Warner Instruments
Intracellular Electrometer
Model IE-251A

Warner Instruments
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CE

Table of Contents

The **IE-251A** is a high impedance amplifier/electrometer designed specifically for intracellular studies in neurophysiology and related fields. The unique ‘active bridge’ circuitry of the **IE-251A** provides for simultaneous stimulation and recording using *a single microelectrode*.

Careful instrument design, coupled with quality component selection, particularly in the headstage, results in an excellent amplifier with low noise and wide bandwidth. The **IE-251A** will faithfully reproduce both the AC and DC components of the measured signal.

This instrument features:

- ✓ High input impedance
- ✓ A miniature active headstage
- ✓ Low noise and wide bandwidth
- ✓ Bridge balance circuitry
- ✓ Current injection circuitry
- ✓ DC position (zero offset) control
- ✓ Input capacitance neutralization
- ✓ Electrode resistance test
- ✓ Probe test circuitry
- ✓ Input and current overdrive indicators

NOMENCLATURE

Text conventions

This manual refers to amplifier controls at three functional levels; control blocks, specific controls within a block, and settings of specific controls. To minimize the potential for confusion, we have employed several text conventions which are specified below. Since our goal is to provide clarity rather than complexity, we welcome any feedback you may wish to provide.

- Warner Instrument product numbers are presented using **bold type**.
- References to instrument panel control blocks are specified using UNDERLINED SMALL CAPS. (e.g. METER, CLAMP COMMANDS)
- References to specific controls within a block are specified using NON-UNDERLINED SMALL CAPS. (e.g. MODE SWITCH, TIMER RANGE)
- References to individual control settings are specified in *italic type*. (e.g. *Amplify*, *100 mV*)
- Special comments and warnings are presented in highlighted text.

Any other formatting should be apparent from context.

THIS EQUIPMENT IS NOT DESIGNED NOR INTENDED FOR USE ON HUMAN SUBJECTS

CONTROL DESCRIPTION

Probe Test

The PROBE TEST section is where the probe connects to the **IE-251A.** This section also contains the controls for testing the performance of the instrument.

Probe

The PROBE is a small, lightweight, active headstage designed for placement at the measurement site.

An available electrode holder connects directly to the input male pin. The exposed inner shield is driven at the input potential and can be extended over the electrode holder to reduce the input capacitance.

Mounting of the PROBE in a micropositioner is easily managed with the PROBE MOUNTING HANDLE which can connect to the PROBE axially or at a 90° angle.

Probe Test Controls

The input gate leakage current for the probe, as well as a convenient check of the amplifier zero setting, can be determined using the PROBE TEST jack. The associated two position, TEST-MODE TOGGLE switch allows for selection of a *20 MΩ* resistor at the PROBE TEST PORT or shunts the PORT to ground (*off*) .

Amplifier

The AMPLIFIER section is comprised of the CAP COMP and INPUT OFFSET controls, an INPUT OVERDRIVE LED, a RANGE SELECTOR switch, the BUZZ circuit, an I.G. ADJUST trim pot, the ELECTRODE TEST circuit, and OUTPUT BNC's.

Capacity Compensation (CAP COMP)

The capacity compensation circuitry is used to adjust the 'speed' of the amplifier to aid in charging stray capacitances associated with the PROBE input. This single turn control can compensate input capacitances of up to 40 pF.

Input Offset

The INPUT OFFSET control is a ten turn potentiometer providing a DC adjustment to compensate for offset and junction potentials at the PROBE input. Offset settings of up to *±200 mV* are applied to the X1 OUTPUT, and up to *±2 V* to the X10 OUTPUT.

Input Overdrive LED

Illumination of the INPUT OVERDRIVE LED indicates that the input capabilities of the instrument have been exceeded. An input overdrive condition occurs when a voltage greater than ±1 V is applied to the PROBE input. This condition can be caused by a faulty or blocked electrode. The INPUT OVERDRIVE LED will light *red* under this condition.

Buzz

Efficient cell impalement can be achieved using the BUZZ circuitry which produces an (audible) electrical oscillation at the probe tip. Oscillation frequency and amplitude are screwdriver adjustable via associated FREQ and AMPLITUDE trim pots. The BUZZ circuit is continuously active so long as the BUZZ PUSH BUTTON is *depressed*.

Gate Leakage (I.G.) Adjust

This control is used during initial set-up to adjust the gate leakage from the headstage FET. Its usage is described elsewhere (*see* Instrument Checkout).

Electrode Test

The ELECTRODE TEST circuit allows the quality of the electrode and its resistance to be quickly checked prior to the beginning of an experiment, or anytime during the course of an experiment. While this measurement returns similar electrode resistance information to that acquired using the DC BALANCE circuit, it is not as accurate nor as sensitive.

When the ELECTRODE TEST button is *depressed*, a calibrated (1 nA) DC current is injected through the PROBE microelectrode to ground. This current results in a voltage of *1 mV/MΩ* at the X1 OUTPUT BNC, or *l0 mV/MΩ* at the X10 OUTPUT BNC, respectively, allowing the electrode's performance to be monitored.

Output BNC's

Two output BNC's for monitoring the amplifier voltage are provided. The X1 OUTPUT BNC passes an *unscaled* signal while the X10 OUTPUT BNC passes a *10x scaled* signal. Both voltage outputs can be used simultaneously if needed.

Current Injection

The CURRENT INJECTION section is comprised of a bridge-balancing circuit (DC BALANCE and TRANSIENT BALANCE controls), a CURRENT OVERDRIVE LED, an ELECTRODE RESISTANCE RANGE toggle, a STIMULUS INPUT BNC, and the BRIDGE OUTPUT and I MONITOR OUTPUT BNCs.

DC Balance and Transient Balance

The bridge circuit is provided to allow for subtraction of the voltage drop across the microelectrode resistance leaving only that of the cell. Adjustment of the DC BALANCE and TRANSIENT BALANCE controls allows compensation of both the DC and AC components of the microelectrode resistance.

When balanced, the ten turn dial of the DC BALANCE control allows a reading of the electrode resistance. This reading is calibrated to the ELECTRODE RESISTANCE RANGE toggle.

Current Overdrive LED

Illumination of the CURRENT OVERDRIVE LED indicates that the current capabilities of the instrument have been exceeded. A current overdrive condition is caused by applying a voltage in excess of ±50 V to the stimulus input BNC. The CURRENT OVERDRIVE LED will light *red* under this condition.

Electrode Resistance Range Control

The ELECTRODE RESISTANCE RANGE toggle is used to adjust the sensitivity of the amplifier to the microelectrode resistance. Available electrode resistance ranges are *0-100 MΩ* and *0-1000 MΩ*.

The RANGE toggle selection also determines the scaling of the electrode resistance as read from the DC BALANCE control. Selecting *0-100 MΩ* calibrates the DC BALANCE adjustment to *10 MΩ/turn* while selecting *0-1000 MΩ* calibrates the DC BALANCE adjustment to *100 MΩ/turn.*

Stimulus Input BNC

The STIMULUS INPUT BNC is used apply a stimulus signal to the PROBE electrode via an external waveform generator.

I monitor output BNC

The I MONITOR OUTPUT BNC reports the amplifier current. This output is scaled to 20 mV/nA of output current.

Bridge output BNC

The BRIDGE OUTPUT BNC reports the voltage appearing at the 10X OUTPUT (AMPLIFIER section) minus the voltage drop across the recording electrode as set by the DC BALANCE control (CURRENT INJECTION section). This output reports the true, corrected potential being applied to the cell.

SETUP AND CHECKOUT

Initial settings

1. (Placement) Position the unit in the desired location and plug it in.

   NOTE: Connection to the wrong line voltage could result in damage to the **IE-251A**. Check the serial number label on the rear of the unit to determine the factory settings for your instrument.

2. (PROBE TEST SECTION) Plug the PROBE into its connector. Switch the TEST-MODE TOGGLE to the *off* position. Insert the PROBE tip into the PROBE TEST port.

   NOTE: The probe and amplifier are calibrated as a unit. Swapping of probes between units is not possible unless a recalibration is performed. A recalibration must also be performed when replacing a failed probe. (*See* Appendix: Probe Recalibration)

3. (AMPLIFIER SECTION) Set the CAP COMP control to its *minimum adjustment*.

4. (CURRENT INJECTION SECTION) Adjust DC BALANCE to *0*. Set the RANGE control to *0-100 MΩ*.

5. Turn the instrument *on*.

Instrument checkout

6. Zero the **IE-251A**. Connect an oscilloscope to the X10 OUTPUT BNC. Verify that the TEST-MODE TOGGLE switch is set to *off*. Plug the PROBE into the PROBE TEST port. Turn the CAP COMP control *fully counter-clockwise*. Adjust the INPUT OFFSET control for *zero output* on the oscilloscope.

7. Check the probe input leakage adjustment. Select a *high-gain sensitivity* on the oscilloscope. Set the TEST-MODE TOGGLE switch to *20 MΩ*. Using a small screwdriver, adjust the I.G. ADJ control to *zero output* to eliminate any change when switching the TEST-MODE TOGGLE back and forth between *off* and *20 MΩ*.

   NOTE: Leakage current is affected by changes in temperature. This test is best performed once the instrument has thermally stabilized, usually after a warm-up period of 1 to 2 hours.

8. Check the amplifier output. Connect the X1 OUTPUT BNC to the oscilloscope. Place the TEST-MODE TOGGLE switch to the *20 MΩ* position. Depress the ELECTRODE TEST button. A *20 mV* DC shift (*200 mV* if using the X10 OUTPUT BNC) indicates proper amplifier function.

OPERATION

Electrode Assembly

Electrodes are pulled using a standard puller and filled (usually with the 3 M KCl), taking care to avoid bubbles or air spaces, following commonly published procedures. The electrode shank is then carefully and firmly pushed into the holder through the bored hole and gasket. Finger tighten the threaded end-piece of the holder to obtain a secure seal. Remove any excess fluid from the outside of the holder with a tissue. Connect the electrode/electrode holder assembly to the **IE-251A** headstage. The unit is ready for use.

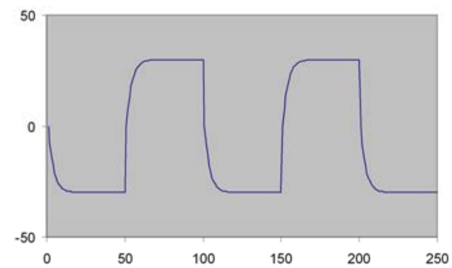
Electrode Test

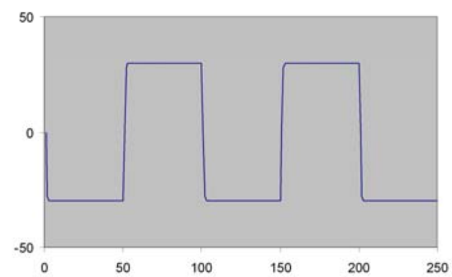
With the PROBE mounted in the micropositioner, the quality and resistance of the electrode can be quickly checked. Connect either the X1 OUTPUT or X10 OUTPUT to an oscilloscope. Place the electrode into the bath and depress the ELECTRODE TEST push button.

The presence of a faulty or blocked electrode which will cause the INPUT OVERDRIVE LED to light and a large voltage (several V) will appear at the X1 or X10 OUTPUT. Alternatively, a working electrode will present a positive DC shift of *1 mV/MΩ* at the X1 OUTPUT (or *10 mV/MΩ* at the x10 OUTPUT). This DC shift allows a rough calculation of the electrode resistance to be made.

Capacitance compensation

Large capacitances connected to the microelectrode can significantly slow the amplifier response to fast potential changes. Proper adjustment of the CAP COMP circuit can optimize the response of the amplifier to experimental conditions.



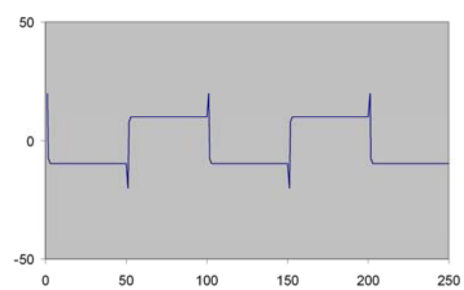


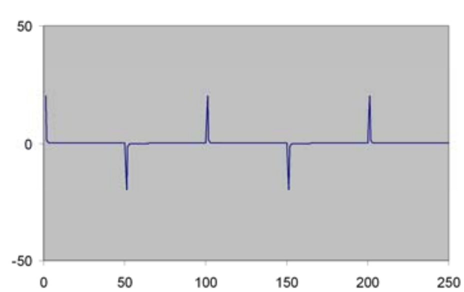
Begin by connecting a waveform generator to the STIMULUS INPUT. Set the generator to produce a square wave. Set the frequency to 100 Hz and adjust the peak-to-peak amplitude to some nominal level (say, 0.5 V of either polarity).

Connect the X1 OUTPUT BNC to an oscilloscope and immerse the electrode immersed in the bath. Set the CAP COMP control to its *minimum* (counter-clockwise) position. You should see an uncompensated waveform on the oscilloscope similar to that shown on the upper image. Adjust the CAP COMP control clockwise until the waveform on the oscilloscope is maximally compensated (see lower image). Over-compensation will induce instrument oscillation with loss of waveform and lighting of the INPUT OVERDRIVE LED.

Bridge balancing

Connect the oscilloscope to the BRIDGE OUTPUT BNC. Set the DC BALANCE to *0*. With an electrode immersed in the bath, select the appropriate RANGE setting as determined above. The initial mixed-mode signal (AC + DC components) should look like the lower image shown above.





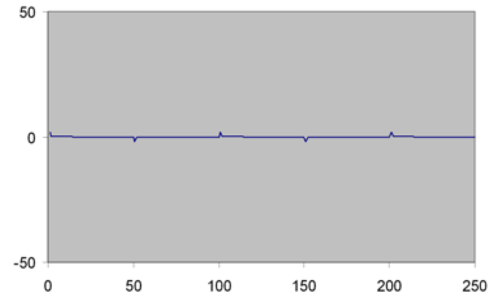
DC Balance

The DC BALANCE control is used to compensate for DC offsets. Adjust the DC BALANCE control until the square wave amplitude is nullified. A properly adjusted DC BALANCE appears as a straight line containing only the AC (spike) component. The two images to the right show the DC balance both in mid-adjustment and fully adjusted.

Once the DC balance is fully adjusted, the DC BALANCE dial provides a direct reading of the electrode resistance scaled to the RANGE setting.

Transient balance

The TRANSIENT BALANCE control is used to compensate for AC offsets. Adjust the transient balance until the oscilloscope trace appears as a straight line with only a small portion of the AC transient in evidence. (See image to the right.)



Current Injection

A constant current (independent of electrode resistance) is generated by the **IE-251A** current injection circuitry by applying a voltage waveform (square wave, ramp, DC, etc) to the STIMULUS INPUT BNC. An replica (attenuated 20%) of the voltage waveform appears at the I MONITOR OUTPUT as a measurement of the injected current. This output is scaled at 20 mV/nA.

The maximum voltage that can safely be applied to the STIMULUS INPUT is ±50 V, which appears as ±10 V at the I MONITOR OUTPUT BNC. Exceeding this limitation will result in an overdrive condition, nonlinear performance of the current injection circuit, and potential damage to the instrument.

NOTES: Current injection is a widely used and valuable technique but is seriously limited by two difficulties: (1) Fluid filled microelectrodes present inherently non-linear resistances as a function of the injection current. The overall resistance of the electrode should therefore be made as low as possible consistent with the experimental application. (2) The maximum current that can be injected through the microelectrode is limited by the maximum allowable voltage at the probe input (I=V/R), which is ±1 volt. For example with a 20 MΩ electrode, the maximum current will be ±1V/20 MΩ = ±50 nA

Cell Penetration

With the circuitry in balance, cell impalement and current injection may proceed. Cell penetration is observed as a sudden negative DC shift in the output voltage. The BUZZ feature produces an oscillation at the electrode tip and has been found to be an aid in penetrating difficult cells.

APPENDIX

Specifications

Amplifier	Input Impedance	> 10^{11} Ω shunted by 0.5 pF
	Noise (0.1 Hz to 10 kHz)	25 μV p-p, input shorted 250 μV p-p, 20 MΩ at input
	Output Resistance	100 Ω
	Gain	x1, x10
	Rise Time (10 to 90%)	10 μs, measured with 20 mΩ resistor
	Capacity Compensation	0 to 50 pF
	Probe Input Voltage Range	±1 V
	DC Positioning Range	±200 mV, referred to input
	Leakage Current	Adjustable to zero
	Electrode Test	
Buzz	Amplitude variable	0 to 15 V
	Frequency variable	100 Hz to 10 kHz
Current Injection	DC Balance Ranges	0-100 MΩ and 0-1000 MΩ
	Current Injection Limits	0.5 μA or ±1 V divided by electrode resistance, whichever occurs first
	Stimulus Input Resistance	3.3 kΩ
	Maximum Stimulus Input Voltage	±50 V
	DC Balance Output Resistance	100 Ω
	I Monitor Output Resistance	1 kΩ
	I Monitor Output Scale Factor	1 V = 50 nA
Physical Dimensions	Case: 12.5 x 20.3 x 25.4 cm (H x W x D) Probe: 9.5 x 65 mm (D x L) with 1.8 m cable	
Power	100-130 or 220-240 V, 50/60 Hz, 10 VA	
Probe Handle	6.5 x 65 mm (D x L)	
Shipping Weight	4.5 kg	
Warranty	Two years, parts and labor	
Operating Conditions	Equipment is intended to be operated in a controlled laboratory environment. Temperature: 0-40 °C, Altitude: sea level to 2000 m, Relative humidity: 0-95%	

Maintenance

Probe

The probe is nickel plated and epoxy sealed to prevent corrosion from saline solutions and mild chemicals. However, it is good practice to keep the surfaces dry, both when in use and during storage. Use only water or alcohol to clean the probe. Avoid solvents which can attack the epoxy. Store in a dry environment when not in use.

Probe Cable

The probe cable is of small diameter and very flexible. To avoid damage to the cable jacket and internal wires, take care to route the cable in a way to avoid kinks, abrasions and strain on either the connector or probe ends.

Electrode Holders

Correct storage of electrode holders will prolong their useful life. After each use, the holder should be rinsed by injecting it with distilled water. Dry the inside by injecting air with a clean syringe. Wipe the external surface dry and store in a dry place. Do not use alcohol or solvents on the electrode holders as this will seriously degrade the polycarbonate body..

Theoretical considerations

Electrode Connection

Fluid filled glass microelectrodes have very high resistances, typically 20 MΩ or more. The input resistance of the recording amplifier must be greater than this by 100 times or more to faithfully record the measured potentials. The input resistance of the **IE-251** is on the order of 10^{11} Ω which more than satisfies this requirement.

Almost of equal importance in electrode connections is that of minimizing input capacitance. Input capacitance has the effect of reducing the amplifier bandwidth (slower rise-time) which can be compensated using the CAP COMP control. However, the use of capacitance compensation results in an increased noise level. Therefore, when designing an experiment, efforts to minimize input capacitance can be rewarding.

Minimizing Input Capacitance

The following considerations will aid in reducing input capacitance:

1. Use an electrode holder. Electrode holders available for use with the **IE-251A** have a 2 mm diameter female jack which plugs directly onto the PROBE male pin. This direct connection eliminates the need for cabling which often increases input capacitance.

2. The probe is constructed of two concentric tubes with the outer shell connected to circuit ground. The inner shield (approximately 5 mm, exposed at the input end) is driven at the input potential and is exposed for the purpose of extending the driven guard over the electrode holder. Use of a compression spring with an ID slightly smaller than the inner shield diameter works well.

   CAUTION: Care must be exercised to insure that the extension does not contact either the probe outer shield or the bath.

Electrode Holders

Two electrode holder styles are available for the **IE-251A**; the straight **EH-25** and a right angle **EH-2R**. Both styles incorporate a Ag/AgCl pellet or optional Ag^{2+} wire at the interface between the fluid filled glass micropipette and the probe input.

Ag/AgCl half cells typically measure only a fraction of a mV potential difference between pairs in a Cl^- containing medium. We strongly recommended using a second half cell, such as Warner's **REF-11**, for connection from the bath to the **IE-251A** circuit ground.

Electrode part numbers		
Catalog Number	**Model Number**	**Description**
64-1301	REF-11	Reference cell
64-0964	ESP/W–F10N	Straight Holder with 2 mm Jack for 1.0 mm Capillary
640965	ESP/W–F12N	Straight Holder with 2 mm Jack for 1.2 mm Capillary
64-0966	ESP/W–F15N	Straight Holder with 2 mm Jack for 1.5 mm Capillary
64-0967	ESP/W–F20N	Straight Holder with 2 mm Jack for 2.0 mm Capillary

Service and Warranty

We recommend all questions on service be referred to our Engineering department. Normal business hours are 9:00 A.M. to 5:00 P.M.. Monday to Friday. Telephone (203) 776-0664 or fax (203) 776-1278

Service Notes

1. If the instrument POWER light fails to illuminate, check the fuse at the rear panel. If needed replace with a 3AG 1/2 A normal blow. If the replacement also fails, please call for help.
2. Occasionally, a knob on the front panel can loosen. These are 'collet' style knobs and are tightened with a screw located under the knob cap. To gain access to the screw, pry the cap off using a thin bladed screwdriver or similar tool.

Warranty

The **IE-251A** is covered by our warranty to be free from defects in materials and workmanship for a period of two years from the date of shipment.

If a failure occurs within this period, Warner Instruments will either repair or replace the faulty component(s). This warranty does not cover failure or damage caused by physical abuse or by electrical stress (e.g., inputs exceeding specified limits).

Shipping charges to the factory are the customer's responsibility. Return charges will be paid by Warner Instruments. This warranty does not extend to electrode holders.

NOTE: Contact Warner Instruments for Return Merchandise Authorization (RMA) prior to returning equipment to the factory.

Certifications

Declaration of Conformity

CE MARKING (EMC)

Application of Council Directive: 89/336/EEC

Standards To Which Conformity Is Declared:	EN55022 Class A EN61000-3-2 EN61000-3-3 EN50082-1:1992 EN61000-4-2 EN61000-4-3 ENV50204 EN610000-4-4 EN610000-4-8 EN610000-4-11
Manufacturer's Name:	Warner Instruments, LLC
Manufacturer's Address:	1125 Dixwell Avenue Hamden, CT 06514 Tel: (203) 776-0664
Equipment Description:	Instrument Amplifier
Equipment Class:	ITE-Class A
Model Numbers:	IE-251A

I the undersigned, hereby declare that the equipment specified above, conforms to the above Directive(s) and Standard(s).

Place: Hamden, Connecticut USA

Signature:

Full Name: Burton J. Warner

Position: President

Declaration of Conformity

CE MARKING (LVD)

Application of Council Directive: 73/23/EEC

Standards To Which Conformity Is Declared:	EN61010-1:1993
Manufacturer's Name:	Warner Instruments, LLC
Manufacturer's Address:	1125 Dixwell Avenue Hamden, CT 06514 Tel: (203) 776-0664
Equipment Description:	Instrument Amplifier Safety requirements for electrical equipment for measurement and laboratory use
Equipment Class:	Class I
Model Numbers:	IE-251A

I the undersigned, hereby declare that the equipment specified above, conforms to the above Directive(s) and Standard(s).

Place: Hamden, Connecticut USA

Signature:

Full Name: Burton J. Warner

Position: President

WEEE/RoHS Compliance Statement

EU Directives WEEE and RoHS

To Our Valued Customers:

Harvard Apparatus is committed to being a good corporate citizen. As part of that commitment, we strive to maintain an environmentally conscious manufacturing operation. The European Union (EU) has enacted two Directives, the first on product recycling (Waste Electrical and Electronic Equipment, WEEE) and the second limiting the use of certain substances (Restriction on the use of Hazardous Substances, RoHS). Over time, these Directives will be implemented in the national laws of each EU Member State.

Once the final national regulations have been put into place, recycling will be offered for those Harvard Apparatus products which are within the scope of the WEEE Directive. Products falling under the scope of the WEEE Directive available for sale after August 13, 2005 will be identified with a "wheelie bin" symbol.

Two Categories of products covered by the WEEE Directive are currently exempt from the RoHS Directive – Category 8, medical devices (with the exception of implanted or infected products) and Category 9, monitoring and control instruments. Most of Harvard Apparatus' products fall into either Category 8 or 9 and are currently exempt from the RoHS Directive. Harvard Apparatus will continue to monitor the application of the RoHS Directive to its products and will comply with any changes as they apply.

- **Do Not Dispose Product with Municipal Waste.**
- **Special Collection/Disposal Required.**