



# **EVC-4000**

*Multi-Channel voltage/current clamp*

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## **INSTRUCTION MANUAL**

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

032707

***World Precision Instruments***



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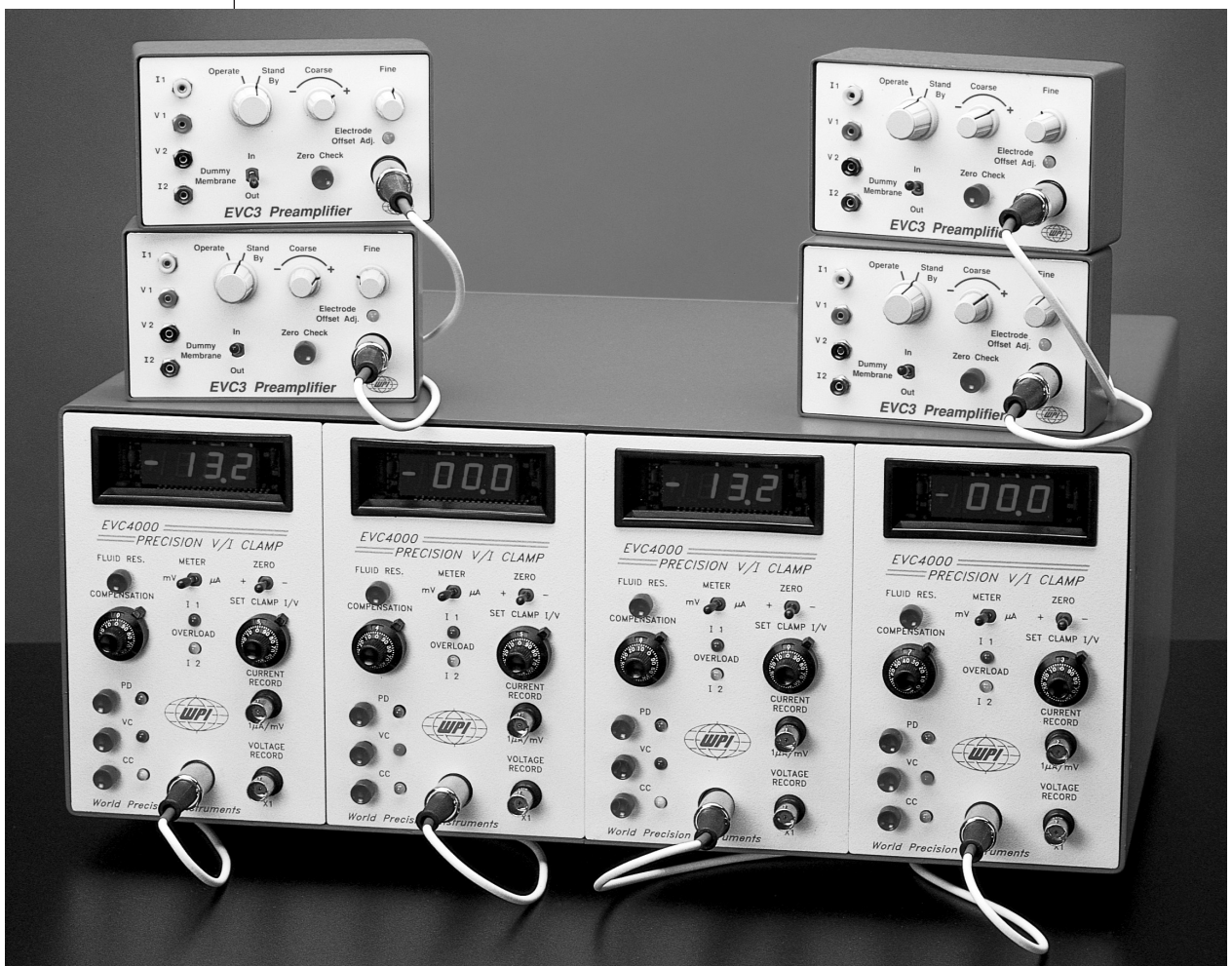
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## INTRODUCTION

A multi-channel Voltage/Current clamp apparatus for the study of the electrical properties of epithelial tissues, EVC-4000 employs the voltage clamp technique to study temporal changes in membrane permeability as a function of membrane voltage or applied chemical agents. This instrument can measure up to four tissue samples simultaneously.

Each module, with its companion preamplifier, can operate independently in one of three different modes: Voltage Clamp (VC), Current Clamp (CC) or Open Circuit Potential (PD) measurement. Operation of the EVC-4000 can be effected manually from the front panel of the instrument or from computer generated commands applied at the rear panel of the instrument.



## SYSTEM ARCHITECTURE

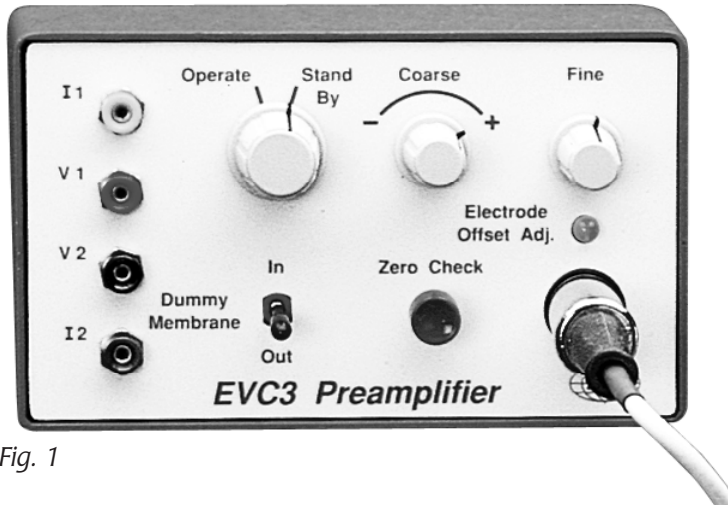


Fig. 1

### Preamplifier

Each channel module of EVC-4000 provides power to its own remote preamplifier (see Figure 1) connected by a flexible cable to the front panel. Preamplifiers should be mounted close to the site of the experiment. To avoid accidental wetting of its electronic circuits, the preamplifier should be supported on a ring stand mount so that its front face is in a vertical position. Membranes to be tested are normally placed into

Ussing chambers or similar devices. One chamber of a test membrane can be arbitrarily designated as the "1" side and the transmural chamber as the "2" side. Four electrodes are positioned so that current electrode  $I_1$  and voltage electrode  $V_1$  are placed in chamber 1; electrodes  $I_2$  and  $V_2$  in chamber 2.

**A unique feature of the EVC-4000 preamplifier is a local circuit that maintains the potential of the V2 electrode invariant at zero potential relative to the system ground.** Thus the preamplifier apparatus will actively maintain the "2" surface of the test membrane close to ground potential under all operating conditions. This is accomplished by incorporating an electronic potentiostat in the preamplifier box.

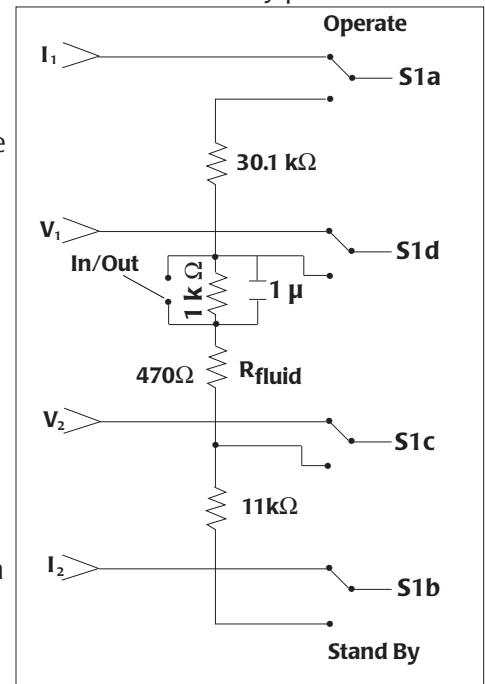


Fig. 2 – Resistance in Stand By Mode – 41.57 KΩ



### **Preamplifier Panel Controls and Connectors**

**Operate/Stand By:** In **Operate** mode, input electrode connectors  $I_1$ ,  $V_1$ ,  $I_2$ , and  $V_2$  are connected to the preamplifier. In **Stand By** mode, the preamplifier is disconnected from external electrodes and is connected instead to a resistor network (see Figure 2).

**Electrode Offset Adj:** Two variable controls, Coarse and Fine, with which to adjust any small voltage asymmetry between voltage electrodes  $V_1$  and  $V_2$  to zero. Once adjusted, the user should not move these controls during an experiment.

**Zero Check:** Amplifier input is grounded.

**Dummy Membrane:** In the Stand By operating mode when the toggle switch is toggled to "In", a resistor and shunt capacitor, simulating a membrane, is inserted between the inputs to the membrane voltage amplifier. "Out" removes this circuit.

Fig. 3



### **Clamp Modules**

Up to four clamp modules (Figure 3) can be housed in the main control housing which supplies power for all modules and preamplifiers.

#### **Clamp Module Front Panel**

**Digital Panel Meter:** The digital meter on each control section is a 3½-digit light emitting diode (LED) display. Using the Meter Select switch, membrane voltage in millivolts or trans-membrane current flow in microamperes will be displayed.

**FLUID RES COMPENSATION:** This ten-turn dial allows the user to dial a resistance value, empirically determined, that enables the voltage clamp to compensate the voltage error that is caused by clamp current flowing through the electrolyte path between the voltage electrodes and the membrane surface. The push button injects an arbitrary DC current through the chamber (in the absence of the membrane) to allow the user to determine fluid resistance.



**SET CLAMP I/V:** This ten turn dial allows the user to set: a) the clamp voltage or b) a constant current. Each full rotation of the dial is equivalent to 10 millivolts or 100  $\mu$ amperes of current. The **ZERO,+/-** toggle switch above the potentiometer selects the polarity of the clamping voltage (or current) . Mid position of the toggle switch results in zero volts or microamperes. Polarity Convention: Membrane Potential is defined as plus if  $V_1$  of the voltage clamp apparatus is positive relative to  $V_2$ . Current is defined as plus if + ion current flows from electrode  $I_1$  to  $I_2$ .

**PD, VC, CC:** Push buttons, adjacent to indicator lamps, initiate one of three different operating modes; PD (open circuit membrane potential difference), CC (constant current or "current clamp") or VC (voltage clamp).

**VOLTAGE RECORD:** An output connector for display of membrane voltage on a recorder, computer or oscilloscope.

**CURRENT RECORD:** An output connector for display of current magnitude and polarity ( 1 millivolt = 1 microampere).

**OVERLOAD:** Lamps  $I_1$  and  $I_2$ , when lit, indicate that a limit has been reached in the circuit serving the current electrodes. This may be due to excessive clamp current, a defective electrode or an open circuit in the current electrode pathway. An audible alarm sounds if either lamp is lit.

### **Clamp Module Rear Panel**

**POWER Switch:** When the Power Switch is turned on, the instrument automatically assumes the open circuit potential difference , **PD**, state as indicated by the green lamp next to the PD button.

**REMOTE LOGIC:** Positive 5 volts logic level applied to either **VC** or **CC**, **REMOTE LOGIC** input connectors, will assert the VC or CC operating mode respectively for **as long as the logic level** is sustained. When the logic level applied to VC or CC ceases, **PD operating mode will be automatically asserted.**

**EXT CLAMP COMMAND:** Externally applied voltages can be applied to either set transmembrane voltage in the VC (voltage clamp) mode or to set transmembrane current in the CC (current clamp) mode. The amplitude and polarity of the command determines the amplitude and polarity of the resulting membrane voltage or current.

**Panel selected SET CLAMP values (of current or voltage) are superimposed upon the externally applied command unless the front panel SET CLAMP I/V toggle switch is in the OFF position. See specifications for command factors converting applied voltage to clamp voltage or current.**



## **USING THE EVC-4000**

### **Dry Run**

An excellent way to become familiar with the operation of the EVC-4000 is to simulate voltage and current clamping. The electrical performance of the instrument can also be determined.

Connect the preamplifier cable to the receptacle of any available module on the front panel of the instrument. Place the Operate/Stand By switch in Stand By position and the Dummy In/Out toggle switch In. This causes dummy electrodes composed of resistors, as shown in Figure 2 to be inserted across the probe input connector array. Connect the instrument power cord to the power line and turn the instrument power on using the rear panel power switch. Two green lamps should light; one adjacent the PD push button and another on the preamplifier panel. Set the METER switch to mV. Push the ZERO CHECK button on the probe and note that the digital panel meter will display zero mV. Releasing the button allows the Coarse and Fine adjustment knobs to control the input offset voltage. Note that rotating these knobs will cover a range of approximately  $\pm 30$  mV allowing the user to adjust small voltage electrode asymmetries to zero. Adjust the offset voltage so that the meter reads 0.0 mV; do not adjust the offset voltage further.

### **Voltage Clamp**

Push the VC button. The red lamp next to the button should light, indicating that the instrument is in the voltage clamp operating mode. Keep the FLUID RES. COMPENSATION knob at zero. Place the polarity toggle switch in the + position and rotate the SET CLAMP I/V knob. Note that one full turn indicates 10.0 mV on the digital meter. Reversing the polarity toggle switch will yield -10.0 mV. Rotate the SET CLAMP I/V knob until a voltage of about 14.7 mV is reached. Switch METER SELECT to microamperes (mA), a clamping current of 10 (+/-1) microamperes will result. To test the alarm for maximum clamp current of 1 mA, place the Dummy In/Out toggle switch at Out. This essentially connects EVC4000 to a 470 Ohm resistor, which is used to simulate fluid resistance, as shown in Figure 2 on page 2. Rotate the FLUID RES. COMPENSATION dial knob for approximate four full turns until a reading of 4.0 is displayed on the dial. Now, turn the METER SELECT back to mV position and then rotate the SET CLAMP I/V knob to increase the set voltage. At about 70 mV of set voltage, at which a clamping current of 1 mA is required, alarm lamps and audible signals are activated.



## **Fluid Resistance Compensation**

The purpose of the procedure below, in a real experiment, is to prevent varying transmural current (during voltage clamp) from causing an error in the measured transmural voltage. It allows the user to experimentally determine the resistance of bathing fluid normally between the voltage electrodes and the surfaces of a membrane. The measurement, performed in the absence of a membrane, allows the experimenter to exactly compensate errors in voltage clamping caused by fluid resistance.

### **1. Using FLUID RES button:**

Push the PD button. The green lamp next to the button should light. Place the METER switch on mV. Switch Dummy Membrane to Out. Push the FLUID RES. switch. This causes the voltage reading to increment. Advance the FLUID RES COMPENSATION control so that the meter voltage reads 0.0 mV. The dial reading of the FLUID RES COMPENSATION knob assembly will then read the “fluid resistance” which exists between electrodes  $V_1$  and  $V_2$ . In this case, 470 Ohms  $\pm$  10 %. Once fluid resistance has been measured, the control is left at that value and the user may then be assured that little error will result as a consequence of current flow during a voltage clamp experiment.

### **2. Using Constant Current mode:**

The constant current, CC, mode enables the user to cause a known current to flow through the membrane sample (in this case the dummy resistor circuit). Polarity and magnitude are determined by the polarity toggle switch and the SET CLAMP I/V control respectively.

On the preamplifier, switch Dummy Membrane to Out. Push the CC button. The yellow lamp adjacent to the CC button should light. Place METER switch on  $\mu$ A. Set SET CLAMP toggle switch polarity switch to either + or - and dial SET CLAMP I/V control to approximately 50 microamperes as indicated on the digital panel meter. Switch METER to mV. Advance the FLUID RES COMPENSATION control so that the meter voltage reads 0.0 mV. The dial reading of the FLUID RES COMPENSATION knob assembly will then read the “fluid resistance” which exists between electrodes  $V_1$  and  $V_2$ . In this case, 470 Ohms  $\pm$  10 %. Once fluid resistance has been compensated, the user may then be assured that little error will result as a consequence of current flow. Notice that rotating the SET CLAMP I/V knob (i.e., varying the DC current) will not significantly vary “membrane volts” when the FLUID RES COMPENSATION has been optimally adjusted to exactly equal the “fluid “ resistance.



## **Operation**

***Maintain the EVC-4000 preamplifiers in Stand By when the instrument is not in use or while electrodes are being connected.***

### **Connecting the Chamber**

Assemble the membrane test chamber without a membrane. Two voltage electrodes\*, one on either side of where the membrane is normally located are inserted into the appropriate ports on the (Ussing) chamber. Two current electrodes\* are also connected at ports located at each distal end of their respective chambers. Note that "1" electrodes, **i.e.**,  $V_1$  and  $I_1$  are connected on the same chamber half; similarly for "2" chamber electrodes,  $V_2$  and  $I_2$ . Fill the chamber with electrolyte through the chamber circulation ports.

### **Compensate for Voltage Electrode Asymmetry**

In the PD operating mode, switch the Stand By/ Operate switch to Operate. Adjust the Electrode Offset Adj. controls on the preamplifier so as to reduce the asymmetry potential to 0.0 mV.

### **Compensate Fluid Resistance**

Depress the FLUID RES. test button and at the same time advance the FLUID RES. COMPENSATION knob so as to reduce the panel meter reading to 0 mV. Return Stand By/Operate switch to Stand By. Disassemble the chamber, install the tissue sample, reassemble and refill with electrolyte. Proceed with V/I clamp experiments.

### **Voltage Clamping**

Maintain the Preamplifier in Stand By mode while making front panel adjustments. It is always good procedure to first check the open circuit transmural potential difference (PD). If the reading polarity and DC amplitude is normal for the membrane sample, one can continue confidently to Voltage Clamp. If the green PD lamp is not lit, push the PD button. Switch from Stand By to Operate on the preamplifier. Return to Stand By after observing the potential (mV) on the panel meter. Use the SET CLAMP I/V controls to set the polarity and amplitude of the clamp voltage. For example, if a "short circuit" clamp is desired place the toggle switch to its center ZERO position. Push the VC button, red lamp will light. Voltage reading should be 0 for short circuit clamp. Switch meter from mV to  $\mu\text{A}$  and observe the membrane clamp current. Voltages proportional to clamp voltage and clamp current are continuously

*\*WPI models EKC and EKV, current and voltage electrodes.*



available at VOLTAGE RECORD AND CURRENT RECORD output connectors respectively.

**Alarm**

The maximum internal voltage available with which current can be forced to flow through the test chamber is  $\pm 32$  volts. If, during voltage clamping, the external current path is accidentally disconnected or if the resistance of this path is too high for the appropriate clamp current to flow, I<sub>1</sub> lamp will light and an audible alarm will sound. Alternatively, if the current exceeds the maximum current available (i.e., 1000  $\mu$ Amperes) I<sub>2</sub> lamp will light and the alarm will sound.

**Current Clamp**

In the Stand By mode, set the polarity toggle switch and value of current desired on the ten turn knob. Set the meter to display either current or membrane voltage as desired. Switch to Operate mode and observe and record results.

**Computer Interface Pinout for EVC-4000**

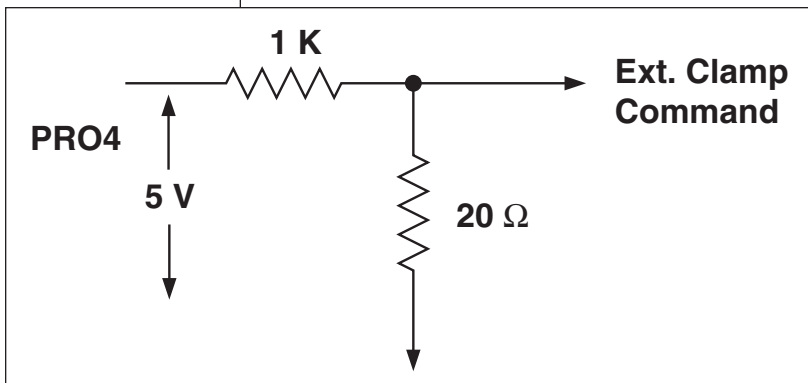
|                  | <b>CH1</b>                         | <b>CH2</b> | <b>CH3</b> | <b>CH4</b> |
|------------------|------------------------------------|------------|------------|------------|
| <b>Command</b>   | 30                                 | 18         | 14         | 2          |
| <b>V Monitor</b> | 31                                 | 19         | 15         | 3          |
| <b>I Monitor</b> | 32                                 | 20         | 16         | 4          |
| <b>V Clamp</b>   | 26                                 | 23         | 18         | 7          |
| <b>I Clamp</b>   | 27                                 | 24         | 11         | 8          |
| <b>GND</b>       | 1,5,6,9,12,13,17,21,22,25,28,29,33 |            |            |            |



## **APPLICATION EXAMPLES**

1. To deliver a 10 mV voltage clamp pulse (1 second) while keeping the preparation in open circuit mode before and after the 1 second command:
  - i. On the front panel of the EVC-4000, push the VC button and dial in 10 mV voltage on “SET CLAMP I/V”. Then push the PD button. Now the system is in open circuit mode while 10 mV command is waiting to be delivered.
  - ii. Use PRO4 to program a one-second 5V TTL pulse with appropriate delay and connect it to the “Voltage Clamp” BNC connector on the back of the EVC-4000. This TTL signal serves as a trigger and the EVC asserts the voltage clamp (VC) mode for as long as the duration of the TTL pulse. During the assertion of VC mode, the 10mV command is also asserted.
2. To deliver a 10 mV 1 second voltage clamp pulse and keep the preparation in voltage clamp of zero mV before and after the 10 mV pulse:
  - i. On the front panel of the EVC-4000, push the VC button and dial 0 mV voltage on “SET CLAMP I/V”. Leave the EVC in VC mode.
  - ii. Connect an external command of 100 mV pulse (1 second) with 0 mV before and after it to the “EXT CLAMP COMMAND” BNC connector on the back of the EVC. This external command is superimposed with front panel command (in this case 0 mV).

For this external command, if you do not have a stimulator with adjustable voltage output, then use PRO4 – (but its 5V output has to be modified (scaled down to 1/50th) with a voltage divider consisting of two resistors. The suggested values for the resistors are 1 K and 20 ohm.





## **EVC-4000 SPECIFICATIONS**

### **Preamplifier:**

|                                |                       |
|--------------------------------|-----------------------|
| Input Resistance               | 10 <sup>12</sup> Ohms |
| Input Leakage Current          | 100 pA, max.          |
| Absolute Maximum Input Voltage | + 15 volts            |

### **Voltage Clamp:**

|  |                 |
|--|-----------------|
| Panel setting range                      | + 200 mV        |
| Clamp V. per volts applied ext           | 100 mV per volt |
| Max. Voltage range of current electrodes | +32 Volts       |
| Max. Clamp current*                      | 1 milliampere   |
| Fluid Resistance Compensation            | 0 to 1000 Ohms  |

### **Current Clamp:**

|                               |                    |
|-------------------------------|--------------------|
| Maximum Current               | + 1 milliampere    |
| Current per Ext volts applied | 1 mA per millivolt |



## 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

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

**Warning: This equipment is not designed or intended for use on humans.**

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**DECLARATION OF CONFORMITY**

We: World Precision Instruments, Inc.  
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as the manufacturers of the apparatus listed, declare under sole responsibility that the product(s):

**Title: EVC4000-4**  
(also covering models EVC4000-1, EVC4000-2 and EVC4000-3; complete with Probe Preamplifier Type EVC3)

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

**Safety:** EN 61010-1:1993 (IEC 1010-1:1990)

**EMC:** EN 50081-1:1992  
EN 50082-1:1992

and therefore conform(s) with the protection requirements of Council Directive 89/336/EEC relating to electromagnetic compatibility and Council Directive 73/23/EEC relating to safety requirements.

**Issued on: 18<sup>th</sup> February 2000**

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