



# Ussing System

*For investigation of epithelial transport*



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## **INSTRUCTION MANUAL**

Serial No. \_\_\_\_\_

040302

**World Precision Instruments**



## 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 ten (10) days after receipt of shipment. Claims for lost shipments must be made within thirty (30) days of receipt of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim is settled. 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.

Do not return any goods to us without obtaining prior approval and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. Goods accepted for restocking will be exchanged or credited to your WPI account. Goods returned which were ordered by customers in error are subject to a 25% restocking charge. Equipment which was built as a special order cannot be returned.

## Repairs

Contact our Customer Service Department for assistance in the repair of apparatus. Do not return goods until instructions have been received. Returned items must be securely packed to prevent further damage in transit. The Customer is responsible for paying shipping expenses, including adequate insurance on all items returned for repairs. Identification of the item(s) by model number, name, as well as complete description of the difficulties experienced should be written on the repair purchase order and on a tag attached to the item.

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

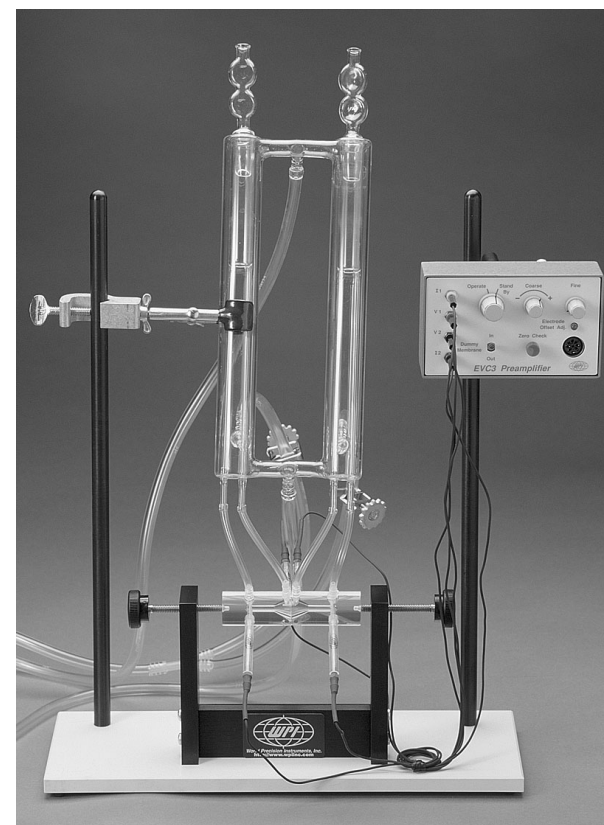
## Ussing Chamber

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**, and **CHM5** 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.

Mount the tissue by stretching and pressing it onto the pins (CHM 1, 2, 3, 5) or on the O-ring (CHM4). For best results, apply a small amount of vacuum grease (silicone) to the O-ring to ensure a good seal. Align the red dots and press chamber halves together.

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 leak) 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



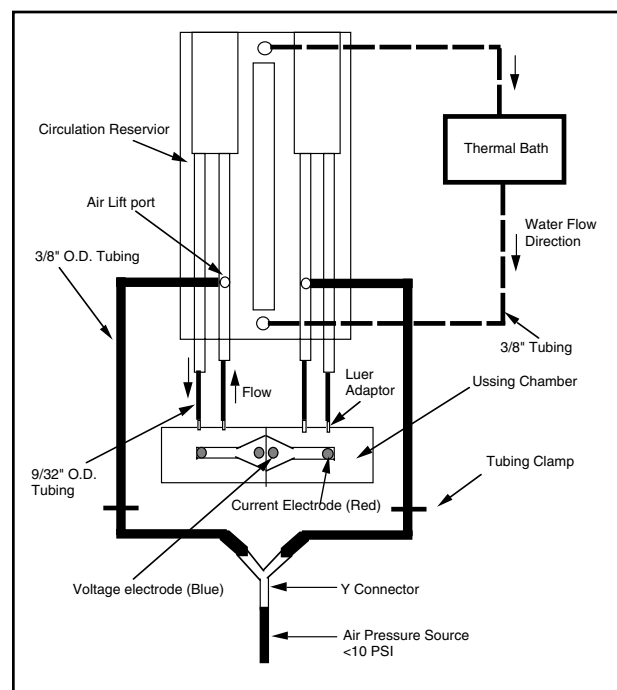
**Fig. 1**

amount of sealant such as silicone grease on the inside rim of the Ussing chamber will help to 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.

The Ussing chamber has eight luer ports. The four ports on top of the chamber are connected to the solution reservoir, as shown in Figure 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. 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.

## Circulation Reservoir

The T connections between the solution columns are used to connect a thermally regulated water flow. In flow is connected to the bottom T and return flow is connected to the top T using the supplied 3/8-inch OD Tygon tubing. The large connectors on



each inside solution column are connected to an air lift system. Normally, a 95% O<sub>2</sub> and 5% CO<sub>2</sub> air mixture is used as the lifting air, which also supplies the necessary gases to keep the tissue oxygenated. Two pieces of 3/8-inch OD Tygon tubing are connected to the plastic Y fitting provided and the two clamps provided are placed between the solution column and the

**Fig. 2**

- 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)
- DRIREF-L** Reference Electrode w/ Luer Tip
- 5210** Large Glass Circulation Reservoir, (20 mL per side)
- 5233** Replacement Stopper for 5210
- 5362** Small Glass Circulation Reservoir, (10 mL per side)
- 5361** Replacement Stopper for 5362
- 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

## Ordering Information

### USSING SYSTEMS, LARGE RESERVOIR

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

### USSING SYSTEMS, SMALL RESERVOIR

- USS1S** Medium Chamber, Stand, Reservoir, Electrodes, Tubing
- USS2S** Small Chamber, Stand, Reservoir, Electrodes, Tubing
- USS3S** Large Chamber, Stand, Reservoir, Electrodes, Tubing
- USS4S** Extra Small Chamber, Stand, Reservoir, Electrodes, Tubing
- USS5S** Snap Chamber, Stand, Reservoir, Electrodes, Tubing
- USS6S** Small Rectangular Chamber, Stand, Reservoir, Electrodes, Tubing
- USS7S** Large Rectangular Chamber, Stand, Reservoir, Electrodes, Tubing
- USS8S** Extra Small Chamber, Stand, Reservoir, Electrodes, Tubing

**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
- MP100WS** MP100 Data Acquisition Starter System (Macintosh)
- MP100WSW** MP100 Data Acquisition Starter System (Windows)

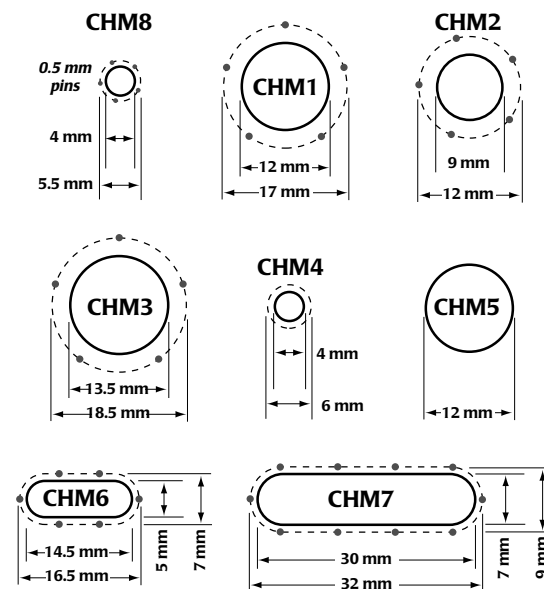
### System components also available separately:

- xxxxD** Drain option (add "D" to part number of chamber or system)
- CHM1** Medium Chamber
- CHM2** Small Chamber
- CHM3** Large Chamber

plastic Y and fully tightened. 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.

The chamber may also be used for perfusion without electrical recording. In that case, the electrode ports can be blocked with luer plugs.

## Volume of the Chamber



**Fig. 3**

	<b>CHM1</b> (medium)	<b>CHM2</b> (small)	<b>CHM3</b> (large)	<b>CHM4</b> (extra small)	<b>CHM5</b> (snap)	<b>CHM6</b> (rect., small)	<b>CHM7</b> (rect., large)	<b>CHM8</b> (extra small)
<b>Reservoir Opening</b>	12 mm	9 mm	13.5 mm	4 mm	12 mm	5 x 14.5 mm	7 x 30 mm	4 mm
<b>Half-Chamber Volume</b>	1.0 mL	0.75 mL	1.2 mL	0.5 mL	1.7 mL	0.8 mL	5.5 mL	0.5 mL
<b>Pin Circle Diameter</b>	17 mm	12 mm	18.5 mm	6 mm*	N/A	7 x 16.5 mm	9 x 32 mm	5.5 mm

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

## Electrodes

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. 3 M KCl will give the lowest resistance when passing large currents. However, the highly concentrated KCl will slowly diffuse into the chamber (the total diffusion rate for all four electrodes is about 0.00004 mole 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 (~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 $\Omega$  to 11 K $\Omega$ , 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.

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 2. Connect the 2-mm pin of each electrode to the proper jack on the **DVC3** preamplifier box if **DVC1000 Voltage/Current Clamp** is used, or to the electrode adaptor (WPI Part No. **3993**) if **EVOM** is used.

## Cleaning and Maintenance

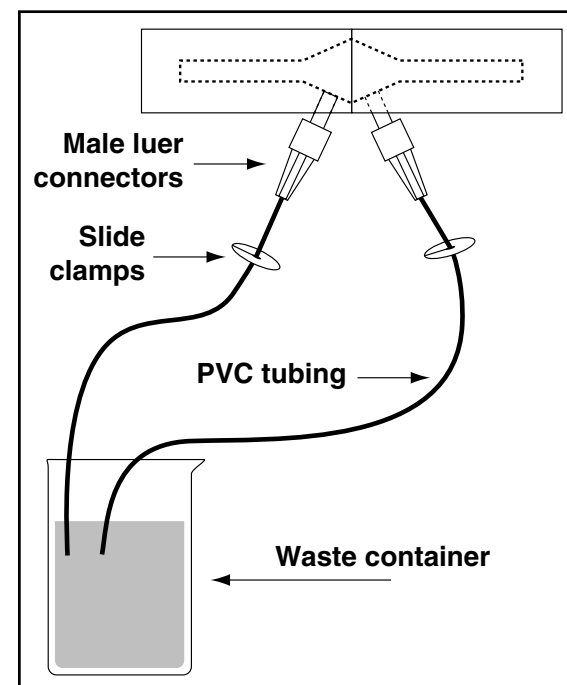
The two red EKC current-passing electrodes should be swapped at least daily to avoid excessive buildup of AgCl at the anode and depletion of AgCl at the cathode. If the voltage readings start to drift, the blue EKV voltage sensing electrodes may be very gently sanded using a very fine emery cloth (800 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 and water. Store the chamber dry. Any rust on the pins may be removed by gentle abrasion.

The chamber made of polysulfone can be sterilized in an autoclave, while the acrylic chamber may only be sterilized chemically (note that any residue left from chemical sterilization may adversely affect live tissue).

## 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 (to be cut by the user), 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. 4**