

Oocyte Clamp Amplifier User's Manual



Warner Instruments OC-725D

WARNER
INSTRUMENTS

a division of **Harvard Bioscience, Inc.**

Table of Contents

| SUBJECT | PAGE # |
|---------------------------------|---------------|
| Warranty | 4 |
| Safety | 5 |
| Introduction | 6–7 |
| Unique Features | 6 |
| High Voltage Compliance | 6 |
| Bath Clamp Headstage | 6 |
| Voltage Headstage Probe | 6 |
| Voltage and Current Meters | 6 |
| Additional Features | 7 |
| Buzz controls | 7 |
| Electrode Test | 7 |
| Overload Alarm | 7 |
| DC Offsets | 7 |
| Nomenclature | 8 |
| Text conventions | 8 |
| Control Description | 9–15 |
| Front panel | 9 |
| Voltage electrode | 9 |
| Bath electrodes | 10 |
| Clamp section | 11 |
| Commands | 12 |
| Current electrode | 12 |
| Rear panel | 13 |
| Additional components | 13 |
| Voltage recording headstages | 13 |
| Bath headstage | 14 |
| Current electrode cable | 14 |
| Model cell | 14 |
| Comments | 14 |
| Connecting to line power | 14 |
| High voltage outputs | 15 |
| Using the Model Membrane | 16–19 |

Table of Contents

| SUBJECT | PAGE # |
|---|---------------|
| Initial instrument settings | 16 |
| Test procedures | 17 |
| Offset controls | 17 |
| Voltage electrode test | 17 |
| Buzz | 18 |
| Current electrode test | 18 |
| DC clamp test | 18 |
| AC clamp test | 19 |
| Setup | 20–26 |
| Pipettes | 20 |
| Electrode holders | 21 |
| Bath probe | 21 |
| Electrode placement and grounding | 21 |
| Bath clamp electrode placement | 22 |
| Cable connections | 23 |
| Resting position of the controls | 24 |
| Using the gain select | 24 |
| Other gain range selection considerations | 25 |
| A Procedure for Recording from Oocytes | 26–29 |
| Initial electrode placement | 26 |
| Voltage electrode placement | 26 |
| Current electrode placement | 27 |
| Impaling the cell | 27 |
| Clamping the cell | 28 |
| Clamping high conductance cells | 28 |
| Unclamping the cell | 29 |
| Removing the electrodes | 29 |
| Comments and Recommendations | 30–31 |
| Membrane damage | 30 |
| Repeated recordings | 30 |
| Electrophysiology | 30 |

Table of Contents

| SUBJECT | PAGE # |
|--|--------|
| Appendix | 32–35 |
| Specifications — Equipment is intended to be operated in a controlled laboratory environment | 32 |
| Gain telegraph outputs | 34 |
| Gain select settings | 34 |
| Noise from bath clamp feedback resistor | 34 |
| References | 35 |

Warranty & Repair Information

Serial Numbers

All inquiries concerning our product should refer to the serial number of the unit. Serial numbers are located on the rear of the chassis.

Calibrations

All electrical apparatus is calibrated at rated voltage and frequency.

Warranty

Warner Instruments warrants this instrument for a period of one year from date of purchase. At its option, Warner Instruments will repair or replace the unit if it is found to be defective as to workmanship or material.

This warranty does not extend to damage resulting from misuse, neglect or abuse, normal wear and tear, or accident.

This warranty extends only to the original customer purchaser.

IN NO EVENT SHALL WARNER INSTRUMENTS BE LIABLE FOR INCIDENTAL OR CONSEQUENTIAL DAMAGES. Some states do not allow exclusion or limitation of incidental or consequential damages so the above limitation or exclusion may not apply to you. THERE ARE NO IMPLIED WARRANTIES OF MERCHANTABILITY, OR FITNESS FOR A PARTICULAR USE, OR OF ANY OTHER NATURE. Some states do not allow this limitation on an implied warranty, so the above limitation may not apply to you.

If a defect arises within the one-year warranty period, promptly contact Warner Instruments, Inc. 1125 Dixwell Ave, Hamden, Connecticut 06514-5014 using our toll free number 1-800-272-2775. Goods will not be accepted for return unless an RMA (returned materials authorization) number has been issued by our customer service department. The customer is responsible for shipping charges. Please allow a reasonable period of time for completion of repairs, replacement and return. If the unit is replaced, the replacement unit is covered only for the remainder of the original warranty period dating from the purchase of the original device.

This warranty gives you specific rights, and you may also have other rights which vary from state to state.

Repair Facilities and Parts

Warner Instruments stocks replacement and repair parts. When ordering, please describe parts as completely as possible, preferably using our part numbers. If practical, enclose a sample or drawing. We offer a complete reconditioning service.



CAUTION

This instrument is not registered with the FDA and is not for clinical use on human patients.

Safety Information

Please read the following safety precautions to ensure proper use of your valve controller. If the equipment is used in a manner not specified, the protection provided by the equipment may be impaired.

To Prevent Hazard or Injury

Use Proper Line Cord

Use only a line cord that is certified for country of use. The operating voltage range is 110-130 VAC~, or 200-250 VAC~, 14VA.

Ground the Product

This product is grounded through the grounding conductor of the line cord. To avoid electric shock, the grounding conductor must be connected to earth ground. Before making any connections to the input or output terminals of the product, ensure that the product is properly grounded.

Make Proper Connections

Make sure all connections are made properly and securely. Any signal wire connections to the unit must be no longer than 3 meters.

Observe All Terminal Ratings

Review the operating manual to learn the ratings on all connections.

Use Proper Fuse

Use only specified fuses with product.

Avoid Exposed Circuitry

Do not touch any electronic circuitry inside of the product.

Do Not Operate with Suspected Failures

If damage is suspected on or to the product do not operate the product. Contact qualified service personnel to perform inspection.

Orient the Equipment Properly

Do not orient the equipment so that it is difficult to manage the connection and disconnection of devices.

Place Product in Proper Environment

Review the operating manual for guidelines for proper operating environments.

Observe all Warning Labels on Product

Read all labels on product to ensure proper usage.



CAUTION
Refer to Manual



Protective
Ground Terminal



CAUTION: FOR RESEARCH USE ONLY.
NOT FOR CLINICAL USE ON PATIENTS.

Introduction

The model OC-725D Oocyte clamp is designed for two-electrode, whole-cell voltage clamping of *Xenopus* oocytes, as well as for other large cells and cell structures such as squid axons. The instrument has several features making it ideal for these purposes.

Unique Features

High Voltage Compliance: The OC-725D combines high AC and DC gains and a voltage compliance of ± 180 V to insure fast, nonsaturating clamp performance under nearly any condition. The AC clamp gain is variable up to 2000. An additional DC gain of 10^6 may be employed for high conductance cells or leaky oocytes.

Two clamp speeds are available. The Slow mode is used for screening oocytes or for applications not requiring rapid response times. The Fast mode is used for accurate voltage clamping of fast whole cell currents. Response time in the Fast mode is 550 μ s (10-90% rise time) when applying a 100 mV step to a model cell with a 1M ohm in parallel with 500nf and 1M ohm current and voltage electrodes.

Bath Clamp Headstage: The current measuring range of the OC-725D bath clamp headstage is extended at both ends by a 3-position range multiplier. This allows smaller currents to be amplified to usable levels while larger currents (up to 1 mA) can be recorded without saturation.

The unique design of the bath clamp eliminates the need for series resistance compensation. It provides an accurate measurement of bath current by creating a virtual ground in the bath while simultaneously clamping the bath potential at zero.

Voltage Headstage Probe: The voltage measuring headstage is a single-ended, high-impedance probe. Its small size, convenient mounting rod, and two meter cable make for easy attachment to a micropositioner.

Voltage and Current Meters: Independent meters provide simultaneous displays of membrane voltage V_m and membrane current I_m . To assure proper impalement of the current electrode, the current meter displays membrane potential, V_e , from the current electrode before the clamp circuit is activated.

Introduction

Additional Features

Buzz controls for each electrode aid in penetration of cell membranes with a minimum of leakage.

Electrode Test for voltage and current electrodes.

Overload Alarm serves as a reminder when the feedback amplifier reaches its maximum output voltage, a condition which could result in damage to the oocyte.

DC Offsets for voltage and current electrodes.

Nomenclature

Text Conventions

This manual refers to amplifier controls at four functional levels; operational sections, 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 a **bold type**.
- References to instrument panel control blocks are specified using UNDERLINED SMALL CAPS. (e.g., COMMANDS, CLAMP)
- References to specific controls within a block are specified using NON-UNDERLINED SMALL CAPS. (e.g., MODE SWITCH, DC GAIN)
- Finally, references to individual control settings are specified in *italic type*. (e.g., *slow, fast, 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

The OC-725D is comprised of three functional channels: A high impedance voltage sensing channel with input offset to measure membrane potential, a current sensing channel with bath clamp to clamp the bath and measure the membrane current, and a high voltage amplifier to deliver the clamping current.

The complete voltage clamp system consists of the OC-725D, the voltage recording probe with electrode holder, the current sensing bath probe with silver wire electrodes, and the current cable with electrode holder.

Front Panel

The instrument front panel is divided into five control blocks titled VOLTAGE ELECTRODE [V_m], BATH ELECTRODES [I_m], CLAMP COMMANDS, and CURRENT ELECTRODE. The instrument rear panel has BNC connectors for GAIN TELEGRAPH OUTPUT and $V_e \times 10$ output, an ALARM *on/off* switch, and binding posts for CIRCUIT and CHASSIS GROUND.

Voltage Electrode

The VOLTAGE ELECTRODE control block contains the VOLTAGE PROBE CONNECTOR, the VOLTAGE METER, the V_m OFFSET control, the ELECTRODE TEST and BUZZ pushbuttons, and the V_m OUTPUT BNC.

The VOLTAGE METER reports the membrane voltage (V_m) with a full scale range of ± 199.9 mV.

The VOLTAGE PROBE CONNECTOR is a 7-pin DIN connector for attachment of the voltage probe to the instrument.

The V_m OFFSET control is a 10-turn potentiometer providing up to ± 200 mV at the VOLTAGE PROBE input for offset of membrane junction potentials.

The V_m ELECTRODE TEST control is used to determine the internal resistance of the voltage probe. When the pushbutton is depressed, a constant 10 nA current is passed through the voltage electrode producing a voltage drop of 10 mV/M Ω of probe resistance. The measured potential is displayed by the meter or reported at the $V_m \times 10$ output BNC (at 100 mV/M Ω).

The V_m BUZZ push button facilitates penetration of the voltage electrode by producing a 1 kHz square wave at the pipette tip.

The $V_m \times 10$ OUTPUT BNC reports the membrane voltage in mV multiplied by 10.



Control Description

Bath Electrodes

The **BATH ELECTRODES** control block contains the **BATH PROBE** connector, the **CURRENT METER**, the **GAIN SELECT** and **FILTER SELECT** switches, and the **I MONITOR** output BNC's.

The **CURRENT METER** reads the voltage (V_e) of the current electrode when the **CLAMP MODE SELECTOR** switch is *off* (see **CLAMP** section). A lit LED indicates voltage readings in mV.

When in clamp mode (**CLAMP MODE SELECTOR** switch set to *slow* or *fast*), the **CURRENT METER** displays the current (I_m) sensed by the bath electrode. A lit LED display on side of the meter indicates current readings in μA .

Instrument gain is set by the two **GAIN SELECT** controls. Gain is selected by a 8-position **GAIN SELECTOR** switch, which ranges from *0.1* to *20* in 1-2-5 steps, and a 3-position toggle switch which selects the gain multiplier (*x0.1*, *x1.0* and *x10*). LED's indicate the gain multiplier selection. The combination of these controls allows gain to be set from *0.01* to *200*.

Instrument bandwidth is set by the **FILTER** switch. This filter is a 4-pole Bessel low-pass filter that sets the upper frequency from 50Hz to 10KHz, or full bandwidth (**BYPASS**).



Control Description

Current output is available from the I MONITOR BNC at the bandwidth set by the FILTER switch.

Clamp Section

The CLAMP control block contains the MODE SELECTOR switch as well as the GAIN and DC GAIN controls.

The CLAMP MODE SELECTOR switch selects for slow and fast clamp speeds, or for off. These choices are described below.

off — In the off position, the clamp amplifier is disconnected from the current electrode. The voltage difference between the current electrode and the bath electrode [V_e , mV] is read on the METER in the BATH ELECTRODES control block. This information is also available at the $V_e \times 10$ output BNC on the front or rear panel of the instrument.



NOTE: All CLAMP controls are disabled by turning the CLAMP MODE SELECTOR switch to the off position. Be sure to switch this control to the off position before handling electrodes!

SLOW — The slow clamp mode is useful for screening of oocytes or where high clamp speeds are not required. The slow clamp speed is approximately 0.5 ms when measured with the model membrane (1 M Ω shunted with 0.47 μ F). In this mode, measured currents are displayed on the BATH ELECTRODES METER in units of μ A.

FAST — Most oocyte clamping is performed in the fast mode. The clamp speed is limited by the resistance of the current electrode and the oocyte membrane capacitance. Therefore, the current electrode resistance must be kept as low as possible to obtain the fastest clamp speeds. Currents are read on the BATH ELECTRODES METER in units of μ A. Fast clamp speeds are \sim 550 μ s when measured with the model cell as described above.

The GAIN control is a single turn potentiometer which varies the full-bandwidth open-loop gain from 0 to 2000. A high DC GAIN (106) can be switched in with the DC GAIN toggle switch to provide a hard clamp when passing large currents from high expression oocytes.

Control Description

Commands

The COMMANDS control block contains the HOLD controls and COMMAND IN $\div 10$ input BNC.

HOLD controls - HOLD potential is set with a TEN TURN POTENTIOMETER and RANGE toggle switch. Ranges are 0- \pm 100 and 0- \pm 200mV depending on the scale multiplier selected (*x10 or x20*). Signal polarity, or OFF is selected with the associated toggle switch. A GREEN LED will light whenever the INTERNAL HOLD COMMAND is on.

COMMAND IN $\div 10$ input BNC- Command signals from an external generator or computer connected to this input are attenuated by 10. Maximum input is ± 10 V.



Current Electrode

The CURRENT ELECTRODE block includes the V_e OFFSET, ELECTRODE TEST, and V_e BUZZ controls. This section also contains the OVER VOLTAGE indicator and a DIN connector for the current electrode.

V_e OFFSET — With a range of ± 200 mV (center zero), this control is used to adjust the offset voltage of the current electrode. Use this control to establish a zero reference before impaling the oocyte. Once the oocyte has been pierced, the resting potential can be read from V_e x10 output BNC or on the CURRENT METER (BATH ELECTRODES section).

ELECTRODE TEST — A voltage proportional to the resistance of the current electrode [$10 \text{ mV}/\text{M}\Omega$] will be displayed on the meter by depressing the ELECTRODE TEST push button when the CLAMP MODE SELECTOR switch is in the *off* position.

V_e BUZZ — The V_m BUZZ pushbutton facilitates penetration of the voltage electrode by producing a 1 kHz square wave at the pipette tip.

CLIPPING LEDs — If the voltage at the current electrode exceeds ± 160 V, the OVER VOLTAGE LEDs will light. An alarm will also sound when the rear panel ALARM switch is in the *on* position.

The V_e x10 output BNC monitors the voltage of the current electrode (x10) when the CLAMP MODE SELECTOR switch is *off*. A V_e x10 output is also available at the rear panel.



Control Description

Rear Panel

The line power connector and fuse are located on the rear panel. Operating voltage is specified on the MODEL/SERIAL NUMBER sticker applied to the rear of the instrument. The rear panel also contains $V_e \times 10$, GAIN and FILTER TELEGRAPH output BNC's, the ALARM switch and instrument GROUND'S..

NOTE: Verify that the instrument is wired for the proper voltage before connecting the line cord.

The $V_e \times 10$ output BNC monitors the voltage of the current electrode ($\times 10$) when the CLAMP MODE SELECTOR switch is off

The GAIN TELEGRAPH output BNC provides a DC voltage indicating the gain setting of the instrument. The output varies from 0.2 to 2.8 volts in 200 mV steps as shown in the appendix.

FILTER TELEGRAPH output 0.2 to 1.8 VDC in 0.2 V steps.

ALARM switch — Activates or deactivates the OVER VOLTAGE current electrode audible alarm.

GROUND'S — Both CIRCUIT and CHASSIS grounding posts are located on the rear panel. CHASSIS is common with the instrument enclosure and connected to earth through the power line cord. A shorting link allows for interconnection of the two grounds. In most experimental setups, separating the grounds will result in minimizing 50/60 Hz signal interference from ground loops. However, trial and error will determine the best results.

NOTE: For safe operation, the ground pin on the power plug must not be removed and the use of "cheater" plugs must be avoided.

Additional Components

Voltage Recording Headstages

7250V PROBE

The voltage probe is an active headstage housed in a 1.25 x 5 cm cylinder (dia. x length).

The probe body is nickel plated and epoxy sealed for corrosion resistance.



NOTE: The outer shell is electrically driven at the input potential.

The microelectrode holder mates directly to the 2 mm input pin on the probe body. A mounting block and handle are also supplied and facilitate attachment of the probe to a micromanipulator. The handle can be mounted either axially or perpendicular to the probe body.

Control Description

Bath Headstage

The BATH PROBE is housed in a 2.8 x 3.5 x 4.2 cm aluminum enclosure. Inputs are two 1 mm pin jacks labeled I SENSE and I OUT. The case is electrically grounded and a pin jack is located on the side for connecting to shields.

A plastic plate with two screw mounting slots is attached to the probe base. The BATH PROBE connects to the control unit with a 6 pin connector



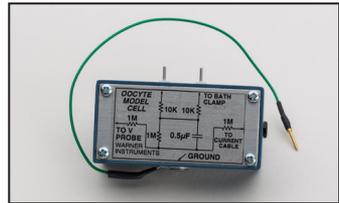
Current Electrode Cable

A two meter shielded cable is supplied with a 2 mm pin jack on one end to mate with the electrode holder and a 3-pin connector on the other end to mate with the instrument. The electrode holder has a handle for mounting in a micromanipulator.



Model Cell

The model cell supplied with the OC-725D is useful as a training aid and as a calibration and test device. It has connections for the voltage and current probes and to the bath clamp allowing all aspects of the amplifier's function to be tested.



Comments

Connecting to Line Power

The model OC-725D is supplied with a 3-conductor power cord. One conductor provides a connection between the instrument housing and the earth ground. Safe operation of the instrument will be assured provided that the ground circuit in the power outlet is wired correctly and is connected to earth.

Control Description

NOTE: If the ground pin of the power cord is removed for any reason the instrument chassis must be directly connected to earth ground

High Voltage Outputs

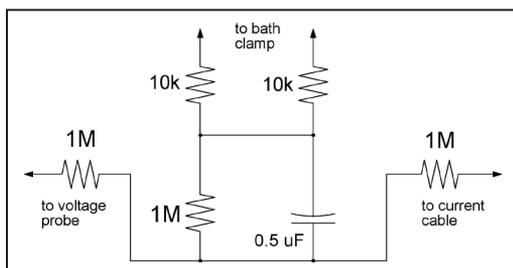
When handling the current electrode cable, be sure to set the GAIN CONTROL fully clockwise and the CLAMP MODE switch to off.

CAUTION!: The current clamp is capable of high power output (10 mA @ ± 180 V) and can cause serious injury if not properly handled.

Using the Model Membrane

The model cell supplied with the OC-725D can be used for two purposes. First, novice users will find it a convenient tool for gaining experience in the operation of the instrument. Additionally, it is a convenient tool for trouble shooting since the function of the instrument can be quickly checked.

A schematic of the model cell is shown below. The oocyte is represented by a 1 M Ω resistor shunted by a 0.5 μ F capacitor. The voltage and current electrodes are each represented by 1 M Ω resistances and the bath probes are represented by the 10 k Ω resistors.



Initial Instrument Settings

Connect the model cell to the OC-725D as shown on its cover. Be sure to connect the ground wire to the ground mini-jack on the side of the bath probe. Connect the V_m x10 BNC and the I MONITOR BNC on the OC-725D to an oscilloscope.

Set the instrument panel controls as follows and turn the POWER SWITCH on.

| Control | Control block | Setting |
|---------------------------|------------------------------------|---|
| POWER SWITCH | | <i>Off</i> |
| V _m OFFSET | <u>VOLTAGE</u> <u>ELECTRODE</u> | Center of rotation (Approximately 5 turns) |
| OUTPUT GAIN | <u>BATH ELECTRODES</u> | 0.1 V/ μ A |
| GAIN SELECT | <u>BATH ELECTRODES</u> | x1.0 |
| CLAMP MODE SWITCH | <u>CLAMP</u> | <i>Off</i> |
| DC GAIN TOGGLE | <u>CLAMP</u> | <i>Out</i> |
| GAIN | <u>CLAMP</u> | CCW |
| HOLD POTENTIAL | <u>COMMANDS</u> | 00 mV |
| HOLD POTENTIAL MULTIPLIER | <u>COMMANDS</u> | x20 |
| HOLD POLARITY | <u>COMMANDS</u> | <i>Off</i> |
| V _e Offset | <u>CURRENT</u> <u>ELECTRODE</u> | Center of rotation (Approximately 5 turns) |

Using the Model Membrane

Test Procedures

In the following testing procedures, allow a tolerance of $\pm 1\%$ on the readings taken. For example, if the test response is indicated as 100 mV, a reading from 99.0 to 101.0 mV would be within tolerance.

Offset Controls

V_m OFFSET (VOLTAGE ELECTRODE section): The full range of this control is ± 200 mV. This can be verified by rotating the control first fully clockwise and then fully counterclockwise while observing the VOLTAGE ELECTRODE METER. The displayed readings will indicate off scale at the extremes of the control's manipulation since the meter is only capable of displaying ± 199.9 mV.

V_m x10 output BNC (VOLTAGE ELECTRODE section): This output can be monitored using an oscilloscope. The reported voltage will swing between ± 2 V as the V_m OFFSET control is manipulated throughout its full range. Set the V_m OFFSET to 0.0 reading on the meter and verify that the V_m x10 reading on the scope also reads 0 V.

V_e OFFSET control (CURRENT ELECTRODE section): The V_e OFFSET control is tested in the same manner as the V_m OFFSET control. This control is adjustable when the CLAMP MODE switch is set to off. The voltage of the V_e OFFSET is read from the BATH ELECTRODE METER when the CLAMP MODE switch is set to off.

V_e x10 output BNC: This BNC is located on both the front and rear panels of the instrument and reports the setting of the V_e OFFSET control when the CLAMP MODE switch is set to off.

Return all controls to their initial settings when done with this test.

Voltage Electrode Test

In actual practice, the voltage electrode test is used prior to entering the cell and indicates the resistance of the electrode. When used in conjunction with the model cell, it measures both the electrode and membrane resistance ($2 \text{ M}\Omega$). This test is performed using controls in the VOLTAGE ELECTRODE section.

With the model cell in place, depress the V_m ELECTRODE TEST pushbutton and observe a reading of 20 mV on the VOLTAGE ELECTRODE METER. This corresponds to a $2 \text{ M}\Omega$ reading at a calibrated response of 10 mV/ $\text{M}\Omega$.

On the oscilloscope, the V_m x10 output will read 200 mV which is x10 the applied test voltage. Since the test current is being passed through $1 \text{ M}\Omega$ (R_m), the I MONITOR output will indicate 1 mV, which corresponds to 10 nA of current.

With the CLAMP MODE switch set to off, the BATH ELECTRODE METER monitors V_e (voltage at the current electrode). In this case, V_e will be a measure of the voltage across R_m and the meter will indicate 10 mV ($1 \text{ M}\Omega$). The V_e x10 output BNC on the rear panel can also be checked to see that it reads 100 mV (meter reading x10).

Using the Model Membrane

Buzz

This test is performed using controls in the VOLTAGE ELECTRODE section.

Set the oscilloscope sensitivity to 5 V/div and depress the BUZZ pushbutton while monitoring the $V_m \times 10$ output. A 1KHz square wave of approximately 24 V p-p will be generated as long as the button is depressed.

Current Electrode Test

This test is performed using controls in the CURRENT ELECTRODE section.

With the model cell in place, depress the V_e ELECTRODE TEST pushbutton and observe a reading of 20 mV on the CURRENT ELECTRODE METER. This corresponds to a $2 \text{ M}\Omega$ reading at a calibrated response of $10 \text{ mV}/\text{M}\Omega$.

With the CLAMP MODE switch set to off, the BATH ELECTRODE METER monitors V_e (voltage at the current electrode). In this case, V_e will be a measure of the voltage across R_m and the meter will indicate 10 mV ($1 \text{ M}\Omega$).

DC Clamp Test

This test is performed using controls in the CLAMP ELECTRODE section.

Place the CLAMP MODE switch in the fast position and adjust the V_m OFFSET control for a reading of 100 mV on the VOLTAGE ELECTRODE METER.

Turn the GAIN control on and slowly turn the control clockwise until the meter reading (V_m) decreases to zero. The CURRENT ELECTRODE METER should read $-0.10 \mu\text{A}$.

Set the HOLD POTENTIAL (COMMANDS section) to 100 mV (5.0 on the ten turn dial and MULTIPLIER toggle to $\times 20$). Select positive (pos) polarity. The VOLTAGE ELECTRODE METER should read 100 mV and the CURRENT ELECTRODE METER should read $0.00 \mu\text{A}$.

Switch to negative (neg) polarity. The VOLTAGE ELECTRODE METER should read (-100mV) and the CURRENT ELECTRODE METER should read ($0.2\mu\text{A}$)

Return the GAIN control fully CCW and turn the CLAMP MODE switch

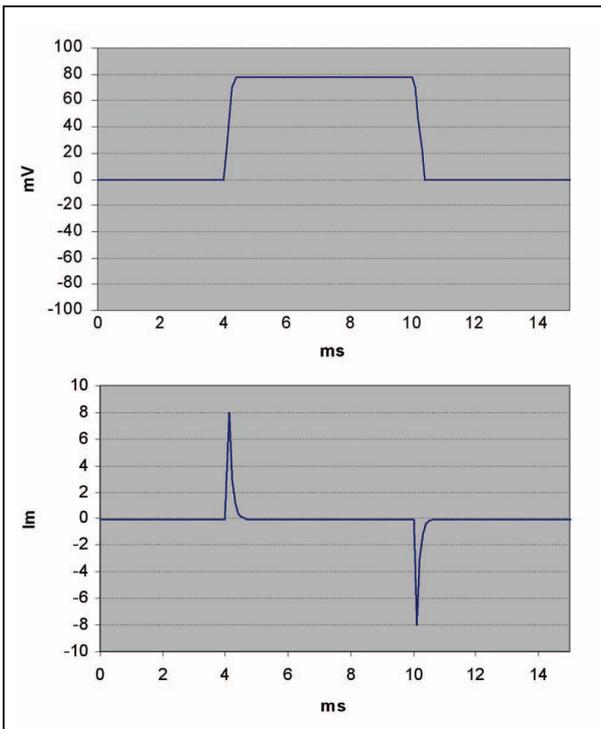
Using the Model Membrane

AC Clamp Test

Adjust the Vm OFFSET control to 0.0 V. Apply a 0.8 V, 100 Hz square wave to the COMMAND IN $\div 10$ BNC. Monitor the Vm x10 and I MONITOR outputs on the oscilloscope.

Switch the CLAMP MODE switch to fast and increase the GAIN until Vm reads 80 mV. Verify that the Vm x10 BNC reports 0.8 V. As you further increase the GAIN control, you will see the rise time of the oscilloscope trace become faster since the speed of the clamp is limited by the resistance of the current electrode and the capacitance of the oocyte. If ringing (oscillation) is observed, decrease the GAIN setting to obtain the fastest clean waveform as shown below. (The current signal (I MONITOR) shown in the figure below displays the high current spikes required to charge the oocyte capacitance.)

Set the HOLD control to a reading of 50 mV and switch the POLARITY toggle to pos. The square wave will be displaced 50 mV in the positive direction. Switching the polarity to neg will produce a -50 mV offset.



Setup

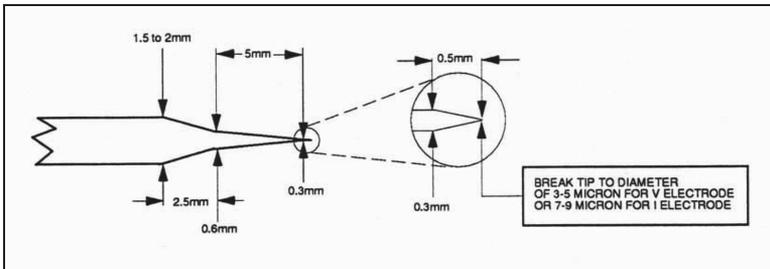
The following instructions are designed to guide the user, step-by-step, through a typical recording session involving a *Xenopus* oocyte. It is assumed that the user is already familiar with the techniques of *Xenopus* oocyte excision and microinjection (for a review of those techniques, see Colman, 1984). It is also assumed that the user has some familiarity with the basic circuitry of a two-electrode voltage clamp (for review, see Hille, 1984).

Pipettes

Microelectrodes can be made using the same glass (tubing and dimensions) as those used for a typical patch pipette and are usually filled with 3 M sterile filtered KCl. Unlike the pipettes used as patching electrodes, microelectrode pipettes do not require fire polishing nor coating with Sylgard. They will need to be broken off, however, to a relatively large diameter to insure a fast response time by the clamp.

For the voltage electrode, the pipette tip should be broken back to an O.D. of 3-5 μm . The current electrode pipette should be broken back to an O.D. of 7-9 μm . The resistances of these pipettes should be about 2 M Ω and

1 M Ω (or less), respectively.



When installed, the current electrode pipette should be shielded from the voltage electrode and that shield should be grounded to the circuit ground. This can be accomplished by wrapping the current pipette with aluminum foil or by mounting a metal screen or plate between the two pipettes. In either configuration, the shield can be grounded by connecting it to the "ground" mini-jack on the side of the bath probe. When using the aluminum foil method, care must be taken to prevent the foil from touching the surface of the bath solution at the bottom end of the pipette or the silver electrode wire at the top end.

Setup

Electrode Holders

Voltage Electrode — The voltage recording electrode holder uses a silver wire for the electrical coupling between the pipette and holder connector. Any silver wire contacting the KCl solution in the pipette must be chlorided to reduce junction potentials (see Chloriding Procedure in Appendix). The pipette should contain just enough KCl so that approximately 1/2 inch of the chlorided wire is submerged. The pipette/holder assembly is attached directly to the voltage headstage prior to mounting in a micropositioner.

NOTE: Do not fill the voltage holder with KCl solution.

Current Electrode — The electrode holder supplied for the current electrode also uses a silver wire for coupling. In a manner analogous to the voltage electrode, the current electrode wire must also be chlorided prior to assembly and use. The pipette/holder assembly is mounted in a micropositioner with the mounting rod supplied.

NOTE: Do not fill the current holder with KCl solution.

Bath Probe

The bath clamp is designed to maintain a virtual ground in the oocyte perfusate. The bath probe should be positioned so that the silver electrode wires can be inserted into the recording chamber or into the agar bridge wells. Sticky wax or tape is usually sufficient to secure the unit when positioned on a flat surface or, alternatively, the unit can be held in place on a separate stand. The bath probe electrodes should also be chlorided before use, as described above.

Electrode Placement and Grounding

Three drawings (shown on pages 19 and 20) have been included to illustrate the various ways a bath circuit can be configured. Most applications involve only a single oocyte and Figures A and B illustrate these setups. Figure C shows a setup for recording from 2 oocytes in a common bath with the use of dual clamps

NOTE: Cable routing must be performed with care. Bundle cables together rather than routing them individually and keep them as far as possible from sources of 50/60 Hz interference (e.g., line cords, transformers, etc.).

Setup

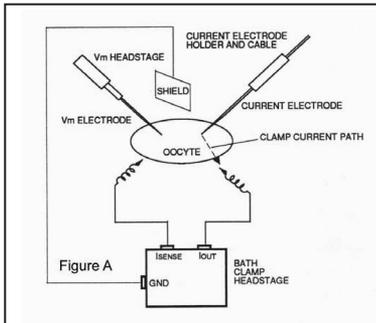
Bath Clamp Electrode Placement

Proper placement of the bath electrodes (I_{out} and I_{sense}) is important for obtaining optimum performance. The I_{sense} electrode (or the agar bridge associated with it) should be placed as close to the oocyte as possible since this point is the virtual ground node, and on the same side as the voltage recording electrode. The I_{out} electrode (or the agar bridge associated with it), on the other hand, can be placed at a greater distance from the oocyte and should be on the same side as the current electrode.

It is recommended that the user not directly expose the electrode wires to the perfusate if the recording session is to last for more than a few minutes. Instead, agar bridges should be employed to provide a circuit between these electrodes and the bath. This protects the cell membrane from the potential adverse effects of the silver wire.

Single Oocyte Setup with Indirect Ground

Single oocyte studies are best accomplished with the setup shown in Figure A. This method uses the bath clamp headstage to establish the bath ground and is preferred for two reasons: (1) current readings with the bath clamp will have the lowest noise level, and (2) properly placed bath clamp electrodes will negate the need for series resistance compensation.



Setup

Cable Connections

Bath clamp headstage: After positioning the probe as described above, connect it to the BATH PROBE socket (BATH ELECTRODES section).

Voltage electrode headstage: The high impedance probe for recording membrane potential should be mounted on a micro-manipulator and connected to the VOLTAGE PROBE socket (VOLTAGE ELECTRODE section).

High voltage current electrode: The holder should be mounted on a micro-manipulator and the cable connected to the I ELECTRODE socket (CURRENT ELECTRODE section).

Command potential: If a computer or external generator is used for controlling the clamp command potential, its signal should be connected to the front panel COMMAND IN ± 10 input (COMMANDS section).

External monitoring: To monitor the microelectrode's potentials on an oscilloscope, computer or a chart recorder, the following connections should be made:

V_m: Membrane potential may be recorded from the V_m x10 connector (VOLTAGE ELECTRODE section).

V_e: The voltage of the current electrode can be monitored from the V_e connector on the REAR PANEL.

The output will be the same as that reported on the CURRENT ELECTRODE METER, showing the voltage across the current electrode when the CLAMP SELECTOR switch is in the off position. (Recall that the meter indicates the current (I_m) when the CLAMP SELECTOR switch is in either the slow or fast position).

I_m: The current signal is available from the I MONITOR and I MONITOR FILTERED outputs (BATH ELECTRODES section).

Gain telegraph: Automatic monitoring of the I_m gain can be achieved by connecting the rear panel GAIN TELE BNC to the appropriate input on your analog-to-digital converter.

Filter Telegraph: Automatic monitoring of the Filter Setting can be achieved by connecting the rear panel FILTER TELEGRAPH BNC to the appropriate input on your analog-to-digital converter.

Power: The power cord should be connected to a properly grounded AC receptacle with the line voltage specified on the instrument nameplate (REAR PANEL).

Setup

Resting Position of the Controls

To begin, set the instrument controls to the following positions:

| Control | Section | Setting |
|----------------------|-----------------------|-------------------------------|
| POWER | | <i>off</i> |
| I MONITOR OUTPUT | <u>BATH ELECTRODE</u> | <i>1 V/μA</i> |
| GAIN SELECT | <u>BATH ELECTRODE</u> | <i>x1</i> |
| HOLD | <u>COMMANDS</u> | <i>0 mV</i> |
| POLARITY TOGGLE | <u>COMMANDS</u> | <i>off</i> |
| MODE SELECT | <u>CLAMP</u> | <i>off</i> |
| CLAMP DC GAIN TOGGLE | <u>CLAMP</u> | <i>out</i> |
| CLAMP GAIN | <u>CLAMP</u> | <i>off (fully CCW)</i> |
| ALARM | <u>REAR PANEL</u> | <i>off or on (as desired)</i> |

Turn POWER on.

Using the Gain Select

A wider range of bath current (I_m) measurements is now possible with the addition of the GAIN SELECT toggle switch located above the GAIN control. The switch has 3 positions; x0.1, x1 and x10. Resistance values shown below each LED indicate the bath clamp feedback resistor used for the current measurement.

The chart on the next page shows the effect of the range selection on the I_m output. Note also that range selection changes the sensitivity of the current meter. Typically, measured currents will fall in the x1 range. The lower and higher ranges are intended to cover those applications where currents are beyond the x1 range. Currents below $1 \mu\text{A}$ should be monitored in the x10 range. Large currents above $100 \mu\text{A}$ require the x0.1 range. Since there is overlap in the ranges, the current being measured may be monitored in one of two ranges in which case the choice may be made on the basis of noise or clamp speed.

Setup

| Gain Select | Headstage Resistor | I_m output range (V/ μ A) | I_m max output (μ A) | Maximum meter reading |
|-------------|--------------------|---------------------------------|-----------------------------|-----------------------|
| x0.1 | 10 k Ω | 0.01 - 2.0 | 5 - 1000 | 199.9 μ A |
| x1.0 | 100 k Ω | 0.1 - 20 | 0.5 - 100 | 19.99 μ A |
| x10 | 1 M Ω | 1.0 - 200 | 0.05 - 10 | 1.999 μ A |

Other Gain Range Selection Considerations

The intrinsic noise of the current measuring circuit is a function of the bath clamp feedback resistor with a larger resistor offering lower noise and greater signal resolution. For comparisons, the following measurements were made with a shorted input, with the standard 0.5 μ F capacitance model cell, and with a 0.22 μ F capacitance modified model cell. All readings were recorded at 1 kHz (8-pole Bessel) and are RMS.

| Feedback Resistor | Noise | | |
|-------------------|---------------|-----------------------------------|------------------------------------|
| | Shorted Input | Standard Model Cell (0.5 μ F) | Modified Model Cell (0.22 μ F) |
| 10 k Ω | 75 pA | 6.0 nA | 4.4 nA |
| 100 k Ω | 28 pA | 5.5 nA | 4.4 nA |
| 1 M Ω | 22 pA | 5.0 nA | 4.0 nA |

A lower value feedback resistor increases the speed of the bath clamp and can handle larger currents without saturating, important when recording from high expression oocytes.

A Procedure for Recording from Oocytes

Initial Electrode Placement

- 1) Make sure that the bath electrodes are submerged in the chamber (or in the agar bridge wells with the agar bridges completing the circuit to the bath) and the oocyte is stable on the chamber floor.
- 2) Install the voltage and current pipettes onto their respective holders but do not yet place them in the chamber bath solution.

Voltage Electrode Placement

- 3) Advance the voltage recording electrode into the bath. The VOLTAGE ELECTRODE METER will indicate (in mV) the potential between the electrode and the bath.
- 4) If there is no voltage reading and you are sure that the pipette tip is in the bath solution, perform the following checks:
 - a) make sure that all cables are connected properly.
 - b) inspect the voltage electrode to see if there is a bubble in the pipette which will cause an open circuit.
- 5) Using the V_m OFFSET control, adjust the VOLTAGE ELECTRODE potential to read 0 mV. If the junction potential of the voltage electrode can not be adjusted to 0 mV, the electrode holder may be at fault.

(See Electrode Holders, page 17.)

- 6) To test the resistance of the voltage electrode pipette, depress the ELECTRODE TEST button. This passes a 10 nA current across the voltage electrode. The VOLTAGE ELECTRODE METER will display the resulting potential in mV. The resistance of the electrode can be easily calculated by dividing the current into the potential. The resulting answer will be expressed in Ω . For example, if the electrode test indicates that a potential of 25.0 mV is produced by the 10 nA test current, then

$$R = \frac{V}{I} = \frac{25mV}{10nA} = 2.5 \times 10^6 \Omega = 2.5 M\Omega$$

NOTES:

a) A simpler calculation is to divide the voltage readout by 10 and append the units of M Ω . (e.g., 25 mV = 2.5 M Ω)

b) The calculated resistance value may vary widely from pipette to pipette but should be less than 4 M Ω for the voltage electrode.

A Procedure for Recording from Oocytes

Current Electrode Placement

- 7) Advance the current electrode until the tip is in the chamber bath solution. Adjust V_e OFFSET for a zero reading on the CURRENT ELECTRODE METER. This will establish a null reference allowing the resting potential to be directly read.
- 8) With the CLAMP SELECTOR switch in the off position, the resistance of the CURRENT ELECTRODE pipette is tested in the same manner as the VOLTAGE ELECTRODE. Pressing the V_e ELECTRODE TEST pushbutton will cause a 10 nA current to be passed across the CURRENT ELECTRODE. The resulting voltage (in mV) will be displayed on the METER in the BATH ELECTRODE section. From that value, the resistance of the pipette can be calculated exactly as described above (i.e., divide the readout by 10 to get the resistance in $M\Omega$). Since the current electrode has a larger diameter, its resistance should be less than that of the voltage electrode (about 1.0 $M\Omega$ or less).
- 9) If no voltage display is present during the electrode test procedure and you're sure that the electrode is contacting the bath, perform the following checks:
 - a) Make sure that all cables are connected properly.
 - b) Check to see that the aluminum shield around the current electrode pipette (if used) is not touching the bath solution or the electrode wire.
 - c) Check the current electrode to see if there is a bubble in the pipette causing an open circuit.

Impaling the Cell

- 10) Recheck the VOLTAGE ELECTRODE METER to verify that the potential is correctly offset to read 0.0 mV and readjust the V_m OFFSET control if needed.
- 11) Advance the VOLTAGE ELECTRODE until its tip is slightly depressing the plasma membrane of the cell and depress the V_m BUZZ pushbutton. This will produce a 1 V, 1 kHz oscillation at the voltage electrode, disrupting the membrane and causing the tip of the electrode to impale the cell with no further movement of the micro-manipulator (this technique will work best with "fresh" oocytes, i.e., 1 or 2 days post-excision). If the buzz technique fails to cause electrode penetration, further advance the voltage electrode until it "pops" through the membrane.
- 12) The potential across the membrane will now be displayed on the VOLTAGE ELECTRODE METER.

A Procedure for Recording from Oocytes

- 13) Now, advance the current electrode until its tip is slightly depressing the plasma membrane of the cell and depress the Ve BUZZ pushbutton. Similar to the voltage electrode BUZZ, the current electrode BUZZ produces a 1 V, 1 kHz oscillation across the current electrode. This disrupts the cell membrane and causes the tip of the electrode to impale the cell with no further movement of the micro-manipulator. Once again, if the BUZZ technique fails to cause penetration, further advance the current electrode until it “pops” through the membrane.

Clamping the Cell

- 14) Activate the clamp by switching the CLAMP MODE switch to either the slow or fast mode. **NOTE: slow mode is useful for initial screening.**
- 15) Adjust CLAMP GAIN control clockwise as far as possible without illuminating the OVER VOLTAGE LED (located in the CURRENT ELECTRODE section).

NOTE: If the ALARM signal switch on the back panel is on, an alarm will sound when the OVER VOLTAGE LED illuminates.

- 16) The clamped membrane potential can now be observed over time or it can be manipulated by selecting the desired polarity and amplitude with the controls located in the COMMANDS section. Alternatively, you can control the COMMAND voltage externally from a computer by leaving the POLARITY toggle switch in the off position and connecting the appropriate analog output from your computer DAC to the COMMAND IN ± 10 BNC connector. (See Cable Connections, page 20.)
- 17) Depending on the amplitude of the response you wish to record, you may adjust the instrument GAIN to a higher or lower position. The CURRENT ELECTRODE METER should now be displaying the current (in μA) that is delivered to hold the cell at the designated command potential.

Clamping High Conductance Cells

- 18) DC GAIN mode (CLAMP section) may be required to clamp high conductance (low resistance) cells. This condition will be evidenced by the inability of the instrument to maintain a DC holding potential (to within 1% or better of the set value) and the maximum instrument gain is not sufficient to provide a hard clamp. DC GAIN mode provides an additional DC gain greater than 10^6 while the AC gain remains at 2000 maximum for stability.

A Procedure for Recording from Oocytes

Unclamping the Cell

- 19) To unclamp the cell, turn the GAIN control (CLAMP section) fully counter-clockwise.

NOTE: If the control is not fully off and the DC GAIN is left on, the preparation will not be unclamped.

Removing the Electrodes

- 20) It is very important that the CLAMP GAIN be returned to the off position (fully counter-clockwise) as described above and the CLAMP MODE selector switch be placed in the off position before removing the current electrode from the cell.

Failure to perform the above steps will overload the feedback amplifier (due to the large current generated when the membrane resistance between the current electrode and the bath virtual ground goes to zero). This will damage the oocyte! For this reason we recommend that the user enable the audible overload alarm to provide a warning when the potential for such damage exists.

Comments and Recommendations

Membrane Damage

Recording from the same cell at a later time requires that the cell remain healthy during the interim incubation. The less damage done to the membrane during handling and impaling the cell, the happier it will be. Use of the BUZZ function should help minimize the trauma from electrode penetration. Membrane damage can be further reduced by properly isolating the experimental platform from vibration. Finally, hydraulically driven micro-manipulators will also reduce membrane damage while the electrodes are in the cell.

Repeated Recordings

Most recording sessions will involve repeating the above steps several times with many different cells. Unless there is a concern about contamination of the bath solution by something carried over from previous experiments, the pipettes can also be used repeatedly. They should be free of debris and should have approximately the same resistance as they had in the previous recording. A significantly higher resistance could indicate that the pipette is partially plugged with cellular debris.

Make the following control settings before the next recording is carried out:

| Control | Section | Setting |
|-------------|----------------|----------------------|
| POLARITY | <u>COMMAND</u> | <i>off</i> |
| MODE SELECT | <u>CLAMP</u> | <i>off</i> |
| GAIN | <u>CLAMP</u> | <i>0 (fully CCW)</i> |

Electrophysiology

If you are well versed in setting up electrophysiological equipment, you can safely skip over the rest of this section. If, however, this is your initiation into electrophysiology (as it may well be for some of you molecular biologists), then you may find the following recommendations helpful.

While the whole cell electrophysiological configuration is more forgiving than the patch clamp, it is still important to minimize mechanical motion. The platform for your experimental setup, therefore, should be mechanically well isolated. This will reduce leakage around the electrodes, making the clamp more effective and reducing noise in your recordings. The latter is especially important when recording responses of certain ligand-gated channels where membrane potential changes may only be a few mV

Comments and Recommendations

In addition to mechanical isolation, the setup must also be isolated from external electrical noise sources. These include motors, lamps, and wiring. The platform should be shielded from these sources of electrostatic radiation with a Faraday cage. All equipment within the Faraday cage should be grounded to the rear panel instrument circuit ground and is best achieved by connecting everything (including the cage) to a ground bus within the cage. Then only one wire is run from the setup to the instrument ground.

You will need to mount the voltage recording electrode headstage and the current injecting electrode on micro-manipulators. They need not be hydraulically driven but such drives will minimize the damage to the cell during and after penetration, and will make for better seals around the electrodes. Another advantage gained by reducing membrane damage by electrodes is to enhance the possibility of making subsequent recordings from the same cell. These suggestions are also important for minimizing mechanical noise in the recorded data

You will need a dissecting scope for viewing the placement of the electrodes. Anything more powerful than 40x will just get in the way. The light source for your scope should be DC and may need to be IR and UV filtered if you plan to use it during recording.

Minimally, the recording chamber can be a stable surface on which the oocyte will not roll around. A disposable petri dish with a piece of nylon mesh on the bottom has been successfully used for this purpose. The dish can be stabilized by a holder or by some wax placed around its perimeter. The diameter of the dish needs to be large enough to accommodate the oocyte and the two bath electrodes. The walls of the recording chamber should be low enough to not interfere with electrode placement. Perfusion of the chamber can be accomplished using a gravity fed system. Perfusate from the dish can be evacuated using gentle vacuum. Use as small an aperture as possible to avoid disturbing the surface of the perfusate in the dish.

NOTE: Specialized equipment suitable for oocyte studies (oocyte chambers, perfusion systems, a Faraday cage, a noise-free DC lamp, and micro-manipulators) are available from Warner Instruments.

Although it is not required, a computer can be employed to control the command voltage. Acquisition and display of data is also usually handled by computer. The OC-725D is fully compatible with all commercially available software packages designed for electrophysiological research.

Finally, a microelectrode puller is necessary for making appropriately sized voltage and current electrodes. Usually, the microinjection pipette puller can also be used to make microelectrodes. You will need use of a microscope to break off the pipette tips.

Appendix

| SPECIFICATIONS | |
|---|---|
| Voltage recording channel (V_m) | |
| Input Impedance | 5 x10 ¹¹ Ω , shunted by 3 pF |
| Output Resistance | 100 Ω |
| V_m OFFSET | \pm 200 mV at V probe input |
| Noise* | 50 mV RMS at 1 kHz |
| Electrode Test | 10 mV/M Ω |
| V_m Meter Range, full scale | \pm 199.9 mV |
| Bath electrode channel (I_m) | |
| V_e OFFSET | \pm 200 mV |
| Noise* | |
| I_m Clamp (clamp on) | 5.5 nA RMS at 1 kHz [x1 range] |
| Open loop (clamp off) | 28 pA RMS at 1 kHz [x1 range] |
| I MONITOR | 0.01 - 200 V/mA in 3 ranges, 8 steps per range |
| I FILTERED | Filtered at 0.05, 0.1, 0.2, 0.5, 1, 2, 5, 10 kHz and Bypass (4-Pole Bessel) |
| Gain Telegraph | 0.2 - 2.8 VDC in 0.2 V steps |
| Filter Telegraph | 0.2 to 1.8 VDC in 0.2 V steps |
| Meter Ranges (full scale) | |
| V_e (clamp off) | \pm 199.9 mV |
| I_m (x0.1 range) | \pm 199.9 μ A |
| I_m (x1.0 range) | \pm 19.99 μ A |
| I_m (x10 range) | \pm 1.999 μ A |

Appendix

| SPECIFICATIONS | |
|---|---|
| Current electrode channel (V_e) | |
| Compliance Voltage | ± 180 V |
| Alarm ± 160 V | |
| Gain | |
| Variable | 0 - 2000 AC/DC |
| DC | 1 x106 DC, switch selected |
| Electrode Test | 10 mV/M Ω |
| Commands | |
| Hold (internal) | ± 200 mV in 2 ranges |
| External input | 1 V in = 0.1 V command |
| (attenuated by 10) | |
| Maximum external input | 10 V |
| Power requirements | 100 -120 v~, 0.5A; or 220-240 VAC, 50/60 Hz, 14VA |
| Fuse | 0.5A @ 120v (slow blow) |
| | 0.15A @ 240v (slow blow) |
| Dimensions | |
| Enclosure | 9 x 42 x 25 cm (H x W x D) |
| Voltage Headstage | 1.25 x 5 cm (dia. x length); with 1.8 m cable |
| Mounting Handle | 4.8 mm x 6.3 cm (dia. x length) |
| Bath Headstage | 2.8 x 3.5 x 4.2 cm (H x W x D); with 1.8 m cable |

*All noise measurements made with an 8-pole Bessel filter.

Appendix

Gain Telegraph Outputs Gain Telegraph Outputs

| I_m Output (V/ μ A) | Gain Telegraph |
|---------------------------|----------------|
| 0.01 | 0.2 V |
| 0.02 | 0.4 V |
| 0.05 | 0.6 V |
| 0.1 | 0.8 V |
| 0.2 | 1.0 V |
| 0.5 | 1.2 V |
| 1 | 1.4 V |
| 2 | 1.6 V |
| 5 | 1.8 V |
| 10 | 2.0 V |
| 20 | 2.2 V |
| 50 | 2.4 V |
| 100 | 2.6 V |
| 200 | 2.8V |

| Frequency (Hz) | Filter Telegraph |
|----------------|------------------|
| 50 | 0.2 |
| 100 | 0.4 |
| 200 | 0.6 |
| 500 | 0.8 |
| 1K | 1.0 |
| 2K | 1.2 |
| 5K | 1.4 |
| 10K | 1.6 |
| Bypass | 1.8 |

Gain Select Settings

| Gain Select | Headstage Resistor | I_m Output Range (V/ μ A) | I_m max output (μ A) | Maximum Meter Reading |
|-------------|--------------------|---------------------------------|-----------------------------|-----------------------|
| x0.1 | 10 k Ω | 0.01 - 1.0 | 5 - 1000 | 199.9 μ A |
| x1.0 | 100 k Ω | 0.1 - 10 | 0.5 - 100 | 19.99 μ A |
| x10 | 1 M Ω | 1.0 - 100 | .05 - 10 | 1.999 μ A |

Noise From Bath Feedback Resistor

| Feedback Resistor | Noise | | |
|-------------------|---------------|-----------------------------------|------------------------------------|
| | Shorted Input | Standard Model Cell (0.5 μ F) | Modified Model Cell (0.22 μ F) |
| 10 k Ω | 75 pA | 6.0 nA | 4.4 nA |
| 100 k Ω | 28 pA | 5.5 nA | 4.4 nA |
| 1 M Ω | 22 pA | 5.0 nA | 4.0 nA |

Appendix

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Notes



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