EVOM²

Epithelial Voltohmmeter

INSTRUCTION MANUAL

Serial No._____

102711

World Precision Instruments

CONTENTS

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.

INTRODUCTION

EVOM was the first instrument designed to perform routine Trans Epithelial Electrical Resistance (TEER) measurement in tissue culture research. **EVOM²** is the next generation. The confluence of the cellular monolayer is determined by an increase in TEER detected using the unique electronic circuit of the **EVOM²** and **STX2** electrode (included with the instrument). The **EVOM²** qualitatively measures cell monolayer health and quantitatively measures cell confluence. When combined with WPI's **Endohm** chamber, the **EVOM²** can also be used to perform trans *endothelial* electrical resistance measurement.

The **EVOM²** produces an AC current that avoids electrode metal deposits and adverse effects on tissues, which can otherwise be caused by a DC current. In addition, resistance readings are unaffected by membrane capacitance and membrane voltage.

EVOM² is designed with an internal rechargeable 6V NiMH 2200mAH battery pack. The unit is supplied with an external battery charger. A fully-charged battery pack will provide about eight hours of running time. When the battery power falls below the nominal threshold, the meter automatically powers off. While the meter operates safely when it is plugged into an AC power source, WPI recommends disconnecting the meter from the charger when it is in use. Doing so provides for the maximum electrical isolation and reduces the noise to ensure readings of the highest accuracy.

For automation of TEER measurement, consider WPI's **REMS AutoSampler** (WPI # **REMS**), which includes the robotic sampler, data acquisition board, base plate, Windows® based software and an electrode for either the 24- or 96-well plate.

QUICK REFERENCE

For complete instructions on performing measurements with the **EVOM²**, see "OPERATING INSTRUCTIONS" on page 6

For Resistance Measurements:

1. Disconnect the **EVOM²** from the charge and turn the **Power** on (I).
2. Set the **FUNCTION** switch to **OHMs**.
3. Connect the electrodes.
4. Perform the measurements.

For Voltage Measurements:

1. Connect the electrodes to the **EVOM²**.
2. Equilibrate the electrodes with the power off.

   NOTE: Electrodes must be immersed in solution to equilibrate.
3. Disconnect the **EVOM²** from the charger and turn the **Power** on (I).
4. Set the **FUNCTION** switch to **MILLIVOLTS**.
5. Perform the measurements.

INSTRUMENT DESCRIPTION

Parts List

(1) **EVOM²** meter
(1) **STX2** electrode set
(1) **91750** 1000Ω test resistor
(1) 600-grade ultra-fine sandpaper
(1) A/C power cord and charger
(1) Instruction Manual

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 loss or damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 27 of this manual. Please call WPI Customer Service if any parts are missing.

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 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. Please read the section entitled "Claims and Returns" on page 27 of this manual.

Set-up

Test Resistor

The 1000Ω test resistor (WPI **#91750**) is shown in **Fig. 1**. See "Testing the EVOM² Meter" below for instructions on using the test resistor.

Fig. 1—*1000Ω Test resistor*

STX2 Electrode

For resistance measurements only, the **STX2** electrode can be used directly from dry storage without preconditioning. The **STX2** electrode (**Fig. 2**) incorporates a fixed pair of probes, 4mm wide and 1mm thick. Each probe has an outer and an inner

Fig. 2—*STX2 electrode*

electrode. (See **Fig. 4** on page 7) The outside electrodes are small silver (Ag) pads that pass current through the membrane sample. They are referred to as current electrodes. The inner electrodes are small Ag/AgCl pellet voltage sensors. They are referred to as voltage electrodes.

Meter

The **EVOM²** is ready to use, but you will need a slot-head screwdriver (not included) to calibrate the **R ADJ** screw.

CAUTION: Do not leave the meter in direct sunlight for extended periods of time. It will become dark and unreadable.

Labeled items on **Fig. 3** are described below.

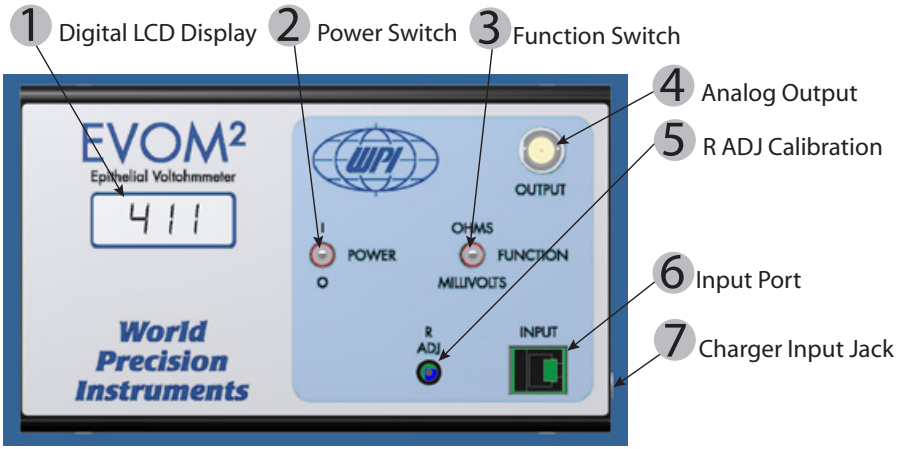


Fig. 3—*EVOM² meter*

① **Digital LCD Display:** The Digital LCD Display registers readings of four and a half digits. When the meter is measuring voltage, it displays the potential difference between the two electrodes in millivolts (mV) up to 999.9mV. When the meter is measuring resistance, it displays the resistance between the two electrode probes in ohms (Ω). Although the meter will provide readings up to 13,000Ω, the accuracy specification is only guaranteed up to 10,000Ω.

② **Power Switch I/O:** The toggle switch turns the meter on (I) and off (O).

③ **Function Switch (Ohms/Millivolts):** When in the Ohms position, the meter is in the resistance measuring mode. When in the Millivolts position, the meter is in the voltage measuring mode.

④ **Analog Output**: This standard BNC connection allows for output of analog data to a recording device.

⑤ **R ADJ Calibration Screw**: When the 1000Ω test resistor is inserted into the input port, this screw is used to calibrate the meter display to 1000Ω while in the **Ohms** mode.

⑥ **Input Port:** Insert the RJ-11 plug of the test resistor, **STX2**, **STX3** or **Endohm** into this port to connect it to the meter.

⑦ **Charger Input Jack:** Insert the charger connector into the Charger Jack and plug the other end into a standard wall outlet to charge the **EVOM²** battery pack. Do NOT take resistance or voltage measurement with the charger connected.

Instrument Diagnostics

It is recommended that EVOM² be put through the diagnostics described below before using it for the first time and then periodically thereafter. If there is a concern that the meter or the electrode is not functioning properly, the following protocols may be used to confirm **EVOM²**'s operating status.

Testing the EVOM² Meter

1. Insert the RJ-11 plug at the end of the test resistor (**Fig. 1**, page 3) into the **Input** port on the meter.
2. Set the **Function** Switch to **Ohms**.
3. Disconnect the **EVOM²** from the charger and turn the **Power** on (**I**). The meter should display 1000Ω. If not, adjust the **R ADJ** screw with a small slot-head screwdriver until the meter shows a reading of 1000Ω.

Conditioning the Electrode For Voltage Measurements

1. Equilibrate the electrode as instructed in the "OPERATING INSTRUCTIONS, 2. Prepare the Electrode," page 6.

   NOTE: When measuring voltage, it is critical that electrodes be equilibrated in solution for at least 12 hours prior to use.
2. Immerse the electrode tips in an electrolyte solution similar to the experimental culture medium or in 0.1-0.15M KCl or NaCl.
3. Set the **Function** switch to **Millivolts**.
4. Disconnect the **EVOM²** from the charger and turn the **Power** on (**I**).

   NOTE: Voltage drift will only be detected if several measurements are made over a period of time.
5. If the meter displays zero, the electrodes are ready for voltage measurements. If the meter still does not display zero, see "Resurfacing/Cleaning the STX2 Electrode", page 10.

OPERATING INSTRUCTIONS

When the **EVOM²** is used for resistance measurements only, the electrode does not need to be equilibrated or preconditioned before use. Any asymmetry of measurement is compensated for by the unit's design.

To measure resistance or voltage, do the following:

1. Disconnect the **EVOM²** from the charger.
2. Connect the electrode to the meter. Insert the RJ plug at the end of the flexible electrode cable into the **Input** port on the meter.
3. Prepare the **STX2** electrode.

   A. Precondition the electrode.

   - For *resistance* measurements, the electrode can be used directly out of dry storage without any preconditioning.
   - For *voltage* measurements, the electrodes need to be equilibrated to eliminate any offset before use. To assure voltage stability and a low inter-electrode potential difference, turn the **Power** off (**O**) on the **EVOM²** and immerse the electrode in electrolyte solution (for example, 0.1 – 0.15M KCl) with the electrodes connected to the **EVOM²**. The **STX2** voltage electrode pairs are shorted together internally when they are connected to the instrument and the **Power** is off (**O**). With the voltage electrode connector pins thus short-circuited for several hours, the asymmetrical potential difference across the two voltage electrodes is reduced. The inter-electrode DC potential will be a few millivolts or less and quite stable. Table 1 (below) lists the recommended equilibration time before using the electrode.

Table 1: STX2 Electrode Equilibration Time

Electrode Condition	Equilibration Time
Stored dry	24 hours
Stored in solution	2 hours

   B. Sterilize the electrodes, if desired. See "Sterilizing the STX2 Electrode" under "INSTRUMENT MAINTENANCE" on page 10.

4. Place the electrode in the sample cup.

   The **STX2** electrode is designed to facilitate measurements of membrane voltage and resistance of cultured epithelia in tissue culture wells. The lengths of the electrodes are unequal allowing the longer (external) electrode to touch the bottom of the dish containing the external culture media while preventing the shorter (internal electrode) from reaching the bottom of the tissue culture

cup or insert (see **Fig. 4**). This feature ensures proper positioning between the electrode and the cell layer in the cup during the trans membrane measurement. In addition, by positioning the longer tip so that it touches the bottom of the dish each time, the reproducibility of the measurements is significantly improved. Place the electrode into the well so the tips just touch the bottom of the wells *without flexing* the electrode. See **Fig. 5**. *Variance of the angle (***Fig. 6***) or depth of immersion (***Fig. 7***) of the electrode will affect resistance measurements.*

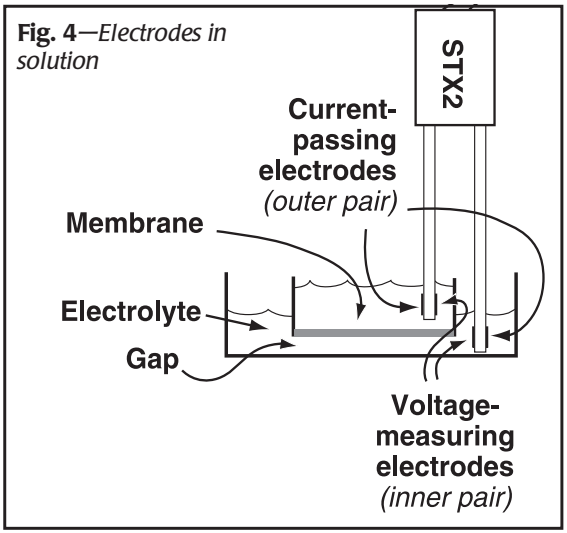


Fig. 4—*Electrodes in solution*

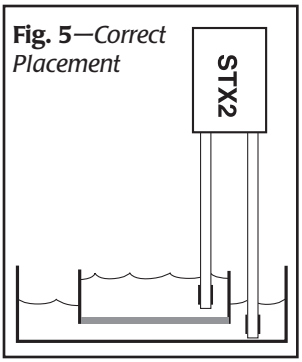


Fig. 5—*Correct Placement*

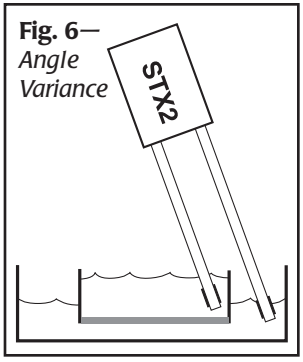


Fig. 6—*Angle Variance*

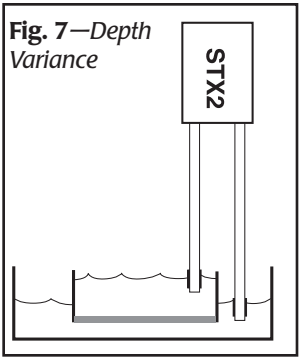


Fig. 7—*Depth Variance*

NOTE: To obtain reproducible results in the same cup, the position of the electrodes must remain constant. To improve the reproducibility and stability of the measurement, it is important to steady the electrode while measuring.

CAUTION: When moving the electrodes from one sample cup to another, it is best **not** to rinse the electrodes with distilled water. Silver/silver chloride electrodes may take several minutes to recover from exposure to distilled water, during which time the potential may drift by a few millivolts. If it is necessary to wash the electrodes between measurements to avoid carryover of one sample into the next, the electrodes should be rinsed with the experimental culture media.

TIP: Transwell inserts made by Corning Costar, in general, have a greater distance between the bottom of the filter cup and the bottom of the plate. (See **Fig. 4**, page 7.) This gap also varies from one lot to the other. It may be large enough to cause the shorter internal electrode to hit the cell layer when the longer electrode touches the bottom of the dish. In this case, the Transwell user can use the optional **STX3** electrodes instead of the **STX2.** They are adjustable. Or, Transwell users can sand *0.5mm* off the shorter probe. See the image at the right. Place the 600-grade sand paper on the edge of a table, and holding the electrodes vertically with the tip of the shorter electrode against the sandpaper and the longer electrode hanging over the edge of the table, drag the electrode lightly over the sandpaper. Check the tip of the electrode, and repeat this procedure until the electrode is the proper length.

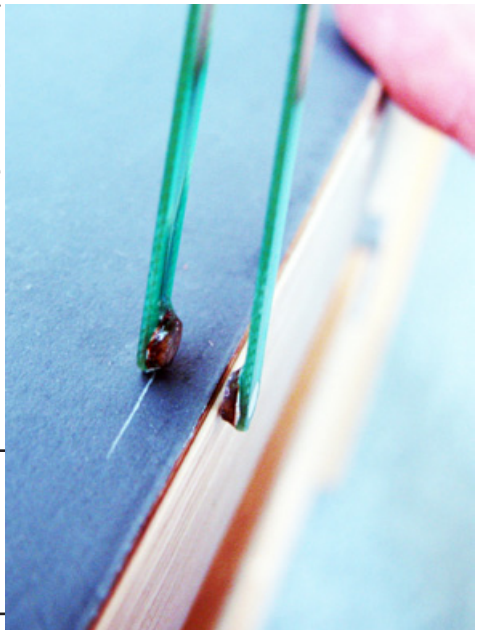


Fig. 8—*Sanding STX2 electrode*

CAUTION: Only the green body of the **STX2** electrode can be sanded. DO NOT SAND TO ANY METAL PART of the electrode or damage to the electrode will result.

5. Measure the resistance.

   A. Set the **Function** switch to **Ohms**.

   B. Disconnect the **EVOM²** from the charger and turn the **Power** on (**I**).

   C. Add electrolyte to a blank cup. (For example, the cell culture insert without cells)

   D. Insert the electrode into the blank cup. A steady ohms reading of the solution resistance should result. The value of the blank always adds to the total resistance measured across a tissue culture membrane. See "APPENDIX A: RESISTANCE CALCULATIONS" on page 21 for a more detailed discussion of the source of the blank resistance and information on calculating true tissue resistance and unit area resistance (the value that is normally reported).

NOTE: The blank resistance must be measured and then subtracted from the resistance reading across tissue in order to obtain the true tissue resistance.

6. Measure the voltage

   A. Set the **Function** switch to **Millivolts**.

   B. Disconnect the **EVOM²** from the charger and turn the **Power** on (**I**).

   C. Insert an equilibrated electrode into the cell culture insert. A steady voltage reading of the trans membrane potential should result. Note that the shorter (internal) electrode is connected to instrument ground and acts as the reference electrode. If the meter reading is positive in the Millivolts mode, the basal side of the cellular tissue (the side adhering to the filter of the insert) is positive with respect to apical side (exposed). Conversely, if the meter is reading negative, it means that the basal side is negative with respect to the apical side.

NOTE: A summary of the operating procedures can be found in the "QUICK REFERENCE" on page 2.

INSTRUMENT MAINTENANCE

Replacement parts can be ordered using the part numbers in Table 2 (below). For additional accessories, refer to the Table 3 on page 13.

Table 2: Replacement Parts

WPI Part #	Description
STX2	Replacement Electrode Set
91750	1,000Ω Test resistor

Electrodes

Electrodes must be properly cleaned, sterilized and stored.

CAUTION: Do not flame electrodes. Doing so will cause them to melt and voids the warranty.

Resurfacing/Cleaning the STX2 Electrode

With use, the electrode surface can become coated with protein or other foreign materials. This build-up, or contamination, will degrade the performance of the system resulting in large voltage offsets (10-20mV) or unstable voltage readings. (Resistance readings will probably not be affected.)

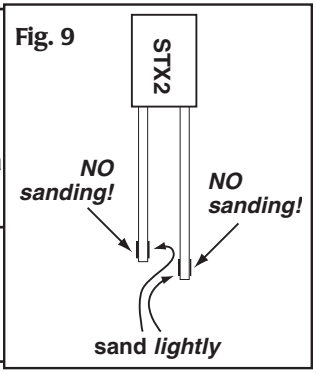
To address these issues, re-chloridize or resurface the electrode:

- RE-CHORLIDIZE – To re-chloridize the electrode, soak the electrode in a solution of 5% sodium hypochlorite (undiluted household bleach) for three minutes. Rinse with water immediately after soaking. Although this cloridizes the electrode surface again, it may not correct the offset.

CAUTION: Ensure that only the electrode tip is exposed to sodium hypochlorite. Do not permit the solution to touch the upper part of the electrode.

- RESURFACE – To resurface the electrode, *lightly* sand the voltage electrode of the **STX2,** which is the silver pellet on the inner surface near the electrode tips. Use the 600-grade ultra fine sandpaper provided. (See **Fig. 9**, right.) Remove only a very thin surface layer of the pellet.

Fig. 9



CAUTION: Repeated sanding will eventually remove the Ag/AgCl pellets. When sanding no longer improves the voltage readings, the electrode needs to be replaced.

TIP: In the absence of 600-grade sandpaper, an ink eraser may be substituted to clean the electrodes.

Sterilizing the STX2 Electrode

The **STX2** electrodes are non-sterile as supplied. They may be sterilized using alcohol, ethylene oxide, UV or a bactericide like Cidex Plus (WPI #**7364**) or Sporicidin.

CAUTION: The electrode cannot be sterilized by autoclaving.

A Typical Sterilization Protocol

In a laminar flow hood:

1. Immerse the electrodes in Cidex Plus (WPI #**7364**) for 15 minutes. Allow them to air dry for 15 seconds.

CAUTION: NEVER leave the electrode in alcohol for more than 30 minutes at a time. Continuously soaking the electrode in alcohol will weaken the protective coating on the electrode and shorten its life.

2. Rinse the electrode in a sterile electrolyte solution similar to the experimental cell culture medium or in 0.1–0.15M KCl or NaCl solution.
3. The electrode is now ready to for *resistance* measurements.
4. For *voltage* measurements, allow the electrode to equilibrate in the sterile electrolyte.

TIP: The electrode can be stored in a UV hood to keep it sterile.

NOTE: When the electrode is exposed to strong visible or UV light, a dark colored oxide film will slowly form on the electrode surface. This film normally will not affect the performance of the electrode. To avoid the formation of the film, shield the electrode from strong light.

Storing of the Electrode

Short term storage (less than two weeks): Immerse the electrode tip in electrolyte solution. Ensure the electrode cable plug is connected to the electrode port on the **EVOM²** meter so that the system is internally short-circuited and electrode symmetry is maintained.

Long term storage (greater than two weeks): When storing for long periods of time, the electrode should be rinsed with distilled water and stored dry and in the dark.

EVOM² Charger and Battery

NOTE: Make sure the EVOM² is powered off when charging the battery.

The **EVOM²** charger plugs into a standard AC wall outlet for recharging the battery pack. It incorporates a universal power supply allowing for use in many countries. The **EVOM²** contains a NiMH 6V 2200mAH rechargeable battery pack.

When the battery charge gets low, "BATT" appears in the digital display. To protect the battery, an automatic power down circuit is used when the battery charge gets too low. To avoid an automatic power down, recharge the battery when the BATT warning appears.

Changing the Battery Pack

The battery pack can be repeatedly charged and discharged for approximately 500 cycles. It may be charged at any time in the discharge cycle, and can be charged continuously without damage.

CAUTION: Use ONLY the **EVOM²** charger. Use of any other charger may damage the battery pack.

Fig. 10–*NiMH replacement battery pack*

The battery life is about 1 to 2 years, depending upon use. NiMH batteries discharge even if they are not being used. The rate of discharge is related to temperature. The cooler the temperature, the slower the discharge rate. If the battery pack is allowed to self discharge for too long, damage may result. To prevent battery damage, charge the battery pack at least once every two months. Should the battery pack fail in your **EVOM²**, replacements are available (WPI **#91736**). See "ACCESSORIES" on page 14.

To change the battery pack:

1. Using a #1 Phillips screwdriver, remove the four screws from the **EVOM²** case.
2. The battery pack is mounted on the bottom chassis plate. Using a #1 Phillips screwdriver, remove the two screws securing the battery pack cover.

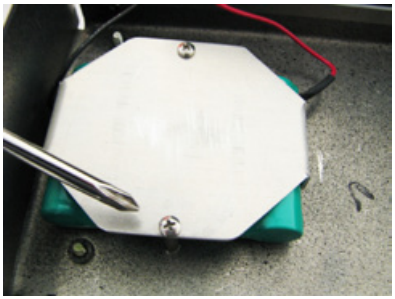


Fig. 11–*Battery pack mounted to bottom chassis plate of **EVOM²***

3. Disconnect the small plastic connector by pulling it straight up at the circuit board (**Fig. 12**). Note the position of the connector and wire color code.

4. Properly dispose of the used battery pack.

5. The battery pack connector will only install one way. If it does not slide easily into place, do not force it. To install the battery pack, line up the locking guide on the circuit board with the tabs on the connector. The locking guide must face the latching ledge (**Fig. 13**). Slide the connector over the pins on the board and push the connector straight down.

WARNING: DO NOT REVERSE POLARITY WHEN CONNECTING THE BATTERY PACK. PERSONAL AND EQUIPMENT DAMAGE MAY RESULT.

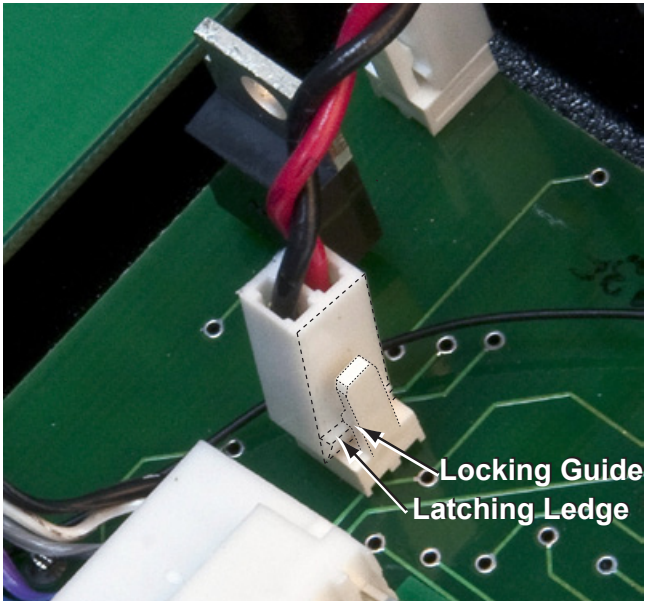


Fig. 12–*Battery pack connector locked in place*

6. Resecure the battery pack cover.

7. Reassemble the **EVOM²** case.

8. Charge the new battery pack for at least 24 hours.

ACCESSORIES

Table 3 (below) lists accessory parts and provides a brief description of the parts. Many of the parts are also described in more detail below the table.

Table 3: Accessory Parts

WPI Part #	Description
METER ACCESSORIES	
3993	Electrode Adapter (for electrodes with 2mm pins)
2851	BNC cable, 6-ft
800496	Battery Charger
91736	NiMH Rechargeable Battery Pack
91750	1000Ω Test Resistor
ELECTRODES FOR TEER (EPITHELIAL) MEASUREMENT	
STX2	Replacement Electrode Set
STX3	Adjustable Electrode Set
ELECTRODES FOR ENDOTHELIAL/EPITHELIAL MEASUREMENT	
ENDOHM-6	Endohm for 6 mm culture cup (24 wells per plate) and the 12 mm Millicell-CM
ENDOHM-12	Endohm for 12 mm culture cup (12 wells per plate)
ENDOHM-24SNAP	Endohm for 24 mm and Costar Snapwell cup (6 wells per plate)
ELECTRODES FOR HTS (High-Throughput) ENDOTHELIAL MEASUREMENT	
STX100C	STX100 for Corning Costar HTS Transwell-24
STX100M	STX100 for Millipore Multiscreen CaCo 96-Well Plate
STX100C96	STX100 for Corning HTS 96-Well Plate
CELL CULTURE CUPS WITH SYNTHETIC MEMBRANES	
CALICELL-12	12 mm Calibration Cell for Endohm-6 and Endohm-12
CALICELL-24	24 mm Calibration Cell for Endohm-24
OTHER	
7364	Cidex Plus (quart)
LAB-TRAX-4	4-Channel Data Acquisition System

Electrode Adapter (WPI# **3993**) converts the four contacts in the RJ-11 into four independent 2-mm jack. It allows the user to utilize the **EVOM²** meter with an Ussing system or other four-electrode system.

STX2 electrode can be used with the **EVOM²** and is designed to facilitate measurements of membrane voltage and resistance (TEER) of cultured epithelia directly in tissue culture wells. The electrode incorporates a fixed pair of probes, 4mm wide and 1mm in thickness. Each probe has an outer (voltage) and an inner (current) electrode.

STX3 is an alternative electrode to the **STX2** that can be used with the **EVOM²**. It differs from the **STX2** in that the distance between the probes of the **STX3** can be adjusted.

STX100C and **STX100F** are optional electrodes for use with the **EVOM²**. Specialized for high throughput screening (HTS) plates, they combine the advantages of the **STX2** and **Endohm**, measuring TEER directly in the culture plate with good reproducibility.

Endohm is an optional electrode chamber for the **EVOM²** for measuring low-resistance tissue culture cells or when increased precision is desired. It can also be used for studying the tight junction changes induced by chemicals and other factors. Concentric pairs of electrodes above and below the insert membrane results in excellent stability and reproducibility. (See "APPENDIX C: Improving the Accuracy and Repeatability of the System" on page 24.) Unlike the **STX2**, the inserts must be transferred from the culture plate to the **Endohm** chamber to make a measurement.

CaliCell™ is a cell culture insert with a synthetic membrane that mimics a confluent epithelial membrane's resistance in fluid. Each **CaliCell** insert has an established resistance measurement determined at the time of manufacture and against which the system resistance can be compared. Used as a control to check the functionality of the system, **CaliCell** is especially useful when working with confluent cells that yield low resistance readings, causing concern that the **EVOM²** system is not working. The high resistance readings obtained with **CaliCell** provide reassurance that the **EVOM²** system is operational. Although the resistance may vary with time, **CaliCell** cups will remain reasonably constant for several years if stored at room temperature. They can be used to test the **EVOM²** (and Ussing) system with a variety of electrode configurations.

TROUBLESHOOTING

EVOM² Meter

Most of the time, system problems are related to the electrode, not the meter itself. At least half of the meter failures result from a failed switch because of the presence of corrosion, and typically corrosion is caused by accidental spillage of saline solution or culture media on the meter. If the meter has been kept free of salt solution and functional testing of the meter demonstrates acceptable performance results, then the meter is working correctly. (See "Testing the EVOM² Meter" on page 5.)

STX2 Electrode

Although the **STX2** electrode is warranted for 30 days, its normal life is 1 to 2 years, depending on its usage. Some issues you may encounter include:

- UNSTABLE OR HIGH VOLTAGE READING: If the electrode fails, the most common symptom is an unstable or unusually high reading.
- LOW RESISTANCE: When the meter displays a lower than expected resistance, but is stable and reproducible, the most likely cause is related to the cell culture, not the electrode or meter.

While there is no quantitative method available to do an in situ or wet test on the electrode, two qualitative methods may assist in determining if the electrode is working and will respond to an increase in resistance.

- Use **WPI**'s **CaliCell** to test the electrode. **CaliCell** is a cell culture insert with a synthetic membrane that mimics a confluent epithelial membrane's resistance in fluid. Each **CaliCell** insert is supplied with an established resistance measurement determined at the time of manufacture and against which the system resistance can be compared.
- Test the resistance differences between a well filled with electrolyte and a blank culture insert filled with electrolyte. The resistance of electrolyte alone should be less than 50Ω and stable, if the electrode is kept stationary. The resistance of the blank insert is normally in the 80 to 200Ω range, depending on the brand and size.

TIP: During normal usage, it is helpful to write down the resistance range of each particular type of blank insert with the specific culture media used. If the electrode is subsequently suspected of having a problem, a comparison of current readings to past readings on the same blank insert and culture media could assist in determining if the electrode is functioning as expected.

Issue	Possible Cause	Solution
Unit runs briefly, and powers off	Unit is insufficiently charged	Recharge the unit for at least 12 hours. **NOTE: Make sure the EVOM² is powered off when charging the battery.**
Unit does not charge	Insecure connection at power input jack on meter	Verify that the power connector is securely connected at the meter power input jack.
	Defective charger	Verify correct output voltage at the power supply connector with a volt meter. Replace the power supply if approximately +12V DC is not present at the center conductor on the connector. If the charger is working and the unit does not power up, contact WPI for service.
Meter will not power on with charger DISCONNECTED	Batteries are discharged	The meter powers down automatically when the batteries are depleted. Power off the meter and connect it to the battery charger. Charge it for at least 12 hours. A full charge may require up to 24 hours. For reliability, the meter should be charged each evening (with the power off) before use the next day.
	Batteries are defective	Replace the battery pack or contact WPI for service. See "Changing the Battery Pack" on page 12.
Voltage reading is unstable	Low battery	Ensure that the charger is connected and plugged into a live wall outlet. Wait one minute and test again. If the voltage output is not zero (in plain culture media) and the battery pack is fully charged, the instrument is malfunctioning. Before contacting WPI Technical Support for assistance, recharge the instrument for 24 hours and repeat the procedure.
	Electrode too close to strong electromagnetic radiation device	Move the system to a different area away from sources of electromagnetic fields. Electromagnetic field sources could include computers, MRI equipment, magnetic stirrers, etc.
	Power line or output jack connected to a recording device causing noise	Remove the connection to the recording device to eliminate that problem.

Issue	Possible Cause	Solution
Voltage reading is not zero in plain culture media or blank cell	Electrode not equilibrated	The Ag/AgCl disks (inner voltage measurement electrodes) on the probe may become polarized due to an uneven distribution of ions between the electrode pair. This ionic charge can be neutralized by immersing the probe into 0.1M KCL for 24 hours with the probe electrodes shorted electrically. The probe is shorted electrically when it is connected to the meter with the power OFF, and the FUNCTION switch in the OHMS position.
	Dirty electrode	Clean the electrode. (See "Resurfacing/Cleaning the STX2 Electrode" on page 10.)
	Conductive contamination between electrodes	Inspect the inter-electrode surface areas for material which could form a conductive bridge between the inner and outer electrodes. If the material cannot be removed, the electrode should be replaced.
	Corrosion has formed on conductive traces	An insulative transparent coating protects the copper conductors that connect each electrode to the main wiring in the handle. When the probe is very old, this coating can deteriorate, allowing the salts in the media solution to leech through to the copper conductors. This is observed as a black discoloration on the copper circuit traces underneath the conformal coating. If this is observed, replace the electrode.
Resistance reading unusually high	Contaminated electrode contacts	With use, the chopstick electrode probe contacts may acquire a buildup of protein or other foreign material that effectively increases the baseline resistance of the electrodes. The electrodes can be cleaned using various methods. See "Resurfacing/Cleaning the STX2 Electrode" on page 10.
Resistance reading unusually low	Cell culture or media problem	If the cell culture has been given sufficient time to achieve confluence, and the reading is stable but significantly lower than expected, then the problem is probably related to the cell culture. Electrode failure will not generally cause a lower than expected yet stable reading. Use the test resistor to verify the meter is functioning correctly. The meter display should read 1000Ω.
Resistance is a negative value	Conductive contamination on electrodes	Use the 1000Ω test resistor to verify that the meter is working correctly. The meter display should show 1000Ω. If a negative value exists only when using the STX electrode, inspect the electrode for the possible formation of a conductive bridge of foreign material that electrically joins the current and voltage electrodes. Remove the foreign material and retest.

Issue	Possible Cause	Solution
Resistance reading drifts or is unstable	Electrodes are not immersed in culture media solution	Use the test resistor to verify that the meter is functioning correctly. The meter display should read 1000Ω. The meter will not provide a stable reading if the electrodes are disconnected from the meter or if the electrodes are not immersed into culture media.
	Electrodes are not held still during measurement	Use the test resistor to verify the meter is functioning correctly. The meter display should read 1000Ω. Handheld electrodes must be kept as motionless as possible during a measurement. Excessive movement will cause the measurement to fluctuate.
	Charger is connected to the meter	The meter reading can become unstable due to the loss of electrical isolation when the charger is connected to the AC power. To ensure stability of readings, always disconnect the charger from the meter when making measurements.
	Meter needs set	Use the R ADJ calibration screw on the front panel to adjust the resistance value to 1000Ω, using the test resistor. See "Testing the EVOM² Meter" on page 5.
	Old electrode probe	Use the test resistor to verify the meter is functioning correctly. The meter display should read 1000Ω. The lifetime of the chopstick electrodes is between 1- 2 years with normal use.

NOTE: If you have a problem/issue with your **EVOM²** that falls outside the definitions of this troubleshooting section, contact the WPI Technical Support team at 941.371.1003 or technicalsupport@wpiinc.com.

SPECIFICATIONS

Membrane Voltage Range	±200.0 mV
Resolution	0.1mV
Resistance Range	0 to 9999Ω
Resistance Resolution	1Ω
AC Square Wave Current	±10µA nominal at 12.5Hz
Power	Internal rechargeable 6V NiMH 2200mAH battery with external 12VDC supply for recharging
Battery Charge Time	12 hours
Nominal Battery Run Time	~8 hours*
Analog Output	1-10V (1mV/ohm)
Dimensions	7.25″W x 4.25″H x 2.30″D (19cm x11cm x 6cm)
Weight	3 lb (1.4 kg)
Electrode Connection	RJ-11connector (telephone style)
Test Resistor	External
Environmental Range	50-100°F (10-38°C) 0-90% non-condensing relative humidity

* When the battery power level falls below a minimum threshold, the unit automatically powers off. A fully-charged battery will provide about eight hours of running time. **Make sure the EVOM² is powered off when charging the battery.**

APPENDIX A: RESISTANCE CALCULATIONS

Resistance

The value of the blank always adds to the total resistance measured across a tissue culture membrane. (See below.) *The blank resistance must be measured and then subtracted from the resistance* reading across the tissue in order to obtain the true tissue resistance.

For example, suppose the resistance through a 0.15M KCl solution and across the membrane support (with no tissue present) of a 12-well cell culture insert measures 130Ω. This is the blank reading for that cell culture insert. (Resistance may vary for culture cups made by other manufacturers.) In this example, using 800 Ω as the sample measurement, the calculated resistance for the tissue itself (R_{tissue}) is:

$$R_{Total} = 800$$

$$R_{blank} = 130\ \Omega$$

$$R_{blank} + R_{true\ tissue} = R_{Total}$$

$$R_{true\ tissue} = R_{Total} - R_{blank}$$

$$R_{true\ tissue} = 800\Omega - 130\Omega = 670\Omega$$

Resistance value of the "blank" insert

When using an **EVOM²** with an **STX2** to measure a blank insert, the resistance value is typically between 120 to 180Ω, depending on the specific brand of the insert. This is not background resistance due to the resistance of the blank filter. Rather, if the filter membrane is removed from the insert, the resistance reading of the insert will remain the same, because the background resistance reading is due mainly to the small gap between the bottom of the cell culture insert and the bottom of the cell culture plate. See **Fig. 4**, page 7.

This gap is about 1mm, with some insert brands having a slightly larger gap than others. The variation in this gap is the cause of the difference between blank readings of different brands. The smaller the gap, the higher the electric resistance. *The resistance of the filter membrane itself is actually negligible.*

If an **Endohm-24SNAP** or **Endohm-12** chamber is used, the blank resistance becomes near zero, because the external electrode is directly underneath the filter and the gap does not exist.

Unit Area Resistance

As the resistance is inversely proportional to the area of the tissue, instead of reporting resistance, typically the product of the resistance and the area is calculated and reported. The unit area resistance is independent of the area of the membrane used and may be used to compare data obtained from inserts of different sizes.

NOTE: Resistance readings for 24 mm or larger diameter inserts obtained by using the **EVOM²** with the **STX2** electrode should *not* be converted to unit area resistance. The **Endohm** is recommended for these larger inserts. (See "TROUBLESHOOTING" on page 16.)

The **unit area resistance** is obtained by **multiplying** the meter readings by the effective surface area of the filter membrane. The dimension is Ωcm². The resistance is **inversely** proportional to the surface area. Thus, the larger the membrane, the lower the resistance.

Resistance of a unit area = Resistance (Ω) x Effective Membrane Area* (cm2)

* See manufacturing specifications for the particular insert

Unit Area = 1 cm²

The unit area resistance is independent of the area of the membrane used and may be used to compare data obtained from inserts of different sizes.

Continuing with the previous example, in which the $R_{true\ tissue}$ = 670Ω, if an effective membrane diameter (d) were 1.05 cm, the unit area resistance would be:

Resistance x Effective Membrane Area = 670Ω x $\pi d^2/4$

= 670Ω x (3.14)*(1.05cm)²/4

= 580Ωcm²

580Ω is the resistance of a unit area of 1 cm².

The larger the membrane, the lower the resistance. The dimension is Ωcm², not Ω/cm². This may be confusing to a new user who might expect to divide to find the resistance of a unit area.

A further illustration may help to reinforce this concept:

Assuming a 1cm² membrane has a resistance of 500Ω, then a 5cm² membrane will have a resistance of 100Ω, not 2,500Ω, because the resistance is *inversely* proportional to the area. Accordingly, if a 5cm² membrane has a resistance of 100Ω, then the resistance of a 1cm² membrane will be 100Ω x 5cm² = 500Ωcm² because the smaller membrane is 1/5 the size and the resistance will therefore be five times greater.

APPENDIX B: 24 MM DIAMETER (6-WELL) INSERTS AND STX ELECTRODES

Note that the resistance readings from 24mm diameter tissue culture inserts (used in 6-well plates) obtained by using WPI's STX series of electrodes will be 1.8 to 2.2 times higher than that obtained using the **Endohm-24**, because the **STX** electrode cannot deliver a uniform current density over the relatively large membrane through the small gap between the membrane and the bottom of the well. *Therefore, the resistance reading of a 24 mm (6-well) diameter insert obtained by* **STX** *electrodes should not be used to calculate the unit area resistance.* **STX** electrodes are only intended for providing a qualitative measurement of cell monolayer health and quantitative measurement of cell confluence. This is not a problem with smaller inserts, such as the 12mm and 6mm diameter inserts, because of the relatively smaller size of the membrane compared to the electrode. When **EVOM²** is used with the **Endohm**, it gives a repeatable and accurate value of membrane resistance consistent with that obtained by using a well-designed Ussing chamber. It is also consistent with that obtained by the **STX2** electrode for the 12mm and 6mm diameter filter inserts.

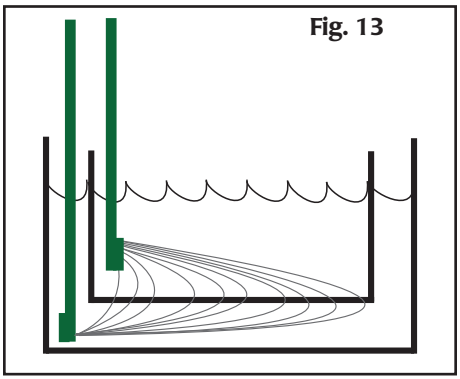
NOTE: The unit area resistance is independent of the area of the membrane and may be used to compare data obtained from inserts of different sizes.

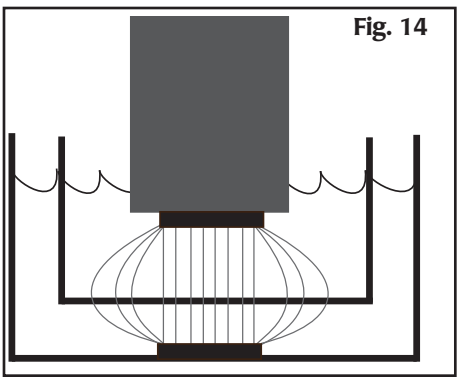
APPENDIX C: IMPROVING THE ACCURACY AND REPEATABILITY OF THE SYSTEM

Whenever possible, operate the **EVOM²** meter with the battery charger disconnected. This ensures maximum electrical isolation for the most accurate readings. When the charger remains connected to the meter during measurements, electrical noise can be introduced into the system. For the highest accuracy in measurement, WPI recommends disconnecting the unit from the AC power source.

For measuring low-resistance tissue culture or for more precise measurement, the user should consider using the **Endohm** chamber instead of **STX2** electrode. Although cells must be transferred from their culture wells to the **Endohm** chamber for measurement, more accurate measurement of membrane resistance can be achieved. By introducing concentric pairs of electrodes above and beneath the membrane, the **Endohm** reduces background resistance (with a blank cup inserted) from 150Ω to less than 5Ω. (**Endohm-6** is higher.) The shape of the current electrodes allows a more uniform current density to flow across the membrane. See Figures 13 and 14, below. Fig. 13 demonstrates how the **STX2** electrode measures TEER, and Fig. 14 shows how the **Endohm** chamber measures TEER. With fixed electrode geometry, the variation of readings on the same sample is 1-2Ω as compared to 5-10% of the total reading using the **STX2** electrodes. The **Endohm**, together with the **EVOM²**, offers the most accurate, convenient and economical solution for trans membrane electrical resistance measurement.

For automation of TEER measurements, consider the **REMS AutoSampler** that integrates a robotic system and computer interface to automatically measure tissue resistance in 24- and 96-well plates. The ability of the **REMS AutoSampler** to precisely locate the electrode results in highly reproducible TEER measurement.





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DECLARATION OF CONFORMITY

WORLD PRECISION INSTRUMENTS, INC.
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DECLARATION OF CONFORMITY

We: World Precision Instruments, Inc.
175 Sarasota Center Boulevard
Sarasota FL 34240-9258
USA

as the manufacturer of the apparatus listed, declare under sole responsibility that the product:

Title: EVOM² Epithelial Voltohmmeter

to which this declaration relates is in conformity with the following standards or other normative documents:

Safety: EN61010-1:2001

EMC: EN61326-1:2006
EN61326-2-3:2006

and therefore conforms with the protection requirements of Council Directive 2004/108/EC relating to electromagnetic compatibility and Council Directive 2006/95/EC relating to safety requirements.

Issued on: January 6, 2009

Mr. Cliff Bredenberg
General Manager
World Precision Instruments, Inc.
175 Sarasota Center Boulevard
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Mr. Glen Carlquist
Vice President of Manufacturing
World Precision Instruments, Inc.
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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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