove all residue of Cidex Plus as recommended in that product's instructions. Failure to remove all residue may adversely affect live tissue.

ORDERING INFORMATION

USSING SYSTEMS

USS1L	Medium Chamber, Stand, Large Reservoir, Electrodes, Tubing
USS2L	Small Chamber, Stand, Large Reservoir, Electrodes, Tubing
USS3L	Large Chamber, Stand, Large Reservoir, Electrodes, Tubing
USS4L	Extra Small Chamber, Stand, Large Reservoir, Electrodes, Tubing
USS5L	Snap Chamber, Stand, Large Reservoir, Electrodes, Tubing
USS6L	Small Rectangular Chamber, Stand, Large Reservoir, Electrodes, Tubing
USS7L	Large Rectangular Chamber, Stand, Large Reservoir, Electrodes, Tubing
USS8L	Extra Small Chamber, Stand, Large Reservoir, Electrodes, Tubing

*For USSING Systems with a small reservoir, substitute an S in the part number for the L (For example, USS1S instead of USS1L)

*Add EVC-4000 at reduced price when buying Ussing system with equivalent number of channels

EVC-4000-1 1-Channel Voltage Clamp & Preamps

EVC-4000-2 2-Channel Voltage Clamp & Preamps

EVC-4000-3 3-Channel Voltage Clamp & Preamps

EVC-4000-4 4-Channel Voltage Clamp & Preamps

System components also available separately:

xxxxD	Drain option (add "D" to part number of chamber or system)
CHM1	Medium Chamber
CHM2	Small Chamber
CHM3	Large Chamber
CHM4	Extra Small Chamber with O-Ring Seal
CHM5	Snap Chamber (fits Costar Snapwell cups)
CHM6	Small Rectangular Chamber
CHM7	Large Rectangular Chamber
CHM8	Extra Small Chamber with Mounting Pins
EK1	Ussing Electrode Kit (2 voltage, 2 current)
EKC	Extra Ussing Current Electrode (red) (each)
EKV	Extra Ussing Voltage Electrode (blue) (each)
5210	Large Glass Circulation Reservoir, (20 mL per side)
5233	Replacement Stopper for 5210 (large reservoir)
5362	Small Glass Circulation Reservoir, (10 mL per side)
5361	Replacement Stopper for 5362 (small reservoir)
3955	EKV Cartridges, 35 mm (pkg of 12)
3960	EKC Cartridges, 58 mm (pkg of 12)
3669	Tubing Kit (flexible hose and luer fittings)
3579-20	Replacement luer fittings for tubing connections (pkg of 20)
5153	Support Stand

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 one year* 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.

** Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.*



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