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EndOhm

Tissue resistance measurement chambers for tissue culture cups

INSTRUCTION MANUAL

Serial No._____

091407

World Precision Instruments

Contents

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.*

EndOhm Chambers

ENDOHM-6 Chamber for 6 mm culture cup, 15.8 mm ID (24 wells per plate)

ENDOHM-12 Chamber for 12 mm culture cup, 23.2 mm ID (12 wells per plate)

ENDOHM-24SNAP Chamber for 24 mm and COSTAR Snapwell™ culture cup, 37.3 mm ID (6 wells per plate)

The **EndOhm** series of chambers, when used with WPI's **EVOM** or **EVOMX** resistance meter or the Millicell ERS, are designed to provide reproducible and accurate resistance measurements of endothelial tissue in culture cups. Resistance values obtained with the EndOhm are consistent with those obtained using a well-designed Ussing Chamber.

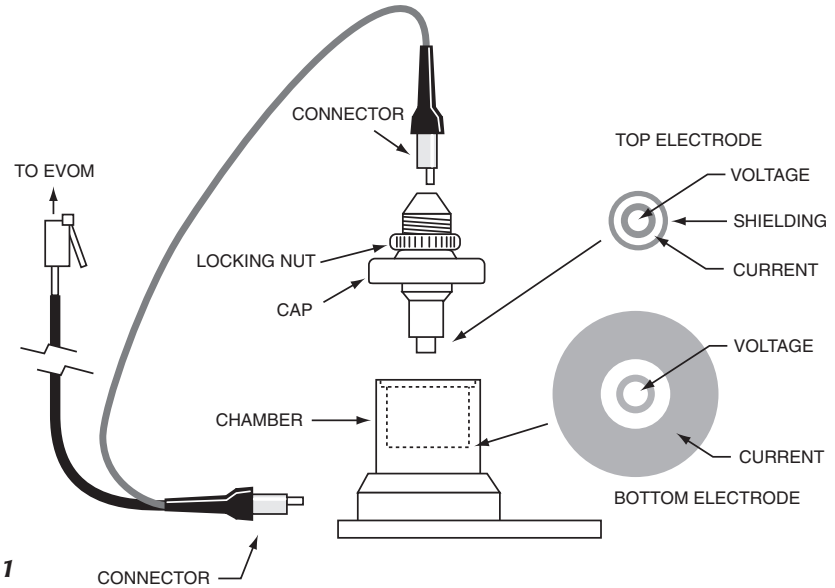

Fig. 1

The EndOhm chamber and cap each contain a pair of concentric electrodes: current flows between these symmetrically opposing circular disc electrodes. This design provides several advantages:

Current density across the membrane is more uniform with EndOhm's circular disc current electrodes than with the **STX2** electrodes.

Most importantly, with EndOhm's fixed electrode geometry, variation between successive resistance measurements of the same sample is reduced from 10-30 Ω (variation dependent on the experience of the user) to 1-2 Ω.

The structure of the EndOhm is pictured in Figure 1. Each pair of concentric electrodes incorporates a voltage-sensing Ag/AgCl pellet in the center and an annular current electrode. The current electrode is made of silver and coated with gray-colored silver chloride. The top cap assembly is composed of three parts — cap, locking nut, and electrode. The cap has two important functions: it helps to center the electrode in the culture cup and, together with the locking nut, fixes the height of the electrode in the chamber. The cable connecting the chamber to the EVOM can be easily disconnected from both the top cap assembly and the bottom chamber.

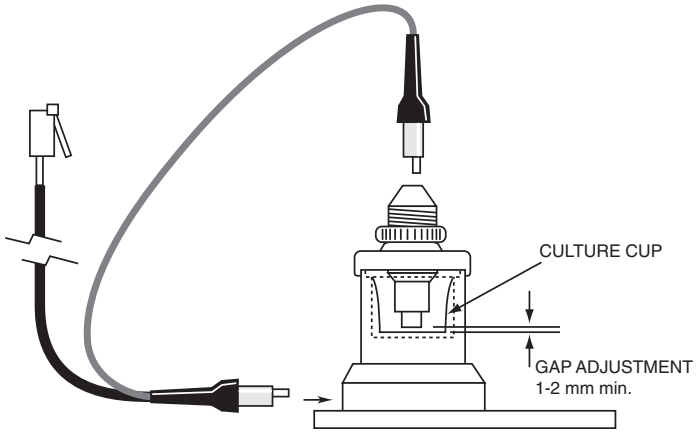

Fig. 2

Adjusting the height of the top electrode

The height of the top electrode of all the EndOhms can be adjusted to fit cell culture cups from different manufacturers.

NOTE: the height of the top electrode of the EndOhm-6 is factory-set for Costar Transwell culture cups; no height adjustment should be made if using these cups.

Before use, place a blank cup into the chamber. Loosen the locking nut on the cap assembly. Turn the cap on the top electrode so that when the cap is in place on top of the chamber, the clearance between the top electrode and the culture membrane is 1 to 2 mm. See Figure 2.

Electrode Preparation

For **resistance measurement**, the EndOhm can be used directly without prestabilizing of the electrode.

Troubleshooting

Unstable or erratic readings

Unplug the ENDOHM from the EVOM meter, then press the test button on the EVOM and watch it for 10 seconds and that it does not drift off of 1000 ohms +/- 2 ohms. This should be stable to 1 ohm and not change after the first two to three seconds. If it does then it may need new batteries, if it does not drift then the electrodes most likely need a cleaning.

Troubleshooting a low or high EVOM reading

1. Moving the upper electrode from one height position to another.
2. The placement of the culture cup is important, some cups have standoffs or "feet" that need to be clear. If you have upper mount cups (usually with a plastic support cone) you need to be certain that there is about 2 mm of clearance over the bottom electrode set.
3. The upper and lower electrodes cannot be in the same contiguous solution, there must be an electrical separation of the upper and lower electrodes by the sides of the cup and the membrane. Sometimes small semi dry salt bridges get across the plastic dish and cause erroneous readings.
4. Changes in the molarity of the buffer or test solutions (a small change of 1% in the 100 mM molarity can cause large reading errors). Continuous low resistance readings (even when dry) may be caused by a bad cable. Replacement cable **63330-01.**
5. The electrodes do absorb bio-matter and get "dirty" and the resistance reading goes up for those cases.
6. Pinholes (areas of no growth) in the monolayer may pass the EVOM current giving a low reading.

Accessories

Part No.	Description
EVOM	Epithelial Tissue Voltohmmeter (includes **STX2** electrode set)
500168	4-way Switchbox (allows **EVOM** to operate four **Endohms**)
CALICELL-12	12 mm Calibration Cell for Endohm-6 or Endohm-12
CALICELL-24	24 mm Calibration Cell for Endohm-24
7364	Cidex Plus

Ammonia cleaning:

Figure 1 shows a cleaned surface with no spots or irregularities. In Figure 4 note the small center tip and the small ring on the top section. These may require the use of a cotton swab to clean.

Re-choriding:

The images in Figure 5 and Figure 6 show two Endohm-12's that have been chlorided; the unit in Figure 5 has not been chlorided enough but the one in Figure 6 has been properly chlorided (note the brighter color).

Fig. 3

Fig. 4

Fig. 5

Fig. 6

For **voltage measurements**, silver/silver chloride voltage electrodes may exhibit a small voltage drift when stored dry and are then newly immersed in electrolyte solution. This drift may affect potential measurement accuracy, but not resistance measurements. In order to stabilize the electrodes, fill the chamber with saline solution, replace the cap, and connect the cable to the EVOM with the power off. In the "off" position, the EVOM connects the voltage electrodes, allowing them to equilibrate more quickly. Equilibration time will vary from approximately 2-12 hours and is mainly dependent on the nature and extent of foreign material on the surface of the electrodes. An offset potential difference between the two voltage electrodes of a few millivolts is normal. This offset can be normalized with EVOM's "Zero V" adjustment. If the offset is over 10 mV, continue with the equilibration procedure leaving the saline solution in the chamber overnight with the cable connected to the EVOM and the power off.

Instructions for Use

Measurement using the EndOhm requires the cell culture cup to be transferred into the chamber. Place a blank culture cup in the EndOhm chamber. Add appropriate electrolyte to the culture cup and the chamber. Make sure that the height of the fluid inside the chamber is no more than 3 or 4 mm so that when the cap assembly is inserted, fluid will not overflow the top of the culture cup. With the cap in place, the height of the fluid in the chamber should be at the same level as inside the cup in order to avoid hydrostatic pressure on the membrane. To obtain reproducible and accurate readings, it is important for the cap to be well seated on the chamber, so that the top electrode will be centered. Through the transparent side wall of the chamber, check to see if any air bubbles are trapped between the top electrode and the surface of the electrolyte. Air bubbles will cause the resistance and voltage readings to be very unstable. A gentle shake or reinsertion of the electrode will usually remove air bubbles.

Voltage measurements

Measure the voltage of the blank cup and adjust the Zero V knob on the EVOM until the voltage reading is zero. This adjustment corrects for any asymmetry between the lower and upper voltage electrodes. Note that the bottom electrode is connected to instrument ground. Thus, the top electrode will produce positive DC voltage readings for positive voltage and negative for negative voltages, relative to the bottom electrode. This is opposite in sign from the STX2 electrode. For example, a culture cup which produces a reading of 2 mV with the STX2 will produce a -2 mV reading with the EndOhm.

Resistance measurements

When making a resistance reading of a tissue culture cup with a membrane, Rb (the resistance value of a blank culture cup) should be subtracted from the total resistance measured.

Measure the resistance of a blank culture cup (no cells) that has been soaked beforehand with electrolyte until thoroughly wet. This resistance value is the resistance (Rb) of the inter-electrode solution and the blank membrane. The Rb depends on the distance between the bottom and top electrodes, the composition of the saline solution, and the temperature at which the measurement is performed. If any of these conditions change, Rb should be re-determined. The Rb value of the EndOhm-6 is slightly higher than that of the EndOhm-12 and the EndOhm-24SNAP because of its construction.

EndOhm Maintenance

Cleaning

Flush the chamber thoroughly with distilled water after use. Long term storage approx. 75 days. Store dry. Additional cleaning may be desirable as described below under Sterilization Methods.

Sterilization

The EndOhm may be sterilized by ethylene oxide (EtO), alcohol, or a bactericide (for example, Cidex or Sporicidin). No other solvents should be used. After sterilization, the electrodes should be thoroughly rinsed with sterile perfusing solution before making membrane measurements.

CAUTION: Long-term storage in the UV hood can cause cracking in the clear plastic chamber.

CAUTION: DO NOT AUTOCLAVE THE ENDOHM. Autoclaving can cause the transparent section of the chamber to become crazed. Repeated autoclaving might also damage the seal of the electrode.

Electrode care

The current electrodes consist of solid silver. The surface is chlorided with gray colored silver chloride to enhance stability and provide a faster response time. Over time, this silver chloride surface may wear off and leave the silver exposed. This should not change the performance of the EndOhm significantly.

Electrode cleaning

Normal cleaning: Re-chloride the tips by immersing in common household bleach (no oils, fragrances) for 10 minutes or until a black-purple layer is formed. Rinse well in DI water and flush with buffer prior to use. Test with a CaliCell-12.

Severe cleaning: Remove the electrode and dip the tips in common ammonia water cleaner (no soap) for about 2 - 5 minutes. This removes biological remnants and the chloride coating of the electrode tip. Rinse well in DI water.

Re-chloride the tips by immersing in common household bleach (no oils, fragrances) for 10 to 20 minutes or until a dark gray layer is formed. Rinse well in DI water and flush with buffer prior to use. Test with a CaliCell-12.

Very severe cleaning: Dry the electrodes and lightly "sand" or buff the ends with a very fine grain Emory paper (600 grit or more) or used an "ink" eraser to lightly buff the electrode ends. Re-chloride the tips by immersing in common household bleach (no oils, fragrances) for 10 minutes or until a black-purple layer is formed. Rinse well in DI water and flush with buffer prior to use. Test with a CaliCell-12.

Ammonia water = 5 to 10% $NH_4OH + H_2O$

Chlorine bleach = 3 to 6% solution of sodium hypochlorite.

CAUTION: The threaded section of the top electrode and the cap are made of black anodized aluminum. Bleach will attack these surfaces. Therefore, the bleach must not contact these surfaces while chloriding the electrodes.