



# EndOhm

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*Tissue resistance measurement chambers  
for tissue culture cups*

## **INSTRUCTION MANUAL**

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

082613

[www.wpiinc.com](http://www.wpiinc.com)

**World Precision Instruments**

# Other WPI Favorites

## EVOM<sup>2</sup><sup>™</sup> Epithelial Voltohmmeter

- **Manual TEER measurements of epithelial cells in 6-, 12-, and 24-well plates**
- **Electrically isolated meter that plugs into a standard outlet for continual readout without push buttons**
- **Compatible with Endohm chambers**
- **STX2 manual electrodes and test electrode included with every meter**



The EVOM was the first instrument designed specifically to perform routine Trans Epithelial Electrical Resistance (TEER) measurement in tissue culture research. EVOM<sup>2</sup> is the next generation, redesigned for ease of use. The EVOM<sup>2</sup> not only qualitatively measures cell monolayer health, but also quantitatively measures cellular confluence. The unique electronic circuit of the EVOM<sup>2</sup> and the included STX2 electrode detect the confluence of the cellular monolayer. When combined with WPI's Endohm chamber, the EVOM<sup>2</sup> can also be used to perform more accurate quantitative measurements or lower resistance measurements like trans endothelial electrical resistance measurements.

The isolated power source of the EVOM<sup>2</sup> was specifically designed to avoid adverse effects on tissue and the formation of electrode metal deposits, even when it is plugged into a standard

wall outlet. Now, the EVOM<sup>2</sup> is always on when you need it. In addition, its rechargeable battery allows up to 10 hours of mobile use. The four and a half digit readout provides a range of 1-9,999  $\Omega$ . The included test electrode lets you calibrate the resistance measurements for an accurate reading every time, and the voltage meter never needs calibration. An analog BNC output is standard with the EVOM<sup>2</sup>, providing an output port for recording data or remote display of the EVOM<sup>2</sup> output. EVOM<sup>2</sup> comes complete with the popular STX2 "chopstick" electrodes, 4 mm wide and 1mm thick. Each stick of the electrode pair contains a silver/silver-chloride pellet for measuring voltage and a silver electrode for passing current. The small size of each electrode is designed to facilitate placement of the electrodes into a variety of standard cell culture wells.

## CaliCell<sup>™</sup>

**Cell culture cups with synthetic membrane for testing STX electrodes, Endohm and Ussing chambers**

It takes a long time and a lot of work to grow a batch of cells, so you will want to make certain that your test apparatus is functioning properly. The CaliCell<sup>™</sup> provides a quick and positive way to test STX electrodes, EVOMs, Endohm, and Ussing chamber.

The CaliCell<sup>™</sup> is a major improvement in TEER electrode calibration. Its membrane makes use of our unique electric current constriction technology to produce resistance readings comparable to those obtained with real cell cultures. The CaliCell<sup>™</sup> does not have to be refrigerated, and can be cleaned and sterilized with alcohol. Readings will not drift over time as long as the unit is kept in good physical condition.



<b>CALICELL-12</b>	12 mm Calibration Cell for Endohm-6/Endohm-12
<b>CALICELL-24</b>	24 mm Calibration Cell for Endohm-24

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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—Endohm-6, Endohm-12 and Endohm-24

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# INTRODUCTION

- ENDOHM-6** Chamber for 6mm culture cup, 15.8mm ID (24 wells per plate)
- ENDOHM-12** Chamber for 12mm culture cup, 23.2mm ID (12 wells per plate)
- ENDOHM-24SNAP** Chamber for 24mm and COSTAR Snapwell™ culture cup, 37.3mm ID (6 wells per plate)

The **EndOhm** series of chambers, when used with WPI's **EVOM<sup>2</sup>** 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.

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 10-30Ω (variation is dependent on the experience of the user) to 1-2Ω.

## Notes and Warnings



**CAUTION:** Long-term storage in a UV hood could 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.

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## Parts List

After unpacking, verify that there is no visible damage to the unit. Verify that all items are included:

- (1) **EndOhm** chamber
- (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 damage should be reported

at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 15 of this manual. Please contact WPI Customer Service if any parts are missing at 941.371.1003 or [customerservice@wpiinc.com](mailto: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 15 of this manual.

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# INSTRUMENT DESCRIPTION

## Instrument Description

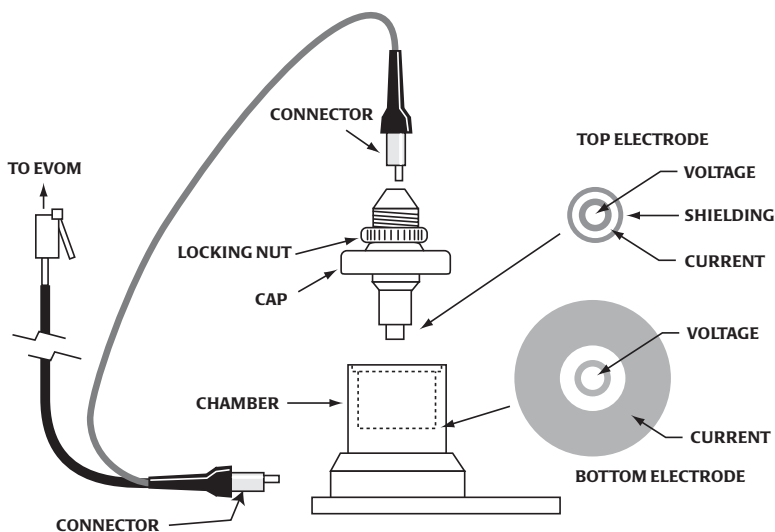


Fig. 2—Schematic diagram of an EndOhm

The structure of the **EndOhm** is pictured in **Fig. 2**. 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.

## Setup

### Adjusting the Height of the Top Electrode

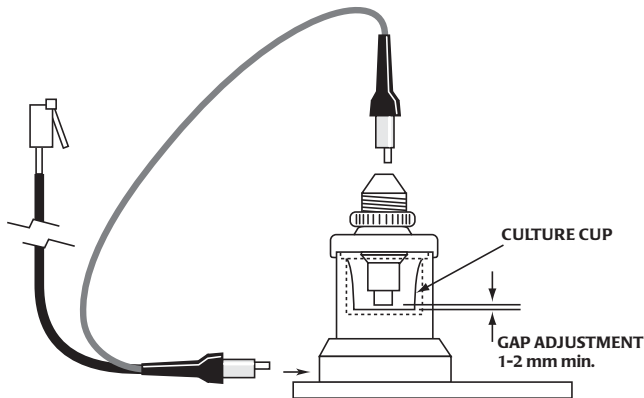
The height of the top electrode of all the **EndOhms** can be adjusted to fit cell culture cups from different manufacturers.

**NOTE:** The height of the top electrode of the **EndOhm-6** is factory-set for Costar Transwell culture cups. No height adjustment is necessary if you use these cups.

1. Before use, place a blank cup into the chamber.
2. Loosen the locking nut on the cap assembly.



3. Rotate the cap on the top electrode to change the height of the electrode. When the cap is in place on top of the chamber, the clearance between the top electrode and the culture membrane is 1–2mm. See **Fig. 3**.



*Fig. 3—Assembled EndOhm showing the appropriate gap between the membrane and the electrode*

## Electrode Preparation

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

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:

1. Fill the chamber with saline solution (for example, 0.1-0.15M KCl).
2. Replace the cap.
3. Connect the cable to the **EVOM<sup>2</sup>** with the power off. The voltage electrodes are shorted together internally when they are connected to the instrument and the power is off. With the voltage electrode connector pins short-circuited for several hours, the asymmetrical potential difference across the low voltage electrodes is reduced. The inter-electrode DC potential will be a few millivolts or less and quite stable. 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. If the offset is greater than 10mV, leave the saline solution in the chamber overnight with the cable connected to the **EVOM<sup>2</sup>** and the power off.

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## OPERATING INSTRUCTIONS

1. Measurement using the **EndOhm** requires the cell culture cup to be transferred into the chamber. Place a blank culture cup in the **EndOhm** chamber.
2. Add an appropriate electrolyte to the culture cup and the chamber. Make sure that the height of the fluid inside the chamber is no more than 3–4mm 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.
3. Position the cap on the chamber. To obtain reproducible and accurate readings, it is important that the cap is well-seated on the chamber, so that the top electrode is centered.
4. Through the transparent side wall of the chamber, look for air bubbles trapped between the top electrode and the surface of the electrolyte. Air bubbles will cause the resistance and voltage readings to be very unstable. Gently shake the **EndOhm** or reinsert the electrode to remove any air bubbles.

## Voltage Measurements

1. Set the Function switch on the **EVOM<sup>2</sup>** to Millivolts.
2. Turn the **EVOM<sup>2</sup>** Power on (I).
3. Connect the **EnhOhm** to the **EVOM<sup>2</sup>**.

**NOTE:** The bottom electrode is connected to instrument ground. 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 2mV while the **STX2** will produce a -2mV reading with the **EndOhm**.

## Resistance Measurements

1. Set the Function switch on the **EVOM<sup>2</sup>** to Ohms.
2. Turn the **EVOM<sup>2</sup>** Power on (I).
3. Add electrolyte to a blank cup. (For example, the cell culture insert without cells).
4. Insert the electrode into the blank cup. Measure the resistance of a blank culture cup (no cells). 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 12 for a more detailed discussion of the source of the blank resistance and information on calculating true tissue resistance and unit areas 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.

**NOTE:** The resistance of the blank for the **EndOhm-6** is slightly higher than that of the **EndOhm-12** and the **EndOhm-24SNAP** because of its construction.

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## MAINTENANCE

### Cleaning

After using your instrument, flush the chamber thoroughly with distilled water, spray it with alcohol and rinse it with distilled water. Do not use anything more abrasive than a cotton swab for cleaning the **EndOhm**.

Between experiments, rinse old growth buffer from the chamber with distilled water and spray with alcohol. Rinse with distilled water before filling the chamber with new growth buffer.

**NOTE:** The silver pellets are porous, and alcohol will soak into them. The final water rinse will pull the alcohol from the pellets. Without the final water rinse, the alcohol that has soaked into the pellet could kill the cell layer.

### Storage

For long term storage (more than 75 days), store the **EndOhm** dry.



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

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### Sterilization

The **EndOhm** may be sterilized with a ethylene oxide (EtO) or a 70/30 mix of alcohol to water. A bactericide like **Cidex** (WPI #7364) can be used, as needed. No other solvents should be used. After sterilization, the electrodes should be thoroughly rinsed with a sterile perfusing solution (growth buffer) before making membrane measurements.



**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.

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### Re-Chloriding the Electrode

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.

Once a week, or as needed, the electrode can be re-chloridized. Immerse the tips of the electrode in common a 5% solution of sodium hypochlorite (bleach) for 10 minutes or until a black-purple layer is formed. Do not use a solution stronger than 3–6% sodium hypochlorite. Rinse well in distilled water and flush with the growth buffer prior to use. Test the electrode with a **CaliCell-12** (WPI #CALICELL-12).

Fig. 4 and Fig. 5 show an **EndOhm** that has been re-chlorided.



Fig. 4—EndoHM-12 shown that has not been chlorided enough



Fig. 5—Properly chlorided EndOhm (note the brighter color)



**CAUTION:** Ensure that only the electrode tip is exposed to the sodium hypochlorite. The threaded section of the top electrode and the cap are made of black anodized aluminum. Sodium hypochlorite will attack these surfaces. The solution must not contact these surfaces while chloriding the electrodes

Once every month or two, as needed, you can remove the biological remnants and the chloride coating of the electrode tip. Remove the electrode and dip the tips in common ammonia/water solution (with no soap) for 2–5 minutes. Rinse well in distilled water. See Fig. 6 and Fig. 7.

**NOTE:** Do not use any solution of ammonia/water stronger than 5%.



Fig. 6—Clean EndOhm surface with no spots or irregularities



Fig. 7—Notice the center tip and the small ring on the top section. These may require the use of a cotton swab to clean.

Rarely you may need to resurface the electrode. Dry the electrodes and lightly “sand” or buff just the tip of the electrode with a very fine grain Emory paper (600 grit or more) or used an “ink” eraser to lightly buff the electrode ends. Remove only a very thin surface layer of the pellet. After sanding the pellet, re-chloride the tips using the instructions above.



**CAUTION:** Repeated sanding will eventually remove the Ag/AgCl pellet. When sanding to longer improves the voltage readings, the electrode needs to be replaced.

## ACCESSORIES

**Table 1: Accessories**

Part Number	Description
<b>7364</b>	Cidex Plus
<b>53330-01</b>	Replacement Endohm cable
<b>CALICELL-12</b>	12 mm Calibration Cell for Endohm-6 or Endohm-12
<b>CALICELL-24</b>	24 mm Calibration Cell for Endohm-24
<b>EVOM2</b>	Epithelial Tissue Voltohmmeter (includes <b>STX2</b> electrode set)

## TROUBLESHOOTING

Issue	Possible Cause	Solution
Resistance reading drifts or is unstable	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.
	Old electrode probe	Use the test resistor to verify the meter is functioning correctly. The meter display should read 1000Ω.
Voltage reading is unstable	Low battery in the <b>EVOM<sup>2</sup></b>	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
Unusually low or high reading	Culture cup is incorrectly positioned	<p>Move electrode to a different height position.</p> <p>If you have upper mount cups (usually with a plastic support cone), verify that there is about 2mm of clearance over the bottom electrode set.</p>
	Conductive contamination between electrodes	Inspect the electrode surfaces for material which could form a conductive bridge between the upper and lower electrodes causing the upper and lower electrodes to be in the same contiguous solution without an electrical separation. If the material cannot be removed, the electrode should be replaced.
	Molarity of buffer solution changed	A 1% change in 100mM molarity can cause large reading errors.
	A bad cable	Continuous low resistance readings, even when dry could be caused by a bad cable. Try a replacement cable (WPI #53330-01).
	Dirty electrodes	Clean the electrodes. (See "Cleaning" on page 7.)
	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.

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

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# 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 ( $R_{\text{tissue}}$ ) is:

$$\begin{aligned} R_{\text{Total}} &= 800 \\ R_{\text{blank}} &= 130 \Omega \\ R_{\text{blank}} + R_{\text{true tissue}} &= R_{\text{Total}} \\ R_{\text{true tissue}} &= R_{\text{Total}} - R_{\text{blank}} \\ R_{\text{true tissue}} &= 800\Omega - 130\Omega = 670\Omega \end{aligned}$$

## Resistance value of the “blank” insert

The resistance measurement of a blank insert 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.

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.

The **unit area resistance** is obtained by **multiplying** the meter readings by the effective surface area of the filter membrane. The dimension is Ωcm<sup>2</sup>. The resistance is **inversely** proportional to the surface area. Thus, the larger the membrane, the lower the resistance.



**Resistance of a unit area = Resistance ( $\Omega$ ) x Effective Membrane Area\* ( $\text{cm}^2$ )**

\* See manufacturing specifications for the particular insert

Unit Area =  $1 \text{ cm}^2$

**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  $R_{\text{true tissue}} = 670\Omega$ , if an effective membrane diameter (d) were 1.05cm, the unit area resistance would be:

$$\begin{aligned} \text{Resistance x Effective Membrane Area} &= 670\Omega \times \pi d^2/4 \\ &= 670\Omega \times (3.14) \times (1.05\text{cm})^2/4 \\ &= 580\Omega\text{cm}^2 \end{aligned}$$

580 $\Omega$  is the resistance of a unit area of  $1 \text{ cm}^2$ .

The larger the membrane, the lower the resistance. The dimension is  $\Omega\text{cm}^2$ , not  $\Omega/\text{cm}^2$ . 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  $1 \text{ cm}^2$  membrane has a resistance of  $500\Omega$ , then a  $5 \text{ cm}^2$  membrane will have a resistance of  $100\Omega$ , not  $2,500\Omega$ , because the resistance is *inversely* proportional to the area. Accordingly, if a  $5 \text{ cm}^2$  membrane has a resistance of  $100\Omega$ , then the resistance of a  $1 \text{ cm}^2$  membrane will be  $100\Omega \times 5 \text{ cm}^2 = 500\Omega\text{cm}^2$  because the smaller membrane is 1/5 the size and the resistance will therefore be five times greater.



## WARRANTY

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

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

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

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

## Claims and Returns

Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within 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.*



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