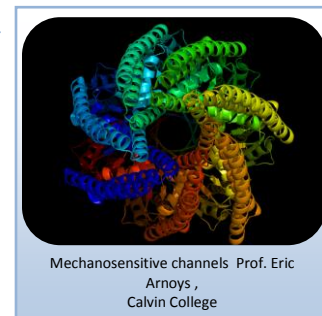
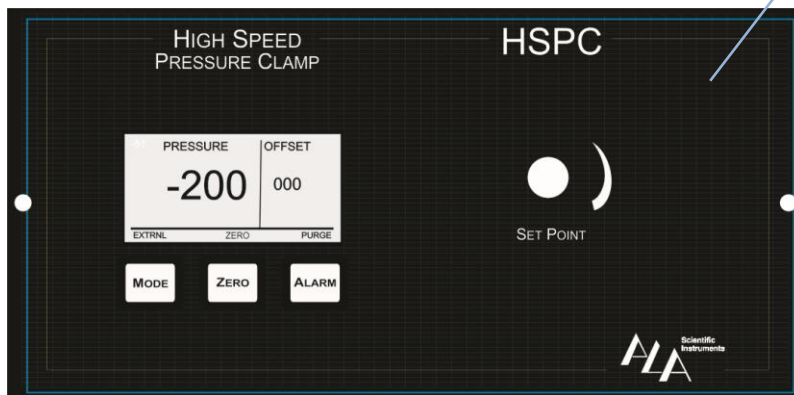




Pressure Clamp

Model# HSPC-2-SB

Ver. 3.0
4.2018



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Introduction

About this Manual

This manual comprises all important information about the installation and the proper usage of the High Speed Pressure Clamp (HSPC-2-SB). It is assumed that you have a basic understanding of technical terms, but no special skills are required to read this manual.

Make sure you read the "Important Information and Instructions" prior to install or to operate this temperature controller.

Important Information and Instructions

Operator's Obligations

The operator is obliged to allow only persons to work on the device, who

- are familiar with the safety at work and accident prevention regulations and have been instructed how to use the device;
- are professionally qualified or have specialist knowledge and training and have received instruction in the use of the device;
- have read and understood the chapter on safety and the warning instructions in this manual and confirmed this with their signature.

It must be monitored at regular intervals that the operating personnel are working safely.

Personnel still undergoing training may only work on the device under the supervision of an experienced person.

Guarantee and Liability

The *General conditions of sale and delivery* of ALA Scientific Instruments always apply.

The operator will receive these no later than on conclusion of the contract.

Guarantee and liability claims in the event of injury or material damage are excluded when they are the result of one of the following.

- Improper use of the device.
- Improper installation, commissioning, operation or maintenance of the device.
- Operating the device when the safety and protective devices are defective and/or inoperable.
- The HSPC-2-SB has an alarm system to prevent accidental suctioning of liquids into the instrument. Circumventing this system voids the warranty.
- Do not apply voltages greater than ± 5 V to the command input.
- Non-observance of the instructions in the manual with regard to transport, storage, installation, commissioning, operation or maintenance of the device.
- Unauthorized structural alterations to the device.
- Unauthorized modifications to the system settings.
- Inadequate monitoring of device components subject to wear.
- Improperly executed and unauthorized repairs.
- Unauthorized opening of the device or its components.
- Catastrophic events due to the effect of foreign bodies or Acts of God.

Safety Information



Warning: Make sure to read the following advice prior to install or to use the device and the software. If you do not fulfill all requirements stated below, this may lead to malfunctions or breakage of connected hardware, or even fatal injuries.

Warning: Obey always the rules of local regulations and laws. Only qualified personnel should be allowed to perform laboratory work. Work according to good laboratory practice to obtain best results and to minimize risks.

This device may only

- be used for its **intended purpose**;
- be used when in a **perfect condition**.
- Improper use could lead to serious, even fatal injuries to the user or third parties and damage to the device itself or other material damage.

Warning: This device is **not** intended for medical uses and **must not** be used on humans.

Malfunctions which could impair safety should be rectified immediately.

High Voltage

Electrical cords must be properly laid and installed. The length and quality of the cords must be in accordance with local provisions.

Only qualified technicians may work on the electrical system.

- Each time before starting up, make sure that the **main power supply** agrees with the specifications of the product.
- Check the **power cord** for damage each time the site is changed. Damaged power cords should be replaced immediately and may never be reused.
- Do not try to insert anything sharp or metallic into the vents or the case.
- Liquids may cause short circuits or other damage. Keep the device and the power cords always **dry**. Do **not** handle it with wet hands. Make sure the device and your experiment are set up in a way that makes it impossible that any liquid spills onto the device or drips into the device from the surface of the table.

Shipping contents

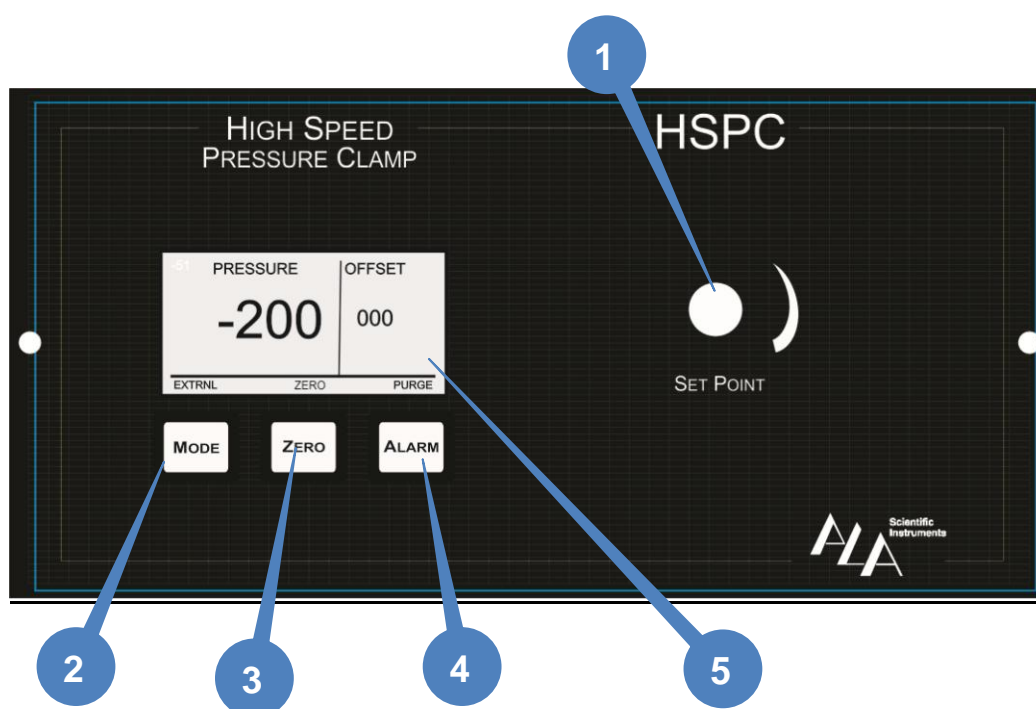
The HSPC-2-SB shipment includes the following items:

- HSPC-2-SB Controller
- HSPC-2-SB Headstage
- Universal Power supply +9V DC
- Power cable
- DB-9 cable M/M 10ft.
- Thick-walled silicone tubing 1ft.
- Set of 1/16 ID PVC tubing 10ft. with male luer fittings.
- Set of female luer fittings.
- Filters 20 micron (x2)
- Instruction manual

Please open the box and the inspect contents immediately upon receipt. If any components appear to be damaged or missing, please contact ALA Scientific Instruments or your local distributor.

HSPC Controller

Front Panel



- 1) **Set Point:** Manual control for setting pressure output from the controller and for manual zeroing of the instrument prior to recording.
- 2) **Mode:** Select between manual or external control. Use Manual mode to use the set point adjust knob to select the pressure output. Use the EXTRN (external) mode to set the pressure output from a command voltage connected to the rear of the unit.

In EXTRN (external) mode the manual set point adjust knob is used as an offset of pressure or vacuum.

In MANUAL mode only the set point knob is functional and is used to manually adjust the vacuum or pressure.

- 3) **Zero:** Is used to control for zero adjustment prior to recording. Press the button to reset the system to zero "0" mmHg output pressure.
- 4) **Alarm:** This alarm feature has two functions. One is when the system detects moisture entering into the headstage an alarm function is triggered. A +200 mmHg of pressure is sent to the headstage to purge any moisture that has entered and the display will blink the message "Alarm Check Electrode".

Press the "ZERO" button to start the internal timer (1minute intervals). Remove all output tubing from the headstage and let air blow out. This helps to dry out the internal moisture sensor. The system will come back automatically once the moisture sensor drops to its correct value.

The second function is as a valve purge feature. After use of the HSPC system one can use this function to send +200mmHg pressure to the valve by pressing

“ALARM” button and then pressing the “ZERO” button to start the above mentioned internal timer. The system will automatically come back to normal after 1 minute or after the moisture sensor comes back to its normal operating range.

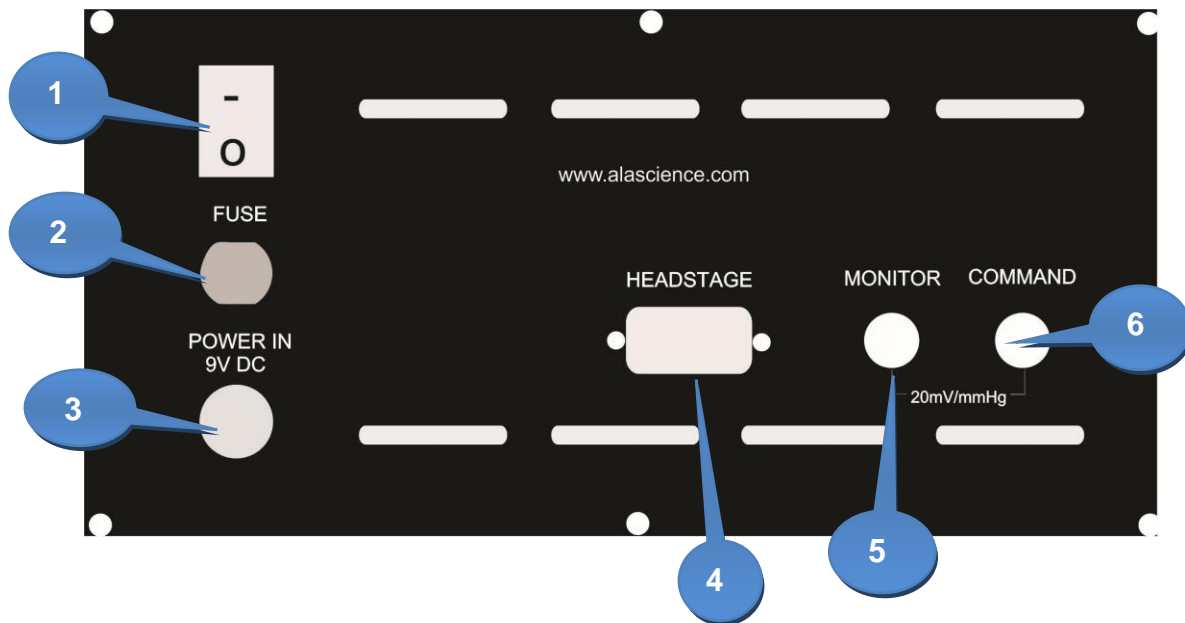
- 5) **Pressure Display:** Will illuminate when power is turned ON.

LCD displays the instrument’s modes and headstage pressure status.

In MANUAL mode the display will show SETPOINT.

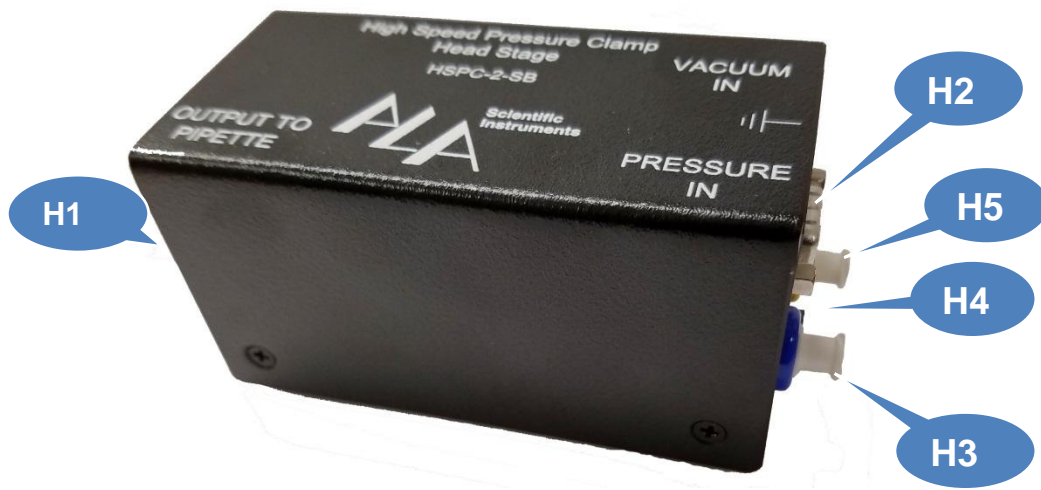
In EXTRNL mode the display will show OFFSET.



Rear Panel

- 1) **Main power switch:** This rocker switch turns power to unit ON or OFF.
- 2) **Fuse:** holder for unit fuse. Replace with 5x20mm 0.5Amp 250v slow blow.
- 3) **Input power:** 2.1mm x 5mm power receptacle. Use provided power supply (9V DC output).
- 4) **Headstage:** DB-9 output that connects controller to headstage.
- 5) **Monitor:** 20 mV/mmHg analog output from pressure sensor. Use to display or record measured pressure. Standard BNC.
- 6) **Command:** 20 mV/mmHg analog input. Use to control pressure from function generator or acquisition software. Standard BNC.

Headstage



H1) **OUTPUT:** Luer connector (Green) for silicone tubing going to electrode holder.

H2) **DB-9 connector:** cable connection between headstage and controller.

H3) **PRESSURE IN:** Luer connector (blue) for positive input pressure from PVPUMP.

H4) **GROUND:** 2mm mini banana plug, use if needed.

H5) **VACUUM IN:** Luer connector (yellow) for negative input pressure from PVPUMP.

Setup of the HSPC-2-SB

Pressure source

The **HSPC-2-SB** requires a stable +/- pressure source to operate properly. For optimal performance from the HSPC, the input and vacuum pressures should not exceed ± 425 mmHg. Higher pressures than this may not damage the valve, but may prevent complete closing of the valve and thus hinder valve performance.

The recommended input pressures are +375 mmHg (7.25psi) and -375mmHg (-7.25psi) or higher simultaneously going into the **HSPC-2-SB** headstage but no more than +/- 425 mmHg.

It is important that the pressure source be filtered for moisture and oils if using house air.

ALA Scientific Instruments manufactures the Pressure/Vacuum system for the **HSPC-2-SB** (catalog number **ALA-PV-PUMP**), that is designed to provide appropriate pressure and vacuum to the respective inlet ports.



The **PV-PUMP** consists of two oil free pumps. One delivers positive pressure and one delivers negative pressure that are configured to be balanced. The **PV-PUMP** will deliver equal pressures to the **HSPC-2-SB** optimizing its performance.

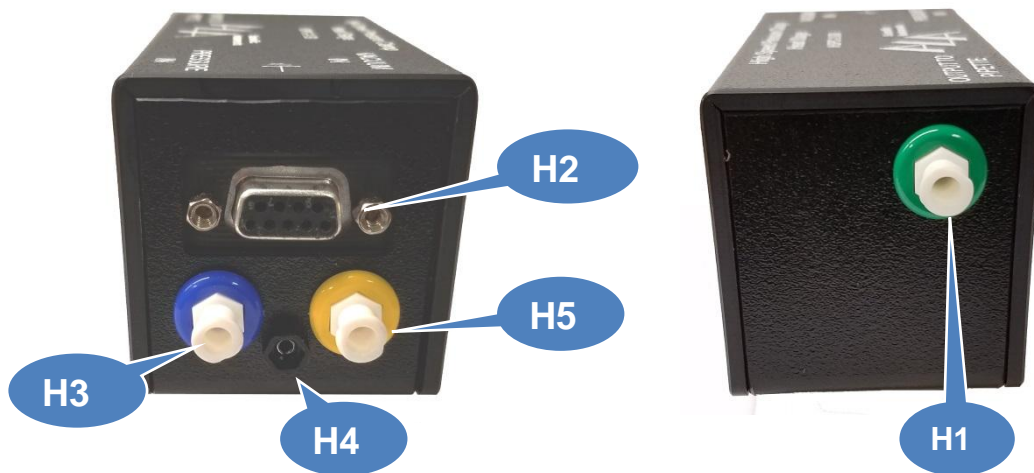
Positioning of components:

ALA Scientific strongly recommends the use of a Faraday cage to minimize electrical noise pickup during patch-clamp recording and the use of an anti-vibration table to minimize damage to seals during recording.

The headstage of the **HSPC-2-SB** should be positioned close enough to the electrode holder of the patch pipette to permit connection to the pipette holder with a 10 cm length of thick wall silicone tubing (provided). Use of long (>10 cm) or distensible tubing (not supplied by ALA Scientific Instruments) will delay and distort the pressure waveform between the headstage and pipette holder. It is not necessary or desirable to bolt the **HSPC-2-SB** headstage to the microscope or table prior to use.

All other electrical components, such as the **HSPC-2-SB** controller and electrically driven pressure or vacuum sources, should be positioned outside of the Faraday cage.

Headstage Connections:



- Connect the output of the **HSPC-2-SB** headstage (H1) to the electrode holder of the patch-clamp with the thick-walled silicone tubing provided. A short length of tubing should be used to minimize compliance. Typically a 10cm length is used.
- Connect the **PRESSURE IN** (H3) to a suitable pressure source (~ +375 to 425 mmHg) with the provided pvc tubing. *
- Connect the **VACUUM IN** (H5) to suitable vacuum sources (~ -375 to -425 mmHg) with the provided pvc tubing. *
- Connect the *headstage* (H2) to the *controller* rear panel (4) using the serial DB-9 cable provided. Secure cables tightly.
- Connect a 2mm mini banana plug to ground (H4) if needed for noise reduction.

*An optional pressure/vacuum pump (**PV-PUMP**) is available from ALA Scientific. It is recommended for use with the **HSPC-2-SB**. See the "**Pressure Source**" section for more information.

With the HSPC-2-SB, it is possible to drive pressures directly from a function generator or acquisition software for gigaseal formation and membrane rupturing (i.e., formation of the whole-cell configuration).

Use of manual methods for application of suction, e.g. by mouth or syringe, can still be used, but the user must provide an air pathway between the HSPC headstage output and input to suction port of the electrode holder.

Controller Connections

All connections are performed from the rear panel of the **HSPC-2-SB** controller..

- Connect the universal power supply (9V DC) provided to the **POWER IN** connector.
- Connect a power cable into the universal power supply. Plug power cable to an AC source. The use of a high quality surge protector is recommended.
- A BNC cable is used to connect the command input to an appropriate analog voltage source. The analog voltage waveform can be derived from any function generator or from commercial electrophysiology experimental control/data acquisition programs (e.g., Axon Instruments pClamp, HEKA Elektronik's PatchMaster, etc.) that generate an output command voltage. However, if the desired experiment requires command waveforms to be generated simultaneously to the voltage-clamp and pressure-clamp, the user should carefully evaluate the specifications of any experimental control/data acquisition software to make certain that the selected software has the required capabilities. Alternatively, a custom signal generator that adheres to the required input limitations (maximum input voltage ± 5 V) may also be used.
- A BNC cable can be connected from the monitor output to an oscilloscope or to an A/D converter to observe or record the pressure measured at the headstage sensor.
- The input command frequency should not exceed greater than 5Hz, otherwise the pressure/vacuum output of the headstage may not keep up with the command signal.

At this point, all electrical and pressure/vacuum connections are complete and the system can be powered on for initial zeroing and use.

Instructions for use during patch recording

This manual presumes a basic understanding of patch-clamp technique by the reader. Many excellent reviews are available (e.g. Reinhold Penner, 1995).

Initial zeroing of the instrument

After the instrument has been set up as described above, attach an appropriately filled electrode to the electrode holder.

The **HSPC-2-SB** may be powered ON by switching the power rocker switch at the rear of the controller.

The HSPC-2-SB display will illuminate and be ready for use.

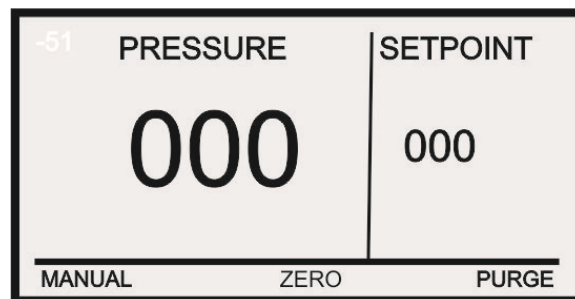
- 1) The **HSPC-2-SB** has an auto zero function that runs upon power ON.

- 2) If the device does not zero.

Press the **ZERO** button located below the display.



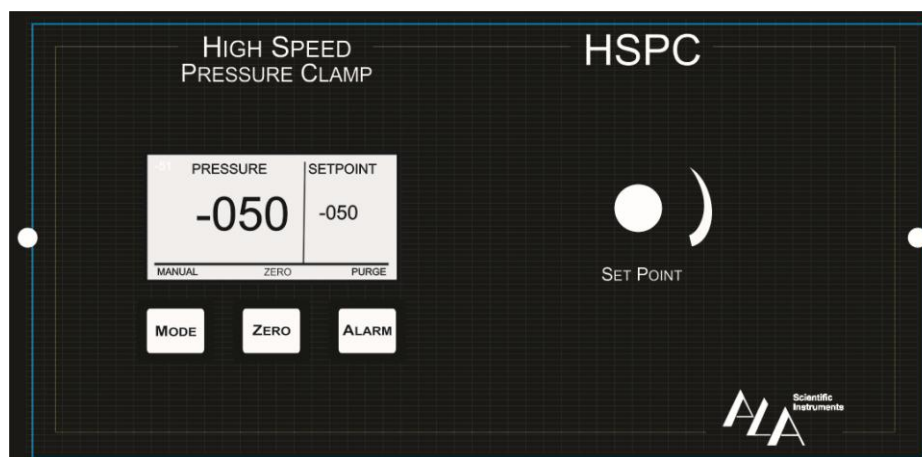
- 3) Once the zero function is complete the display will read as shown. At this point the instrument is zeroed properly and ready to receive an input command voltage or be manually adjusted with the set point to control pressure applied to the patch electrode.



Output pressure will follow input command voltages with a proportionality constant of 20mV/mmHg.

Setpoint adjustments

The HSPC-2-SB output pressures can be adjusted via the SETPOINT rotary switch.



Turn the knob clockwise to adjust the pressure setpoint to a positive pressure and counterclockwise to adjust the setpoint to a negative pressure.

Note: Do not to turn the setpoint knob in a fast manner. The rotary encoder might not detect the turns as easily and the display will not change.

Formation of cell-attached and whole-cell configuration.

A positive outward pressure should be applied to the patch pipette when lowering the pipette through the bath medium. This prevents occlusion of the pipette tip by debris. If you are using a function generator to generate the command voltage, the setpoint adjustment can be used for this purpose.

If you are using a commercial software program, such as pClamp (MDC), CellWorks (npi electronic), or PatchMaster (HEKA Electronic), it is generally more convenient to set this positive pressure using the control panel of the respective program.

After the tip of the electrode is close to the cell (determined by visual inspection under the microscope and/or by an increase in measured resistance during application of a test pulse), negative pressure is applied, using the **HSPC-2-SB** (as described above) to achieve the cell-attached configuration. Negative pressure can again be applied to form the whole-cell configuration. Controlled application of pressure or vacuum by the **HSPC-2-SB** achieves the desired patch-clamp configuration more efficiently and reproducibly than does application of pressure or vacuum by conventional methods.

Execution of pressure clamp protocols

After achieving the desired patch-clamp configuration as described above, both pressure and voltage are controlled by independent negative feedback loops. You can execute complex patch-clamp protocols exactly as you would voltage clamp protocols.

Aspiration of moisture into the headstage

When using this instrument, aspiration with a broken pipette or other conditions may cause moisture to enter the headstage. This moisture sensor is a custom designed capacitance sensor that measures the humidity level or water drops in the tubing. When the humidity level increases or water enters the tubing, the measured capacitance increases which the sensor will measure. Once this capacitance goes above the set level the alarm will trigger. When this happens, the emergency moisture sensor circuitry senses the moisture and prevents damage to the piezoelectric valve.

The input command voltage is disabled; the valve is immediately driven to apply 200mmHg positive pressure to the pipette, and the “Alarm check electrode” message is displayed.



After a moisture alarm, **STOP** the experiment, determine the source of moisture entry and rectify the problem. Disconnect the output tubing from the headstage and let the air blow out of the headstage. Carefully dry the pipette holder and tubing connecting the electrode holder to the **HSPC-2-SB** headstage (sometimes it may be simpler and faster to replace this tubing). The **HSPC-2-SB** piezo valve may be damaged if moisture is not cleared out.

After determining the cause of the alarm and rectifying the problem, the **HSPC-2-SB** can be reset.

Press the “OK” button to start the internal timer. This timer will take at least 1 minute. Remove the output tubing connected to the headstage. Also turn the headstage vertically with the output pointing down. This will also help remove any moisture. The air will now come out and dry out the moisture sensor inside the headstage. Once the moisture sensor dries out to the normal value the system will automatically go from “Alarm” mode to standard mode. If after the initial 1 minute period the sensor is not back to its normal value the system will try again to clear and dry out the sensor.

If after a few minutes the system does not automatically come back to normal you can manually clear the alarm state and restore the **HSPC-2B-SB** to normal operation by doing the following. Press the “Mode” and “Alarm” buttons together. You will enter a configuration mode. This mode is to provide the user the capability of adjusting the threshold of the capacitance sensor when it is too sensitive at the user end or give the ability to disable the smoke sensor when it failed for any unknown reason to prevent lock up. The threshold can be changed from 1 to 63 by rotating the set point control on the front. Increasing the threshold will reduce the chance that the system enters into the alarm mode during the normal operation. The moisture sensor will be disabled completely when the threshold is set to 63.

Note: Although the Alarm function is designed to detect small amounts of moisture entering the headstage, it is not perfect. Moisture vapors can still be introduced into the headstage. Care must be taken to observe the pipette tip for damage to prevent large amounts from being pulled in.

WARNING: If the Alarm continues to sound after one or more resets it is probably indicative of a significant salt intrusion onto the head stage and valve. This condition can damage the electronic valve and must be corrected. Continued usage of the HSPC in this condition will void warranty coverage. If the alarm continues multiple times after re-set, please contact the factory immediately and discontinue use of the HSPC to protect the internal Piezo valve and electronics.

Prolonging the longevity of the piezoelectric valve

The piezoelectric valve in the headstage is designed to last for years under normal working conditions. Several simple steps will maximize its longevity:

- 1) Do not defeat the moisture alarm triggering circuitry.
- 2) Make sure that input air pressure is from a cylinder of purified air or from a house air supply filtered with at least a 5 μ filter. (PV-PUMP is recommended)
- 3) When not using the valve (i.e. when pipettes are being changed), set the setpoint to a small positive pressure (ie. +10mmHg). This small volume of air will flow out of the valve and prevents dust or moisture from entering.

Maintenance and Troubleshooting

With normal usage, no routine maintenance procedures are required other than immediately wiping up liquids spilled on the instrument and cleaning or replacing the contaminated components following moisture-triggered alarms. The HSPC-2-SB has no user serviceable parts. If you have any problems with the HSPC-2-SB, please contact ALA Scientific Instruments (if bought within North America) or your local distributor.

Contact ALA Scientific Instruments by phone or email at the number or address below:

Email: support@alascience.com
Phone: (631) 393-6401

Specifications

Max. input pressure/vacuum : +/-8.2psi/425mmHg

Standard output pressure/vacuum range: +/- 200mmHg

Power: 9V DC

Fuse: 1 A

Weight controller: 4.6lbs (2.1kg)

Dimensions controller: 8.5"(21.6cm) x 7.5"(19cm) x 4"(10cm)

Weight headstage: 0.5lbs (0.23kg)

Dimensions headstage: 3.75"(9.5cm) x 1.75" (4.4cm) x 1.75"(4.4cm)

Typical speed of response: 0 to 100mmHg jump in 15ms: 10 to 90% settling time.

Command input: 20mV/mmHg

Monitor output: 20mV/mmHg

Set point control: holding pressure/vacuum control; +/-200mmHg

Zero auto control: automatic reset of valve set point output to zero.

Moisture alarm: Capacitance liquid detection sensor to protect valve; output 200mmHg

Specifications are subject to change without notice

Pressure Clamp Background

The development of the voltage-clamp enabled stepping of excitable membrane preparations to programmed voltages under closed-loop control. This enormously advanced understanding of the function of voltage-gated channels (Bertil Hille, 1992).

Similarly, the “concentration-clamp” technique enabled rapid application of agonists to ligand-sensitive excised patch preparations and advanced understanding of the function of ligand-gated channels (Peter Jonas, 1995). Mechanosensitive ion channels are also important physiologically (Hamill and Martinac, 2001), but the lack of instruments capable of establishing a “pressure-clamp,” to enable stepping of mechanosensitive preparations to programmed pressures under closed-loop control, has impeded progress in understanding their function.

ALA Scientific Instruments is proud to present the HSPC-2-SB, an instrument that enables any investigator familiar with the patch-clamp technique to perform sophisticated pressure-clamp experiments on mechanosensitive preparations in the excised patch or whole-cell patch clamp configuration.

Drs. Frederick Sachs, Thomas Suchyna and Stephen Besch built the earliest version of the HSPC-2-SB at the University of Buffalo (Besch et al., 2002). The HSPC-2-SB is similar in some respects to an earlier device by McBride and Hamill that employed piezoelectric valves to partition pressure and vacuum sources under closed-loop control (McBride, Jr. and Hamill, 1992). The current HSPC-2-SB features a miniaturized piezoelectric with several innovations that improve response time, stability and valve longevity. These innovations include supporting the piezo bimorph at both ends, minimizing valve dead volume, placing the pressure sensor on the valve body and incorporating a piezo element that does not require a static bias voltage. A final innovation, the moisture-sensitive optical sensor, protects the piezoelectric valve from contact with moisture by triggering an automated application of positive outward pressure when moisture is sensed (Besch et al., 2002).

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Warranty

ALA Scientific Instruments agrees to warranty this product for a period of one year from the date of delivery against any and all manufacturer's defects in material and/or workmanship (other than as noted below). Remedy will consist of repair or replacement at ALA Scientific's discretion. Please report any problems promptly so as not to jeopardize the warranty coverage.

ALA Scientific Instruments does not assume any liability based on the use of this product, whether correct or incorrect, except as specified under law

ALA Scientific Instruments will not warranty any of the plastic connectors or tubing. Use of the HSPC-2-SB in any way that contradicts the instructions of this manual may void the warranty.

If the product does need repair, it must be returned to the factory freight prepaid (freight sent collect will be refused) and in clean condition. If returned parts have been in contact with any liquid substance, documentation of substances used must be provided to ALA Scientific.

This product is intended for use in biophysical experiments with membrane or cellular preparations only.

THIS EQUIPMENT IS NEITHER INTENDED NOR APPROVED FOR CLINICAL USE.