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Axoclamp-2B

High-Compliance Voltage Clamp and Current Clamp



The **recently enhanced** (see box below) Axoclamp-2B is a complete microelectrode current-clamp and voltage-clamp amplifier useful for a wide range of intracellular microelectrode recording techniques. This new model retains the functionality of its predecessor, the Axoclamp-2A.

The amplifier's flexibility has been enhanced by increasing the compliance of the two-electrode voltage clamp to ±130 V, making it easier to study large currents from expressed ion-channel proteins in Xenopus oocytes.

- Continuous current clamping with either of two complete bridge amplifiers.
- Discontinuous current clamping.
- Continuous single-electrode voltage clamping for whole-cell patch and macropatch configurations recording currents up to a few nA.
- Discontinuous single-electrode voltage clamping for clamping cells with currents up to several hundred nA.
- High compliance two-electrode voltage clamping for clamping cells with currents that can range from very small (< 1 nA) to extremely large (> 100 mA) without compromising the clamping speed.

Enhanced Two-Electrode Voltage Clamp

Oocyte recordings can yield very large currents as the membrane resistance drops to very low levels. Under these extreme conditions, maximal clamp gain (μ) is imperative for a stable voltage clamp. All new Axoclamp-2B amplifiers are manufactured with enhanced clamp gain. Read this Application Note for more detail. If you own an Axoclamp-2B amplifier manufactured prior to January 2003, and would like to enhance the performance to avoid DC voltage errors under such extreme conditions, contact tech support.

Ease of Use

Built into the front panel are precision current and voltage command sources, meters, filters, offset controls, and features for clearing microelectrodes, assisting in cell penetration and presetting the clamp to the cell's resting membrane potential. Some functions can be controlled via digitizer outputs, a footswitch or a hand-held remote. With a variety of optional headstages, the Axoclamp-2B can be set for several recording configurations.

Individual circuits for each microelectrode make separate but simultaneous current measurements possible. There are four built-in meters for the display of crucial parameters. A digital counter displays the sample rate. Voltages of the two microelectrodes are read from two dedicated meters and any one of three current outputs may be selected for display on the current meter.

Several internally generated commands assist during setup and may be used simultaneously with external commands. In addition to generating precision command currents and voltages, the Axoclamp-2B offers automatic clamping at resting membrane potential, offset compensation and a calibration signal on outputs.

Outputs may be conditioned in three ways: Blanking removes the stimulus artifact; Output Bandwidth Selection provides filtering for the membrane current and voltage output from the primary microelectrode circuit; and an internally generated Calibration Signal can be superimposed onto each of the outputs.

Buzz and Clear features can assist in cell penetration and unclogging microelectrode tips.

A hand-held switch box, a footswitch or the computer interface can be used in place of front panel controls to activate Buzz and Clear or select the operating mode. With an appropriate digitizer generating TTL pulses, your computer may be used to automatically switch between operating modes. Footswitches and computer triggering offer hands-free operation of Buzz and Clear. Buzz may also be triggered with a hand-held remote unit that adjusts the buzz duration.

The Axoclamp-2B is adaptable to a variety of recording configurations including bath clamp, bath potential measurement and compensation, or virtual-ground current measurement.

The optional bath-clamp headstage can be used to clamp the potential of the bath to zero, thereby eliminating the voltage error due to current flowing through the bath-grounding electrode. This configuration also compensates for changes in the bath potential resulting from alterations in the temperature and ionic composition of the cell's bathing solution. A universal power supply allows the Axoclamp-2B to operate from any AC power source.

User-Friendly Layout

The layout of the Axoclamp-2B was designed to provide a user-friendly instrument for optimal flexibility in experimentation. The logical grouping of commands makes functions easy to locate and engage. Color coding helps identify command groupings and those controls whose adjustment is most crucial to the cell's survival.

Quality

The excellence of the components and construction will be obvious to you from the high quality of the cabinet and controls, precision ten-turn potentiometers and reliable switches abound. Gold-plated connectors are used throughout, ultralow-drift operational amplifiers are used in all critical positions, integrated circuits are socketed for easy maintenance, and the circuit designs and operation have been well tested in

laboratories throughout the world. All this adds up to low-noise, low-drift, reliable and accurate operation.

Current Clamping

The Bridge mode can be used with one or both microelectrodes for continuous current clamping, or the discontinuous current clamp (DCC) mode can be used with one microelectrode while the other is used for continuous current clamping. DCC has the advantage of not requiring re-balance to cope with small changes of tip resistance, but it requires a faster microelectrode response. Bridge current clamp has better resolution.

Voltage Clamping

Voltage clamp with one or two microelectrodes -- your choice is dictated by the needs of your investigation; the Axoclamp-2B does both. Discontinuous single-electrode voltage clamping (dSEVC) is based on the technique of sampling the membrane potential while zero current flows and then retaining this sampled value while current is injected into the cell. This procedure is rapidly repeated to produce a smooth response. Because it is the actual membrane potential that is sampled, there is no voltage error due to series resistance. However, dSEVC may not be best for small currents. Continuous single-electrode voltage clamping (cSEVC) uses a patch pipette to continuously record membrane potential and inject current. The error caused by the voltage drop across the microelectrode resistance can be partially reduced by series resistance compensation. With two-electrode voltage clamping (TEVC), one microelectrode is used to continuously record membrane potential while the other is used to inject current. Gain for each voltage-clamp mode is set by a single potentiometer. The improved high compliance (±130V) further extends the capability of the Axoclamp-2B beyond that of its predecessors. A Phase Lag potentiometer and Multiplier switch determine the time constant of the phase lag. When adjusted correctly, stability is ensured even when the cell membrane has a non-ideal phase shift (*i.e.*, less than 90°) due to the distributed series resistance. RMP balance indicator LEDs assist in presetting the holding potential to the resting potential, thereby minimizing the transients and the holding current when voltage-clamp mode is selected.

Continuous Single-Electrode Voltage Clamp

cSEVC mode is suitable for both whole-cell and macropatch recording of signals that are slow or moderate in speed. Usually, currents up to a few nA can be recorded with minimal voltage error by using the built-in series resistance compensation circuitry. For instance, when a 5 MW pipette is used to record a 1 nA peak current and series resistance compensation of 70% is achieved, the voltage error is only 1.5 mV.

Discontinuous Single-Electrode Voltage Clamp

The Axoclamp-2B allows very fast discontinuous single-electrode voltage clamping with microelectrodes and patch pipettes. In a test cell (see Specifications) the 10-90% rise time is only 100 µs. In a real setup the response speed is limited by the microelectrode characteristics; even so, membrane potential rise times (without overshoot) of less than 1 ms have been regularly achieved in a variety of cell types. For example, it has been possible to reliably record currents up to 30 nA using a 1 MW pipette and achieve temporal resolution equal to that of a continuous clamp. Thus,

dSEVC mode is an excellent alternative to the cSEVC mode in a conventional patch clamp amplifier when the series resistance error becomes limiting. A unique, variable Anti-Alias Filter helps reduce noise towards the theoretical minimum during dSEVC mode by slowing the response of the sampling circuit to suit the sample rate and the microelectrode response. The Sample Rate can be altered continuously from a low value of 500 Hz to a high of 50 kHz. This enables you to take advantage of the decrease in noise and response times occurring when faster sampling rates are used. A Monitor Output allows the input to the sampling circuit to be observed. It is essential to observe this signal during dSEVC to ensure that the microelectrode voltage due to current passing has time to adequately decay at the end of each cycle. An oscilloscope trigger signal at the sample rate is provided for use with the Monitor signal. Two separate Axoclamps may be used together to record from one preparation. The sample clocks of two Axoclamp-2Bs can be synchronized in a "Master-Slave" configuration. This is useful in experiments in which two cells in the same preparation are independently voltage clamped using dSEVC. Linking the two clocks prevents the generation of spurious signals which would otherwise appear as harmonics at the difference in the two clocks' frequencies.

High Compliance Two-Electrode Voltage Clamp

Output compliance in two-electrode voltage clamp mode has been increased to ±130 V. This allows clamping of even extremely large currents such as those arising in Xenopus oocytes. TEVC provides the fastest clamping with the largest compliance of any clamp mode. With an optional bath clamp, the bath potential is clamped to zero, thus obviating the problem of series resistance in the bath-grounding pathway.

Headstages

HS-2A

Two unity-voltage-gain U-type headstages are provided; you can choose from several current gains that cover the range of cell input impedances from less than 1 MW to greater than 1 GW. Ultra-high input impedance versions are also available for ion-sensitive electrodes. High speed and low noise are achieved by using bootstrapped power supplies for the input circuit of each headstage. These bootstrapped power supplies are derived from special high-voltage circuits so that the headstages will not be saturated by the large voltages that may occur during the passage of current through high-resistance microelectrodes. Capacitance neutralization is also derived from high-voltage circuits so that fast responses are not degraded during large input signals. Current in each microelectrode is continuously measured during both voltage clamp and current clamp. This measurement does not include currents from sources other than the microelectrode (*e.g.*, ionophoresis, the other microelectrode) and indicates zero if the microelectrode becomes blocked. Headstages have a gold-plated 1 mm (0.04") input socket to directly accept the provided pipette holders. To connect wire leads, if desired, optional 1 mm pins are available.

VG-2A

An optional VG-2A-x100 bath clamp allows the bath potential to be clamped to zero, thereby eliminating the voltage error due to current flow across the bath-grounding electrode, which is the major contributor to the series resistance in a two-electrode voltage clamp. This headstage also compensates for shifts in bath potential caused by changing solutions or temperature.

HS-4 and VG-2

In two-electrode voltage clamp mode the HS-4-x1MGU headstage can be used to gain a small benefit when large, ultra-fast voltage steps in big cells must be established. The current-sensing resistor inside the HS-4 series headstage is automatically bypassed, thereby increasing the current that can be delivered by the current-passing electrode. To measure the current, the VG-2A headstage is used in conjunction with the HS-4-x1MGU.

Pipette Holders

The pipette holder, HL-U, is custom constructed to ensure low-noise mechanically stable recording. Two different barrel lengths are provided. HL-U accepts pipettes of 1.0 mm to 1.7 mm OD. It includes a silver wire assembly. Post for suction is 1.0 mm OD. The Axoclamp-2B is shipped with two pipette holders. For further information see HL-U holder in the Headstages and Holders section of this catalog.

Alternate Recording Configurations

A third HS-2 or HS-2A headstage can be used extracellularly to record the bath potential. The bath potential is then subtracted from the potentials recorded by the two intracellular microelectrodes to compensate for shifts in bath potential due to changing solutions or temperature. Since both microelectrode amplifiers are complete, each can perform separate and distinct functions. For instance, one microelectrode can be used for ionophoresis while the other is used intracellularly to record the membrane potential.

Buzz and Clear

Two controls for each microelectrode are devoted to clearing blocked microelectrode tips and assisting cell penetration. One is the Clear switch which can be used to force large hyperpolarizing or depolarizing currents through the microelectrode. The other is a Buzz switch which causes the microelectrode voltage to oscillate. Depending on the microelectrode and the preparation, one of these two methods will often succeed in lowering the resistance of blocked microelectrode tips. When used while the tip of the microelectrode is pressing against the cell membrane, Buzz and Clear may assist in cell penetration. With patch electrodes, Buzz may also aid in breaking the membrane patch in order to achieve a whole-cell configuration.

Blank

A Blank facility can be used to force the voltage clamp system to ignore stimulus artifacts that would otherwise be picked up by the voltage-recording circuit and result in large current artifacts that could damage the cell.

Command Generators

In any mode, level and step commands can be generated internally. Level commands (one for voltage clamp and one for each microelectrode), are set on precision ten-turn potentiometers. The TTL-gated Step Command is set on a 3½-digit thumbwheel switch and can be directed to either one of the microelectrodes or the voltage clamp. An indicator light for each microelectrode illuminates during current commands. External command sources can be used simultaneously with internal command sources.

Outputs

Two dedicated Digital Voltmeters continuously display the microelectrode voltages while a third displays the current in the selected microelectrode or in a virtual-ground circuit, if used. A Sample Rate meter lets you know precisely what sampling rate you are using during discontinuous current clamp and voltage clamp. Offset controls are provided for each microelectrode, and a single-pole low-pass filter is provided for the microelectrode used in single-electrode voltage clamping. Also, an internally generated Calibration Signal can be superimposed onto each of the outputs. Hence, the output signals in many cases can be wholly conditioned within the Axoclamp-2B to suit your recording apparatus. Six outputs are conveniently located on the front panel to connect an oscilloscope or a recording device. Five of these outputs are repeated on the rear panel, where the other outputs, the inputs and the headstage connectors are also located.

Model Cells

Every Axoclamp-2B is supplied with CLAMP-1U and MCW-1U model cells. Each plugs directly into the input sockets of the headstages. Both are useful tools to use while learning the operation of the Axoclamp-2B and to verify its correct operation. The CLAMP-1U model cell simulates recording from small cells with microelectrodes. A switch allows the CLAMP-1U model cell to be configured as Bath mode (two 50 MW microelectrodes to ground), or Cell mode (two 50 MW microelectrodes connected to a 50 MW//500 pF cell). The MCW-1U is configured as two 10 MW patch pipettes connected to a 500 MW//33 pF cell. An optional MCO-1U model cell is useful for simulating large cells, such as amphibian ooctyes.

Remote and Computer Control

Selecting the operating mode can be made remotely for computer sequencing of experiments. Hands-free operation of Buzz is possible using the footswitches supplied with every Axoclamp-2B. The duration of the Buzz oscillation is normally equal to the time that the front-panel switch is pressed. The shortest duration that this switch can be pressed is about 100 ms. For small cells, 100 ms Buzz oscillation is sometimes detrimental. The Remote Buzz Duration Control supplied with the Axoclamp-2B is a hand-held control that contains a trigger switch to buzz either electrode, and a duration control for setting the Buzz duration in the range 1-50 ms. An appropriate duration can be found for most cells that is sufficiently long to allow penetration of the membrane but short enough that the cell is not damaged after penetration.

Universal Power Supply

The universal power supply operates at any international voltage without requiring that the user set a voltage-selection switch. This sophisticated switching power supply virtually eliminates line-frequency interference.

Excellent Comprehensive Manual

We also provide a detailed operator's manual that serves as a handbook of procedures for microelectrode users. Tutorials written by Axon Instruments staff and scientific

consultants provide a useful guide to the operation of the instrument and are informative references for several electrophysiological techniques.

Specifications

The specifications are available in Adobe PDF format.

 Complete Axoclamp-2B description with specs (PDF, 1.4MB).

Get the Axoclamp-2B user manual from the Downloads page.

Ordering Information

For ordering information go to the Sales Information page.

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