900A

Micropressure System

Serial No._______________________

www.wpiinc.com

092820
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ABOUT THIS MANUAL

The following symbols are used in this guide:

⚠️ This symbol indicates a CAUTION. Cautions warn against actions that can cause damage to equipment. Please read these carefully.

⚠️ This symbol indicates a WARNING. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and TIPS contain helpful information.
INTRODUCTION

WPI’s model 900A micropressure system is designed to measure pressures from –200 to +400 mmHg in small blood vessels, cells and other electrolyte-filled micro-cavities. The micropressure system includes a main electronic Control Unit, Probe and Pressure Pod. Fluid-filled glass micropipettes (supplied by the user) pulled to an outside diameter of 2–5 μm act as microelectrodes when paired with the 900A sensing probe to complete a circuit across the measurement location. A pressure source supplying up to +500 mmHg of pressure and a vacuum source supplying up to –300 mmHg vacuum interfaces through the Pressure Pod to maintain a null pressure, that is proportional to the pressure sensed at the tip of the microelectrode.
Parts List

After unpacking, verify that all items are included:

Each 900A system includes the following components:
(1) 900A Micropressure System Control Unit with power cord
(1) 900AP Probe
(1) 900APP100 Pressure Pod or 900APP Pressure Pod
(1) 900A Startup Kit, which includes:

- MF28G-5 MicroFil 28 ga 97mm long (5)
- CAL900A Pressure Calibration Chamber
- 13661 Potentiometer Tweaker
- 300534 Probe Holder
- TIPTW900A Pre-Pulled μTip Micropipettes (10)
- RC1T Silver/Silver Chloride Reference Electrode
900A Micropressure System

13776
Adapters, Reference Electrode to Ground Jack, 2 mm (2)

5332 Vacuum Trap

13157
Luer Fitting, Female (12) for 3/32" ID red tubing

13161
Luer Fitting, Male (12) for 3/32" ID red tubing

13251
Luer Lock Fitting, Male (12) for 1/16" ID clear tubing

14061
Luer Dead-end Fitting (3)

3742
Luer Female T (3)

3744
10cc Syringe (3)

MEH6SF-10
Microelectrode Holder with Pellet (2)

MEH6SRF-10
Microelectrode Holder with Pellet (2)

400195
Polyurethane Tubing, 15 ft. OD 5/32" (0.156"), ID 3/32" (0.094")

3747
Tygon Tubing, 4 ft. OD 1/8" (0.125"), ID 1/16" (0.063")

13010
SILR Tubing, 1 ft. OD 3/32" (0.094"), ID 1/32" (0.031")
Other Required Items (not included)

Other required items (not included):

- Manometer, such as:
  - WPI # SYS-PM01D (1 psi) or WPI # SYS-PM01R (1 psi) Rechargeable
  - WPI # SYS-PM015D (15 psi) or WPI # SYS-PM015R (15 psi), Rechargeable
  - WPI # SYS-PM100D (100 psi) or WPI # SYS-PM100R (100 psi), Rechargeable
- Voltage recorder (oscilloscope or data acquisition system)
- Pressure and vacuum source (See “Pressure and Vacuum Supply” on page 8)
- Salt solutions

Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled “Claims and Returns” on page 33 of this manual. Please contact WPI Customer Service if any parts are missing at 941.371.1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI’s Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100mm (four inches) of shock absorbing material. For further details, please read the section entitled “Claims and Returns” on page 33 of this manual.

Notes and Warnings

NOTE: Use fresh micropipettes each time it is to be used.
NOTE: Be sure the air pressure source provides clean, dry air.
NOTE: Use micropipettes with tips pulled to 2 to 5µm in diameter.
NOTE: Use a short tube (12" or less) to connect the microelectrode holder to the Pressure Pod tubing port.

CAUTION: Always pay attention to the tube between the holder and the tubing port. If you find that solution drops have been sucked into the tube, set to the Zero Set mode. Disconnect the tube, and use the air pressure supply to blow the solution drops out. Leaving solution in the pressure tube may damage the Pressure Pod.

CAUTION: It is important that the pressure and vacuum sources supply clean, filtered, dry air. Air that is not clean and dry damages the piezo valve, and damage of this sort is not covered under warranty.
**CAUTION:** If the microelectrode is connected to the Pressure Pod before the pressure and vacuum sources are connected and turned on, fluid may be pushed through the Fluid Trap and into the Pressure Pod causing damage to the piezo valve. *This damage is not covered under warranty.*

**CAUTION:** Before moving the micropipette from one recording site to another, set the Loop Status switch to Zero Set. This ensures that no pressure is applied to the micropipette while it is being moved. If this is not done, the pressure pod tries to compensate the increased tip resistance (caused by the open circuit) by applying pressure to the micropipette. This may expel the filling solution from the micropipette.

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**INSTRUMENT DESCRIPTION**

**System Overview**

The 900A uses an ion gradient at the tip of sensing electrode to measure pressure. The internal pressure of the microelectrode is continuously monitored and adjusted to equal the pressure outside the tip, to keep the ionic gradient in dynamic equilibrium. The dynamic equilibrium of the ion concentration at the tip of the microelectrode is continuously maintained as part of a loop circuit formed between the microelectrode, an electrolyte-filled bath and the 900A. This circuit serves as an electrical feedback loop that is monitored for deviations. Once the circuit is set, disruption in pressure outside the microelectrode's tip will directly affect the resistance in the circuit by offsetting the ionic concentration at the tip of the microelectrode. The 900A senses the ionic alteration caused by the pressure change.
The 900A then signals the Pressure Pod to maintain the circuit to the equilibrium (null) value.

Within the Pressure Pod, a piezoelectric gas valve actively regulates vacuum and pressurized air in and out of the Pressure Pod. The pressure of the incoming air is greater than the vacuum, creating a net positive pressure when the piezoelectric valve is open. The piezoelectric gas valve within the Pressure Pod is continuously functioning at a 0.5 ms response rate, so when a pressure offsets the ionic equilibrium in the microelectrode, the Pressure Pod promptly reacts to supply an opposing pressure that adjusts the circuit back to its null value. The amount of pressure required by the Pressure Pod to maintain equilibrium of the circuit is equal to the pressure outside the tip of the microelectrode. This applied pressure is sensed by a pressure transducer within the Pressure Pod and is digitally displayed on the 900A's front panel. A voltage signal proportional to the displayed pressure reading is simultaneously sent to the Pressure Signal output connector that can be connected to a recording device, such as an oscilloscope or other data acquisition system.

**Response Rate**

The response rate of the piezoelectric valve (from fully closed to fully open) is 0.5 ms. The response rate of the overall system, when properly configured, is typically less than 10 ms. This figure is affected, however, by the pressure chamber's residual volume, which includes the micropipette, fluid trap, connecting tubing and the pressure transducer piezoelectric valve outlet, due to the compressibility of air. Long lengths of tubing add dead space, which slows overall system response. Keeping the residual volume low by mounting the Pressure Pod close to the microelectrode and using short lengths of small-bore tubing minimizes dead space and contributes to a rapid system response.

**Using Sensitivity and Damping to Correct Oscillations**

All feedback control systems tend to become unstable and oscillate under certain conditions. The 900A provides two controls for combating instability and oscillation—**Sensitivity** and **Damping**. Decreasing **Sensitivity** or increasing **Damping** often reduces or stops oscillations. The proper settings for both controls depends on the particular application.

Keep the **Sensitivity** knob as high as possible to provide close matching and following of the external pressure by the internal pressure in the micropipette. We recommend starting at the 12:00 position, then adjusting in a counterclockwise direction, as needed. Generally, to maintain proper response, **Sensitivity** should not be set below one-third of the full rotation.

The **Damping** knob adjusts the amplitude of feedback. Turning the damping control clockwise reduces the amplitude of the feedback. Turning the damping control counterclockwise increases the amplitude of the feedback. Starting at the 12:00 position, one-quarter to one-third rotation typically produces a stable, quiet baseline.
Circuit Discussion

The 900A consists of an oscillator, head stage, phase detector, buffer amplifier, pressure transducer and Pressure Pod.

Fig. 3—(Right) The circuit is complete when the reference electrode is immersed in the solution at the measurement site, along with the microelectrode, and both the probe and the reference electrode are properly connected to the 900A system.

**OSCILLATOR:** A Wein bridge oscillator generates a 1000 Hz sinusoidal voltage. This is amplitude stabilized by internal negative feedback transistors in the bridge circuit.

**HEAD STAGE** (or **FOLLOWER**): A 1000 Hz carrier constant current is injected through the microelectrode. The resulting voltage drop is compensated in the follower circuit by advancing the Electrode Resistance dial so that balance null is achieved. The microelectrode resistance is then read directly in KΩ from the dial. If the microelectrode resistance changes, a 1000 Hz imbalance signal is amplified and detected by a phase detector.

**PHASE DETECTOR:** This is a synchronously switched detector in a single integrated circuit package. The resulting detected signal is filtered and is available at the output of the amplifier.

**BUFFER AMPLIFIER:** The buffer amplifier lies between the detector and the Pressure Pod driver. It compensates the detector signal with output from the pressure transducer in order to compensate for the inherent lag in propagating the pressure changes transmitted to the micropipette tip. The lag would normally cause the system to oscillate. The Damping knob sets the amplitude of the buffer amplifier.

- Insufficient damping (For example, the Damping knob is turned too far counter clockwise) causes the system to oscillate at higher frequencies.
- Too much damping (For example, the Damping knob is turned too far clockwise) can result in a slow rate of response and low frequency oscillations.

**PRESSURE TRANSDUCER:** The pressure transducer is a silicone strain gauge resistance bridge device. Its volume and pressure displacement are small, and its sensitivity is high. The bridge output is amplified, scaled and displayed on the digital meter. The meter has a resolution of 0.1 mmHg from +199.9 to −199.9 mmHg. (The 900APP100 is capable of measuring ±100 mmHg.) Pressures outside this range can be viewed by toggling the Range switch to High. The pressure output can also be monitored via the BNC connector marked Output on the front panel. The output level of this signal is 10 mV/mmHg.
PRESSURE POD: The pressure pod consists of a driver circuit to operate the piezoelectric controller and an alarm circuit to warn when the maximum pressure of the controller has been reached.

Getting Started
Pressure and Vacuum Supply

The maximum pressure that should be applied to the pressure pod is +500 mmHg. The maximum recommended vacuum is –300 mmHg. In the Auto mode, the system can compensate for small fluctuations in the supply pressures, but large supply pressure changes may cause a pressure reading error. For accurate pressure change readings, both pressure and vacuum sources should be stable.

If your experiment involves pressure measurements consistently lower than the rated maximum, you can use lower supply pressure and vacuum. For example, if pressure readings at the pressure measuring site are fairly consistent and slow-changing at approximately 150 to 200 mmHg, a positive supply pressure of +300 mmHg would suffice. If your pressures are much lower and rapidly changing, a positive pressure of 250 mmHg and vacuum of -60 will be ideal.

To keep the pressure gradient high and ensure a quick, accurate response, plan to provide positive/negative pressures at least 50 mmHg greater than the maximum positive/negative pressure to be measured. Pressure differentials of less than 50 mmHg are inadequate to move air quickly, causing response times to increase considerably.

CAUTION: It is important that the pressure and vacuum sources supply clean, filtered, dry air. Air that is not clean and dry damages the piezo valve, and damage of this sort is not covered under warranty.

Microelectrodes

The microelectrodes used here are glass pipettes filled with an electrolyte solution. The shape of the microelectrode tip significantly affects the 900A’s response. Shorter tipped microelectrodes that do not have an excessively long taper (similar to a patch clamp tip) work best. Microelectrodes with internal tip diameters of 2-5µm are suggested (WPI #TIPTW900A). Within this size range, the microelectrodes will operate within the normal parameters described here. Pipettes can be pulled from glass capillary tubes to these specifications with an appropriate glass puller device (WPI #PUL-1000). Tip size can be estimated based on the resistance value measured on the Electrode Resistance dial.

TIP: Microelectrodes are best utilized if the tip is first soaked in the appropriate electrolyte solution prior to filling.

Salt Solutions

Prepare salt solutions before you begin. To measure a change in resistance, the 900A relies on a steep concentration gradient. This gradient should approximate a 10×
change. For example, if the microelectrode is filled with 1 M NaCl, the external bath should be 0.1 M NaCl. NaCl is a common choice for 900A users. KCl has been used well in this system, though it should be noted that KCl is a well-known agent used to stop the heart. You may alternately choose to utilize a K-free physiological saline, such as a modified phosphate buffered saline (PBS), when working with live animals (e.g. zebrafish, etc.) For example, K⁺-free PBS works well with a 10× solution in the microelectrode and 1× solution in the electrolyte bath. (See recipe below). Traditional PBS with K⁺ is suitable for live cells in culture. Other physiological salines also contain a similar osmolality of electrolytes and can also be well-utilized.

**TIP:** Alcian blue can be added to the microelectrode solution to add a blue tint. Alcian blue is conductive and in small amounts can add excellent contrast to the microelectrode for enhanced viewing. Microelectrode solution should not contact measurement sites. Other solutions will also work well, if the ionic gradient is maintained. Appropriate physiological solutions for experimental scenarios should be determined experimentally to suit the physiology of the organism or recording site, when appropriate.

When working within physiological conditions, it is advantageous to shift the concentration gradient inside the pipette tip such that the tip contains a solution that is more ionically compatible with the fluid outside the tip. (See “Adjusting Electrode Resistance” on page 20.) This will create a longer diffusion path between the high concentration electrolyte bath solution and the outside of the top. This will also create a resistance value that is slightly greater than the intrinsic value and could affect the rate of response to externally-applied stepped pressure, but this is not perceptible in most applications.

**Recipe for 10× K⁺-free Phosphate Buffered Saline**

<table>
<thead>
<tr>
<th>Mass (g)</th>
<th>Chemical</th>
</tr>
</thead>
<tbody>
<tr>
<td>75.97</td>
<td>NaCl</td>
</tr>
<tr>
<td>12.46</td>
<td>Na₂HPO₄</td>
</tr>
<tr>
<td>4.8</td>
<td>NaH₂PO₄</td>
</tr>
</tbody>
</table>

1. Add chemicals to a 1 L container with 850 mL double distilled H₂O.
2. Stir until dissolved.
3. Adjust pH to desired value.
4. Add enough water (QS) to bring the quantity to 1L.
5. Dilute 1:10 to create electrolyte bath solution that will be close to 290 mOsm.

**CAUTION:** High concentration salt solutions will evaporate and crystalize quickly and can damage the glass microelectrode tip.

**WARNING:** Potassium will stop the heart.
Experimental Containers

A sturdy container (petri dish) of adequate depth will be able to hold an appropriate depth of electrolyte bath solution and accommodate the experimental subject. 2-3% agarose filled petri dishes are commonly used to hold organisms. The agarose is covered with electrolyte bath with the reference ground electrode secured within or over the agarose. Z-molds (WPI #Z-MOLDS) can create well defined depressions in the agarose that greatly help when stabilizing organisms or other vessels. The depressions can also allow for conductivity between the microelectrode and the reference electrode so a shallow layer of electrolyte bath can be used.

Fig. 4—Z-MOLDS come in four different patterns. Press into freshly poured agarose and allow the agarose to solidify. Remove the mold. The impressions make it easy to hold embryos.

Micromanipulators

A micromanipulator is required to hold the Probe and microelectrode. Though the probe could conceivably be moved into the recording site by hand, it is unlikely that the small microelectrode tip will remain undamaged without fine movements capable with a micromanipulator.

CAUTION: The experimental platform should be stable. Micromanipulators and measurement sites need to be static and secure. Small movements between the recording site and microelectrode can damage the fine glass tip.

Viewing and Imaging

A stereo microscope is suggested for viewing the movement of the microelectrode as it penetrates into the experimental subject. As the microelectrode begins to penetrate, the circuit is broken if the tip of the microelectrode becomes blocked by tissue or other barricades. This can cause an alarm in the circuit (See “Alarm” on page 11.), which can result in fluid being drawn into the microelectrode tip, or eventually
into the Pressure Pod. Fluid in the Pressure Pod will damage the servoelectric valve. It is further important to monitor the progress of the microelectrode to know when the microelectrode is within the recording site. An imaging system is also advantageous to monitor variation between experimental subjects and for monitoring and recording of timed experiments.

**Alarm**

The 900A alarm sounds:
- When the Pressure Pod valve is completely open to indicate that maximum pressure has been reached.
- When electrical continuity is broken for any reason. For example, if the microelectrode comes out of the solution, the filling solution level drops below the Ag/AgCl pellet, the ground reference is disconnected, etc. or the tip is blocked, or if the sensitivity is adjusted too high.

It is crucial that you respond to alarms immediately. In each scenario, the eventual result is potential damage to the sensitive components of the Pressure Pod. Quick response to the alarm prevents this damage from occurring. When an alarm sounds, return the 900A to the Zero Set position. This will bring the system out of dynamic operation and into a “safe mode.” See “Operating Instructions” on page 12 for more information.

**Liquid Trap**

A Liquid Trap is provided with the 900A and should be installed between the micropipette holder and the 900A Pressure Pod to prevent intrusion of liquid into the Pressure Pod.
Fig. 5—Refer to this diagram when setting up the 900A micropressure system. Be sure the setup is complete before you turn on the power.

**Setting Up and Powering the 900A**

1. Line up the pins and connect the seven-pin cable on the Pressure Pod to the connector labeled Pressure Pod on the right side of the front panel of the 900A.
2. Connect the 900A Probe to the connector labeled Probe on the left side of the front panel of the 900A.
3. Connect the tip of the 900A Probe to CKT and CHAS ground connections using a banana to 2 mm pin adapter (WPI #13776).
   **NOTE:** The CKT and CHAS (circuit and chassis) ground connections should be electrically connected with a metal strap, so either ground connection is suitable.
4. Connect the power cord to the receptacle on the rear panel of the 900A. Plug the other end into a wall socket.
5. Using adapters 13776, connect the reference electrode from the CAL900A or the experiment to the ground terminal marked CKT (or CHAS) on the front panel of the 900A.
6. Place the Loop Status switch in the Zero Set position. This maintains a pressure of 0 mmHg in the pressure chamber.
7. Set the Damping and Sensitivity control knobs to the 12:00 position.
8. Set the **Electrode Resistance** dial fully counterclockwise to zero.

9. Set the **Offset** switch to the **Off** position.

10. Set the **Pressure** switch to **Off** position.

11. Turn the power switch on and let the system warm up for 15 minutes.

12. Using a BNC cable, connect the data acquisition port marked **Pressure Signal** on the right side of the 900A front panel to an oscilloscope or data acquisition system.

### Zero Adjusting the Pressure Pod to Ambient Pressure

1. Verify that the **Loop Status** is set to **Zero Set** and the system has warmed up for at least 15 minutes.

2. In the **Zero Set** mode, applying no pressure or vacuum to the **Pressure Pod**, insert a screwdriver into the recess on the **Pressure Pod** marked **XDCR Zero** and turn clockwise or counterclockwise until the digital meter on the 900A reads 0 mmHg.

![Fig. 6—Using a small screwdriver, adjust the XDCR Zero until the meter reads zero.](image)

### Applying Pressure and Vacuum and Re-zeroing

1. Verify that the **Loop Status** switch is set to **Zero Set**.

2. Use a pressure manometer to regulate the source of pressure and vacuum for the 900A **Pressure Pod** to the desired pressure (e.g. +350 mmHg for the pressure, and −150 mmHg for the vacuum.) If you are using a the WPI #PM015 Pressure Manometer to monitor the pressure, use port B on the meter for measurement.

**CAUTION**: Do not connect the pressure or vacuum until you are sure the values are within the safe pressure ranges for the pod. The maximum pressure is 500 mmHg and the maximum vacuum is −300 mHg. Pressures outside of
the safe operating pressure or vacuum can damage the Pressure Pod. This sort of damage is not covered under warranty.

**NOTE:** The pressure and vacuum sources may be regulated to pressures other than those suggested here, however, IT IS CRUCIAL THAT THE PRESSURE SIDE ALWAYS BE OF A GREATER ABSOLUTE VALUE THAN THE VACUUM SIDE, AND THAT THERE IS A MINIMUM OF 75 mmHg DIFFERENCE BETWEEN THE PRESSURE AND VACUUM SOURCES.

3. Connect these regulated pressure and vacuum sources to their respective input ports on the Pressure Pod.

4. Block the To Micropipette port on the pressure pod. A finger can be used for this operation if it’s held firmly against the port opening. (Fig. 7)

5. Keeping the To Micropipette port blocked, insert a screwdriver into the recess marked Pressure on the 900A front panel, and turn clockwise or counterclockwise until the digital meter reads 0 mmHg. (Fig. 8)

6. Now open the pipette output by removing your finger. The panel pressure meter should remain at 0 mmHg. If the unit has been properly re-zeroed, very little change occurs when the tubing port is opened.

Connect vacuum and pressure with Luer lock fitting 13251 and tubing 3747 — or Luer fitting 13161 and tubing 400195.

**Fig. 7**—Use your finger to block the To Micropipette port on the top of the Pressure Pod.  
**Fig. 8**—Use a screwdriver to adjust the recessed Pressure screw until the meter reads zero.

**Fig. 9**—Remove your finger to open the To Micropipette pressure port.
NOTE: If you are unable to get a 0 reading, turn off the 900A, disconnect the pressure and vacuum supplies and repeat the previous steps in this section.

Seting Up the Measurement Site

NOTE: It is advantageous if the following steps are completed using the CAL900A as your test measurement site. The CAL900A creates a stable platform to test the system and calibrate the voltage output signal to known pressures for data recording. This accessory is sold separately. If you are not using the CAL900A, a petri dish is perfectly adequate to prepare the system as described below. The following directions assume you are using the CAL900A.

1. Prepare the two electrolyte solutions:
   - Microelectrode Solution (e.g. NaCl)
   - Electrolyte Bath Solution (e.g. NaCl)

2. Put the Electrolyte Bath Solution into the CAL900A Pressure Calibration Chamber. The level should be enough to cover the Ag/AgCl pellet in the CAL900A.

3. Connect the integrated blue ground (Ag/AgCl pellet) connection of the CAL900A to the CKT or CHAS ground connection on the 900A using a banana to 2 mm pin adapter (WPI #13776).

NOTE: The CKT and CHAS ground connections are electrically connected with a metal strap, so either ground connection is suitable.

TIP: Connecting the CAL900A to the ground on the 900A will allow a completed circuit when the microelectrode is inserted in the CAL900A chamber. As such, the integrated Ag/AgCl electrode in the CAL900A now serves as the reference electrode, for testing the system. If you are NOT using the CAL900A, fill a petri dish with
Electrolyte Bath Solution, and immerse a dry Ag/AgCl reference electrode. Connect the reference electrode to the 900A through the CKT or CHAS ground connection, as with the CAL900A.

Preparing the Microelectrode and Microelectrode Holder

REMINDER: Use a micropipette pulled to a tip diameter of 2–5 µm. A high concentration microelectrode solution is important in order to maintain a large concentration gradient between the microelectrode’s internal salt solution and the solution outside the tip.

1. Prepare the micropipette for filling by immersing the tip into the microelectrode solution and allowing the solution to wick up into the tip of the micropipette. This decreases the possibilities of air bubbles. Ideally, the pipette tip should be set into solution for several hours or longer prior to operation.

   TIP: Utilize an electrode storage receptacle (WPI #E210) to prepare several micropipettes for filling, ahead of time.

Fig. 10—(Right) The E210 storage jar is perfect for safely storing your pulled micropipettes. You can even fill the jar with your filling solution so you can immerse the tips prior to the study.

2. Backfill the shank of the micropipette using a microelectrode filling needle and syringe. WPI’s flexible MicroFil filling needles (WPI #MF28G67-5) are ideally suited for this application. Do not allow air bubbles to remain in the pipette.

   NOTE: The micropipette, filled with microelectrode filling solution is now a microelectrode.
CAUTION: Protect the microelectrode tip from damage. Limit exposure of the filled microelectrode to the air. Evaporation of the microelectrode solution can cause salt crystals to form in and around the tip. This can damage the tip of the micropipette. It is recommended that the tip be immersed in the microelectrode solution (i.e. in an electrode storage jar) prior to inserting into the electrode holder. Once the microelectrode is connected to the microelectrode holder, the tip should likewise remain immersed in solution whenever possible to prevent the formation of salt crystals and damage to the glass.

3. Fill the microelectrode holder, making sure that no air bubbles remain in the holder, and that the filling solution covers the Ag/AgCl pellet at the back of the microelectrode holder.

4. Insert the microelectrode into the microelectrode holder and tighten the cap.

5. Check again to be sure that no air bubbles remain in the microelectrode or the microelectrode holder. Tightening the cap can expose any otherwise unseen bubbles. Examine them carefully under magnification, if necessary.
Fig. 14—Here you can clearly see a bubble trapped in the microelectrode holder.

**NOTE:** It is crucial that the fluid maintains contact with the Ag/AgCl pellet inside the microelectrode holder to complete the circuit.

6. Install the microelectrode assembly into the CAL900A cap, and secure the cap by screwing it onto the base of the CAL900A for a good pressure seal.

Fig. 15—Carefully slide the microelectrode into the CAL900A chamber.

**NOTE:** Creating a good pressure seal is important for calibration. Be careful not to break the pipette tip when inserting it into the cap or in the CAL900A. Be mindful that the tip of the micropipette can strike the ground electrode or the CAL900A chamber wall.

7. Verify that the Loop Status switch is at **Zero Set**.

8. Insert the 900A Probe into the microelectrode assembly. The Null Detector will go to a hard left position.
Fig. 16—Carefully insert the tip of the probe into the end of the microelectrode.

**NOTE:** If the needle of the Null Detector meter is deflected to the left of zero, then the pipette tip resistance is in fact greater than the value indicated on the Electrode Resistance Dial. Conversely, if the Null Detector needle is deflected to the right of zero, the pipette tip resistance is less than that indicated on the Electrode Resistance Dial. When the Null Detector is at zero, the resistance of the pipette is the value that appears on the 10-turn potentiometer (Electrode Resistance Dial).

- Needle Deflected Left: Tip resistance is greater than the pot reading
- Needle Deflected Right: Tip resistance is less than the pot reading
- Needle On Zero: Tip resistance is equal to the pot reading

Fig. 17—The Null Detector Meter deflection points to the actual value of the tip resistance.
Adjusting Electrode Resistance

1. Begin turning the Electrode Resistance dial clockwise. The needle of the Null Detector display eventually moves from the left to right. Adjust the dial until the Null Detector needle is in the zero position.

2. Take note of the resistance reading on the 10–turn resistance adjustment potentiometer. This is the actual resistance of the pipette tip in proper equilibrium. Each full turn is 1000 KΩ of resistance. The correct electrode resistance for a 1 molar NaCl or KCl solution should be between 250 KΩ to 400 KΩ.

Fig. 18—The resistance shown is the actual resistance at the pipette tip. In this case, that is 300 KΩ.

**TIP:** If the electrode resistance is more than 400 KΩ, it is likely that there is an air bubble somewhere in the pipette between the tip and the Ag/AgCl pellet. If the resistance is less than 250 KΩ, it is likely that the pipette tip inside diameter is greater than 5 µm. This may happen if the tip is broken.

**TIP:** If your electrode solution is of higher conductance (e.g. PBS), the resistance value indicated on the potentiometer may be much lower compared to that of NaCl. For example, a 2 µm tip filled with PBS may read closer to 300 KΩ, instead of 400 KΩ. Likewise a 5 µm tip may read less than 200 KΩ.

**CAUTION:** The system will NOT operate correctly with an air bubble in the system.

Connecting the Pressure Pod to the Microelectrode Assembly

1. Prepare a 12-inch (30 cm) or shorter length of small-bore tubing. This will be used to connect the Pressure Pod to the microelectrode holder.

2. Cut 12” tube into two sections – a short section and a long section. (See to Fig. 19).

3. Install the liquid Trap as shown in Fig. 19.
4. Connect the tubing and fluid trap to the **Pressure Pod**, but **do not connect this tube to the microelectrode yet!**

**CAUTION:** If the microelectrode is connected to the **Pressure Pod** before the pressure and vacuum sources are connected and turned on, fluid may be pushed through the liquid **Trap** and into the **Pressure Pod** causing damage to the piezo valve. This can also occur if there is significant fluctuation in the pressure or vacuum sources. This damage is not covered under warranty.

5. Verify that the pressure and vacuum sources are at appropriate values and re-zero, if necessary.

6. Connect the **Pressure Pod** to the microelectrode assembly.

7. Confirm that the reading on the **Null Detector** is still at zero. Some adjustment to the **Electrode Resistance Dial** may be necessary, but the value should still be within 250-400 KΩ.

### Preparing the Microelectrode for Operation Mode

1. Verify that the **Loop Status** switch is set to **Zero Set** and the **Null Detector** is at zero.

2. Turn the **Electrode Resistance** dial clockwise gradually. The **Null Detector** meter moves to the right.

3. Adjust the **Electrode Resistance** dial until the reading on the **Null Detector** is about 75. Take note of the **Electrode Resistance** dial reading.

**NOTE:** By deflecting the needle of **Null Detector** to the right, you will set the system to a resistance that is less than the **Electrode Resistance** dial reading. You already know your electrode resistance. When you adjust the **Electrode Resistance** dial so the needle goes to the right, the resistance at the tip doesn't change while in the **Zero Set** mode, despite the value indicated on the **Electrode Resistance** dial. However, that is about to change.
CAUTION: If an alarm sounds and continues during the next step, immediately switch the Loop Status switch back to the Zero Set position. A short blip of sound is ok.

4. Switch the Loop Status switch to Auto. The electronic feedback system will signal the Pressure Pod to allow the electrolyte bath solution to be drawn up into the tip of the microelectrode until the Null Detector reaches approximately the zero position (0 µA), but should be slightly negative (pressure will read about –1 to –3 mmHg). This is because the height of the filling solution now exerts pressure on the electrode tip.

NOTE: When switched into the Auto mode, the electronic feedback system automatically adjusts the microelectrode tip resistance to the value noted on the potentiometer, selected in Step 2, which offset the Null Detector to read 75.

Resetting the resistance to a higher value is achieved, because the system automatically adjusts to the Null value (0 µA) by drawing in the electrolyte bath solution, which is less conductive, thus has increased resistance.

If the Null Detector pointer fails to move when the Electrode Resistance dial is adjusted, one of two situations may have occurred:

A. An external pressure applied to the micropipette was large enough to force an excessive amount of dilute solution into the tip, resulting in a resistance greater than 10 MΩ.
   1. To correct this, set the Loop Status back to Zero.
   2. Turn the Electrode Resistance dial down to the original setting, as described above.
   3. Turn the Loop Status switch to Auto. The 900A will then adjust the pressure inside the micropipette back to the original value by expelling the dilute electrolyte bath solution, to decrease the tip resistance to its original value.

Another method of purging the micro-pipette tip is to follow the procedure described below in “Setting Pressure Mode” on page 27.

B. The microelectrode is not making contact with the electrolyte solution, or the reference electrode is not connected. In either case (A or B), the problem is an open circuit.

If adjusting the Electrode Resistance dial or changing the Loop Status switch to Auto has no effect, check the placement of the microelectrode, the reference electrode, the liquid level in the microelectrode holder and all connections.

NOTE: Take care not to leave the Loop Status switch on Auto, to avoid discharging the contents of the microelectrode.

5. View the signal continuously on an oscilloscope or data acquisition system. The pressure signal oscillates.
CAUTION: Never move the microelectrode between measurement sites without first switching the Loop Status to Zero Set. This can cause damage to the Pressure Pod.

The control valve within the Pressure Pod is very sensitive to salt water. If microelectrode solution or electrolyte bath solution gets into the valve, there is a high probability of irreparable damage. This control valve is the most expensive component in the Pressure Pod. Make sure the liquid Trap is in place, and that it is in an upright position. It is a good idea during the setup process to always pay attention to the possibility for migration of liquid into the Pressure Pod by constantly monitoring the tube and liquid Trap! Damage caused by water intrusion is not covered under warranty.

Adjusting the Sensitivity and Damping

REMINDER: Use the Sensitivity and Damping controls to reduce oscillation to lowest level possible. The sensitivity adjustment process can lead to system failure. This is because when the sensitivity is continuously reduced, at some point the signal becomes unstable. This results in the signal going off-scale in the positive or negative direction. When this happens, the phase locked loop amplifier will lose its “lock” and the carrier signal will be lost, causing the control signal to the Pressure Pod to go to its extreme limit. An alarm will sound. If this happens, immediately turn the sensitivity control back in a CLOCKWISE direction to regain control of the signal and re-establish stability. By experimenting with this process a few times, an adjustment for minimum level of oscillation can be achieved while still maintaining control of the signal. During this process a vacuum may be applied to the Pressure Pod, which could draw liquid into the Pressure Pod. Always use the fluid trap and monitor the tubing to mitigate any possible damage to the Pressure Pod.

Fig. 20—(Right) Sensitivity and Damping controls are used to modulate the oscillations.

See “Using Sensitivity and Damping to Correct Oscillations” on page 6.

1. The Sensitivity control should be adjusted first by adjusting the knob slowly counter-clockwise from the 12:00 position, to decrease the sensitivity. This will generally be about one third of a turn from the 12:00 position. As the sensitivity is reduced, the oscillation should become smaller.

Again, as you reduce the sensitivity, the signal will eventually become unstable and go off-scale and must be adjusted to bring the signal back on-scale quickly and with care (see warnings above and in the introduction). The Sensitivity dial will likely never be turned past one third of a turn from 12:00.
2. Use the **Damping** to smooth the signal by adjusting clockwise approximately 1/4 - 1/3 of a turn from 12:00 position. Over-adjustment of **Damping** will not cause system failure, but excessive damping will reduce sensitivity.

3. Once the signal is stabilized, switch on the **Pressure Offset** control and adjust the dial to remove any offset voltage displayed on the digital pressure meter. By adjusting the **Pressure Offset**, you can bring the meter reading on the 900A display to zero. However, it should be noted that the **Pressure Offset** control affects the meter only, not the pressure signal. If you are recording the signal, as most people are, the **Pressure Offset** meter reading is inconsequential.

![Fig. 21—Turn on the pressure offset and use the dial to bring the 900A display to zero.](image)

4. If you are not taking immediate readings, switch the **Loop Status** control to the **Zero Set** position.

**Calibrating the Pressure Signal**

It may be desirable to calibrate the voltage output signal to known pressures within the expected range of your experimental system. This is commonly done when conducting continuous data recordings from the voltage output signal (**Pressure Output** port). While the microelectrode assembly is connected to the CAL900A, any increase (or decrease) in pressure that is applied to the CAL900A chamber will be sensed by the 900A, as long as the **Loop Status** is in **Auto** mode.

The CAL900A is equipped with a Luer fitting on the side that can be connected to a pressure source for calibrating the voltage signal to known pressure values. This is done by adding known pressure pulses into the CAL900A chamber through the Luer fitting. This pressure will be sensed by the 900A circuit and will be output as voltage from the **Pressure Output** BNC port.
Moving the System to the Recording Site

**CAUTION:** Before moving the microelectrode from one recording site to another, set the Loop Status switch to Zero Set. This ensures that no pressure is applied to the micropipette while it is being moved. If this is not done, the Pressure Pod tries to compensate against the increased tip resistance (caused by the open circuit) by applying pressure to the microelectrode. This may expel the filling solution from the micropipette. So, **ANYTIME** measurements are not being made, switch the Loop Status control to Zero Set mode. This is the safest way to leave the system when you must step away.

1. Set the Loop Status knob to Zero Set.

2. Prepare the recording site by filling a container (e.g. petri dish) with electrolyte bath solution.

3. Unplug CAL900A from the ground on the 900A. The needle on the Null Detector will deflect to the far left position upon breaking the circuit. If the Loop Status is in the Auto mode, an alarm will sound.

4. Place reference electrode into the recording site. Be sure the reference electrode is immersed in the electrolyte bath solution and connected to the CKT or CHAS ground on the 900A.

5. Carefully remove microelectrode assembly from the CAL900A without damaging the microelectrode.

6. Attach probe with microelectrode assembly to a micromanipulator.

7. Using the micromanipulator, advance microelectrode until the tip is immersed in the electrolyte bath near the desired recording site. If the reference electrode is also submerged in the electrolyte bath, the circuit should be complete, and the Null Detector should again read near 75. Slight adjustments may be necessary to bring the Null Detector to near 75.
Fig. 23—The Probe is mounted on a micromanipulator, and the tip is advanced into the measurement site. Note the reference electrode (also in solution at the measurement site) completes the circuit.

NOTE: If microelectrode solution leaks from the microelectrode or is otherwise disturbed, it may be necessary to re-zero the system. All steps above can be repeated near the recording site, if the microelectrode tip is immersed in electrolyte bath solution with a reference electrode connected to ground. So, re-zeroing can be done in the electrolyte bath solution without moving the assembly back to the CAL900A. Further, it is advisable that movement between measurement sites be economized to lessen the chances of accidental damage of the tip.

8. Again turn the Loop Status to Auto. The Null Detector should deflect back to near zero, as the 900A controls the pressure in the microelectrode to generate the tip resistance preset on the Electrode Resistance Dial.

9. Monitor the signal on the recording device. If the Null Detector reading or the pressure reading is unsteady, adjust the Damping and/or the Sensitivity dials. If this does not correct the instability, there may be air bubbles in the micropipette/holder system.

10. Advance the microelectrode into the recording site.

NOTE: If the tip becomes blocked, the circuit will be open and the alarm will sound. A short (<1 s) signal is common when advancing across a biological membrane. Signals longer than one second should be mitigated by immediately setting the Loop Status to Zero Set to avoid damage to the system. Confirm that no damage to the microelectrode occurred by checking the resistance and Null Detector as above.

11. Record the pressure at the measurement site.
Setting Pressure Mode

Setting the pressure mode allows you to preset the internal pressure of the micropipette. This is useful for:
- Applying positive pressure to flush the tip
- Applying negative pressure to draw solution into the tip.

This feature is most commonly used to purge the micropipette tip when too much dilute solution has diffused into the microelectrode. Applying a few mmHg of positive pressure gently expels filling solution from the shank of the micropipette into the tip.

The tip resistance can be monitored by watching the Null Detector.
- If the tip resistance is greater than the dial setting, the needle points left.
- If the tip resistance is less than the dial setting, the needle points right.

To enter the Set Pressure mode:

1. Toggle the ± Pressure switch to the center or off position, and rotate the Set knob to 0.

![Set knob and pressure dial](image)

Fig. 24—Set the switch to off (center position), and rotate the dial to zero.

2. Switch the Loop Status control to Set.

3. Select + for positive or – for negative pressure, using the Pressure toggle switch. A selected pressure can be applied to the inside of the micropipette.

4. Turn the Set knob to select the magnitude of the pressure. The selected internal micropipette pressure is displayed in mmHg on the digital meter.
Measuring DC Potential

Although the primary function of the 900A is to measure pressures, it can also be used to measure DC potential at the microelectrode site.

1. Connect a recording device to the **Follower Output** BNC connector.

![Follower Output BNC connector]

Fig. 25—Measure DC potential at the microelectrode site using the Follower Output.

2. To offset background current from the amplifier, insert a small screwdriver into the recess labeled **Follower Zero**.

3. Turn clockwise or counterclockwise until the recording device reads 0 V. Excursion in this mode is limited. You can, of course, provide additional offset correction externally, for example, at the reference electrode or the recording device.

Adjusting the 900A Pressure Transducer

1. Plug the pressure and vacuum ports on the **Pressure Pod** with Luer fitting caps.

2. Set **Electrode Resistance** dial to zero and **Damping** and **Sensitivity** knobs to the 12:00 position.

3. Return probe to the **CKT** or **CHAS** ground.

4. Adjust **XCDR** set screw on the **Pressure Pod** to obtain zero on the 900A display.

5. Attach tubing from a known pressure source to the “to microelectrode” fitting of the **Pressure Pod**.

6. Apply a known pressure to the **Pressure Pod**.

7. With the **Pressure Offset** switch at off (center position), monitor the value in the 900A display.

8. Adjust the pressure.
MAINTENANCE

Cleaning

Carefully wash the holder, beaker and syringes with distilled water before and after use.

**CAUTION:** Always pay attention to the tube between the holder and the tubing port. If you find that solution drops have been sucked into the tube, set to the **Loop Status** knob to the **Zero Set** mode. Disconnect the tube, and use the air pressure supply to blow the solution drops out. Leaving solution in the pressure tube may damage the Pressure Pod.

Fuses

The correct replacement fuse for 120 V operation is 1/4 A slow blow. Use a 1/8 A slow blow fuse for 230 V operation.

**WARNING: BEFORE ATTEMPTING FUSE REPLACEMENT, DISCONNECT THE INSTRUMENT FROM THE AC POWER SOURCE.**

The instrument contains one fuse, located in the fuse holder on the back panel. If necessary, replace the fuse with the type and rating specified on the back panel. Verify that the fuse contained in the fuse holder matches the desired line voltage.

1. Turn the main power switch off (I).
2. Unplug power cord from power cord socket on the back of the unit (Fig. 26).

![Fig. 26—Unplug the power cord to access the fuse housing release.](image)

3. Insert a small flat blade screwdriver under the lip on the right side of the fuse housing cover (Fig. 27).

![Fig. 27—Insert the screw driver under the fuse housing lip and pry the housing open.](image)

4. Pull the fuse housing out as far as it will go and rotate it to the right. There is a catch to keep the housing from coming completely out (Fig. 28).
Fig. 28—Open the fuse housing and rotate it right to remove the fuse.

5. Remove the bad fuse. It is the one on the top. A spare fuse is stored in the bottom slot of the fuse housing.
6. Use the spare fuse provided to replace the bad fuse. Slide it into the top slot of the fuse housing.
7. Rotate the fuse housing and slide it back into position.
8. Reinstall the power cord.
9. Turn the power switch on to verify that the unit has power again.

Service
Contact World Precision Instruments at (941) 371-1003 or wpi@wpiinc.com for all service needs.

ACCESSORIES

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<thead>
<tr>
<th>Code</th>
<th>Description</th>
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<tbody>
<tr>
<td>2851</td>
<td>BNC-to-BNC cable</td>
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<tr>
<td>2933</td>
<td>Rack Mounting Hardware</td>
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<tr>
<td>13776</td>
<td>Banana to 2 mm Pin Adapter</td>
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<td>CAL900A</td>
<td>Pressure Calibration Chamber</td>
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<tr>
<td>DRI-REF2</td>
<td>Dry Reference Electrode, 2 mm Diameter</td>
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<td>Dry Reference Electrode, 4.7 mm Diameter</td>
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<td>Electrode Holders</td>
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<td>MicroFil Filling Needle, 28 g, 67 mm</td>
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<td>Microelectrode Puller</td>
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<td>PicoPump</td>
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<td>SYS-PV830</td>
<td>PicoPump with Vacuum</td>
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<td>Pressure Manometer (1 psi)</td>
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<td>Pressure Manometer (1 psi) Rechargeable</td>
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<td>Prepulled Micropipette for 900A, 2 µm Tip</td>
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<tr>
<td>Z-MOLDS</td>
<td>Microinjection and Transplantation Molds</td>
</tr>
</tbody>
</table>

*Electrode holders may be ordered to accommodate any of the following glass sizes: 1.0, 1.2, 1.5 and 2.0 mm diameter.
SPECIFICATIONS

900APP Pressure Range ........ +500 to −300 mmHg
900APP100* Pressure Range ...... ±100 mmHg
Linearity ....................... < ±0.5% from a straight line
Stability ....................... +/- 0.1 mmHg up to 1 hour or more
Accuracy ....................... ±0.5% of full scale
Risetime ....................... >10ms (10-90%), depending on residual volume
Output ("Pressure Signal") ........ 10mV/mmHg
Amplifier Probe ............... Input Resistance >10^10Ω, Voltage Gain 1.0
Dimensions
  Main Frame .................... 17 x 5.25 x 10 in. (43.2 x 13.3 x 25.4cm)
  Pressure Pod .................. 3.7 x 1 x 2.25 in. (9.4 x 2.5 x 5.7cm)
Power ........................... 110 VAC/220 VAC
Fuse (Older models) ............. 120 V: 0.125 A, slow, 0.25 x 1.25” USA
  230 V: 0.0625 A, slow, 0.25 x 1.25” USA
Fuse (2019 models) ............. 120 V: 0.125 A, slow, 5 x 20 mm metric
  230 V: 0.0625 A, slow 5 x 20 mm metric

*Default module typically sold with the system
WORLD PRECISION INSTRUMENTS, INC.
175 Sarasota Center Boulevard
Sarasota, FL 34240-9258 USA
Telephone: (941) 371-1003 Fax: (941) 377-5428
e-mail wpi@wpiinc.com

DECLARATION OF CONFORMITY

We: World Precision Instruments, Inc.
175 Sarasota Center Boulevard
Sarasota FL 34240-9258
USA

as the manufacturers of the apparatus listed, declare under sole responsibility that the product(s):

Title: 900A Micropressure System

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

EN 55011:1991 – Class B
EN 50082-1:1992


Issued on: 22nd December 1999

Dr. Mark P. Broderick
President and COO
World Precision Instruments, Inc.
175 Sarasota Center Boulevard
Sarasota, FL 34240-9258 USA

Mr. Glen Carlquist
Production Manager
World Precision Instruments, Inc.
175 Sarasota Center Boulevard
Sarasota, FL 34240-9258 USA
WARRANTY

WPI (World Precision Instruments) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of one year* from the date of receipt. WPI’s obligation under this warranty shall be limited to repair or replacement, at WPI’s option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI’s approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within ten (10) days after receipt of shipment. Claims for lost shipments must be made within thirty (30) days of receipt of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim is settled. In some instances, photographic documentation may be required. Some items are time-sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container.

Do not return any goods to us without obtaining prior approval and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. Goods accepted for restocking will be exchanged or credited to your WPI account. Goods returned which were ordered by customers in error are subject to a 25% restocking charge. Equipment which was built as a special order cannot be returned.

Repairs

Contact our Customer Service Department for assistance in the repair of apparatus. Do not return goods until instructions have been received. Returned items must be securely packed to prevent further damage in transit. The Customer is responsible for paying shipping expenses, including adequate insurance on all items returned for repairs. Identification of the item(s) by model number, name, as well as complete description of the difficulties experienced should be written on the repair purchase order and on a tag attached to the item.

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