



INSTRUCTION MANUAL

PV850

Microinjector for Use with External Pressure Source
(Pressure source not included)

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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.



Fig. 1—MIRO-ePUMP Microinjector requires an external pressure source.

INTRODUCTION

Designed to simplify intracellular injection and a variety of other microinjection tasks, the **PV850** uses carefully regulated air pressure for injecting cells with fluid. Injected volumes range from picoliters to nanoliters. The port supplies positive pressure for high-pressure ejection. The pressure port maintains a low positive “compensation” pressure to the injecting pipette between injection pulses to prevent fluid uptake through capillary action.

Timing, injection pressure and compensation pressure are adjusted independently using the touch screen interface. Time intervals can range from 2 seconds down to 10 ms or less, depending on the injection pressure setting. The injection pressure interval is triggered by using a foot switch, manually, or a computer-controlled TTL pulse.

The **PV850** is designed to inject very small quantities of fluids, such as drugs, into cells or small organelles. Pressure injection is an especially useful alternative to electroionophoresis, since it does not mandate the use of charged ions. Two different positive pressures may be applied—one for ejection at high pressure and a second, lower pressure to prevent back filling of the pipette by capillary action.

The **PV850** offers separate regulated compensation (back filling prevention) and ejection pressures with a precision timing circuit that switches from injection pressure to compensation pressure automatically.

NOTE: It is highly recommended to use long-taper style micropipette tips to ensure proper maintenance of your sample versus your bath environment.

Features

- Regulated compensation and injection pressure
- Pressure output: 0.01-87 PSI
- Injection can be triggered manually using the touchscreen, by foot switch, or by a computer-controlled TTL pulse
- Low volume tubing assembly

Benefits

- Intuitive user-interface for injector
- Small footprint takes up very little bench space
- Easy to navigate with touch screen and control knob
- Inject into a single cell in picoliter volumes

Applications

- Microinjection of diverse compounds and biomolecules – DNA, RNA, proteins
- Pre- and post-implantation in embryos of various species – mice, rats, monkeys, bovine, pigs, zebrafish, etc.

Notes and Warnings



IMPORTANT: THIS INSTRUMENT SHOULD BE USED WITH LONG-TAPER MICROPIPETTES TO ENSURE PROPER FUNCTIONALITY.



WARNING: SECURE THE PIPETTE FIRMLY IN THE HOLDER. WHEN HIGH PRESSURE IS APPLIED, A LOOSE PIPETTE CAN BE EJECTED FORCEFULLY. DO NOT APPLY PRESSURES IN EXCESS OF 100 PSI (689.4 KPA).



WARNING: THIS INSTRUMENT IS FOR INVESTIGATIONAL USE ONLY IN ANIMALS OR OTHER TESTS THAT DO NOT INVOLVE HUMAN SUBJECTS.

NOTE: An audible “hissing” sound during operation of the unit is NORMAL. This is a result of air ventilation to regulate system functionality.

Parts List

After unpacking, verify that there is no visible damage to the instrument. Verify that all items are included:

(1) **PV850**

(1) Foot switch

(1) **300753** PV850 Capillary Kit which includes:

- (4) 1.0 mm pipette gaskets (green)
- (4) 1.2 mm pipette gaskets (black)
- (4) 1.5 mm pipette gaskets (red)
- (4) 1.65 mm pipette gaskets (white)
- (1) Pipette handle
- (2) Sealing o-rings
- (1) Pipette holder
- (1) Tubing assembly
- (1) Adapter tubing assembly

(1) **3316** Input Kit for PicoPumps which includes:

- (1) 0.25" NPT Fitting for nitrogen tank regulator
- (1) 10' Hard tubing
- (1) Vacuum fitting

(1) **CBL102** 6' Cable, 3.5 mm mini phone plug to BNC (male)

(1) **803130** Stereo splitter cable, 3.5 mm male to two female

(1) AC/DC 24 V Power Adapter

(1) Instruction Manual (available online at www.wpiinc.com/manuals)

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 31 of this manual. Please contact WPI Customer Service if any parts are missing at (941) 371-1003 or customerservice@wpiinc.com.

Claims and Returns

All RMA claims for evident 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 shipment invoice. In some instances, photographic documentation may be required. WPI assumes no extended warranty or any liability for use beyond the date specified on the container. Goods accepted for restocking will be exchanged or credited to your WPI account if under warranty. Goods returned which were ordered by customers in error are subject to a 25% restocking fee. Non-stock or customized products are final sale.

Repairs

Do not return any goods to WPI without obtaining prior approval, receipt of an RMA # and return instructions from WPI's Returns Department, please contact wpirms@wpiinc.com. Unauthorized goods returned by collect freight may be refused. If a return shipment is required, items must be securely packed with original packaging. The customer is responsible for paying shipping if the item is out of warranty, or received showing excess damage not documented on the original RMA. Identification of the item(s) by model number, and name, as well as the complete description of the difficulties experienced with images (if available) should be supplied once routed to our RMA department.



CAUTION: DO NOT DISCARD ORIGINAL BOX AND PACKAGING. WARRANTY MAY BE VOIDED IF PRODUCT IS NOT IN ORIGINAL CONTAINER UPON RECEIPT FOR REPAIRS/MAINTENANCE.

*If you require a replacement WPI shipping container for a repair/maintenance claim, please contact customer service.

INSTRUMENT DESCRIPTION

Front Panel



Fig. 2—The major features of the PV850 are labeled in this diagram.

Touch Screen Interface – The responsive panel allows for intuitive control. Touch the injection counter to reset it, the tank pressure gauge to refill the tank, Clear Tip to blow a blast of air through the pipette tip, a parameter to modify it using the knob, or the settings area to adjust the settings.

Adjustment Knob – Rotate the adjustment knob to change the highlighted parameter. Press it to toggle between gross and fine tune controls.

Injector Port – Plug the easy connect connector on the tubing into this port.

Rear Panel



Fig. 3—The foot switch connection, power supply connection and power switch are located on the back panel of the PV850.

Foot Switch Connection – Plug the connector from the foot switch into the connection port marked with the foot switch icon. Press the foot switch to initiate a timed sequence (timed mode) or to manually eject pressure (manual mode). This foot switch is a two-stage switch. Depress it half-way to initiate and injection. (See “Foot Switch” on page 7.) Using the **CBL102** BNC cable and the **803130 Y** stereo splitter, you can trigger an injection via a computer-controlled +5 V TTL pulse.

USB Port –This interface is reserved for factory use only.

Power Switch –To power on the unit, press the toggle switch to —. To power off the unit, press the toggle switch to O.

Power Supply Connection –Insert the power supply connector into this port and plug the other end into an A/C wall outlet.

Air Input Port–This is the air supply for the injector. This pressure must be at least 6 PSI higher than the maximum injection pressure used. The maximum pressure must not exceed 100 PSI.

Foot Switch

The foot switch is a two-step switch, but the **PV850** model activates whenever the switch is depressed, either half-way or fully. Both positions have the same result. Both positions trigger an injection.



Touch Panel Display

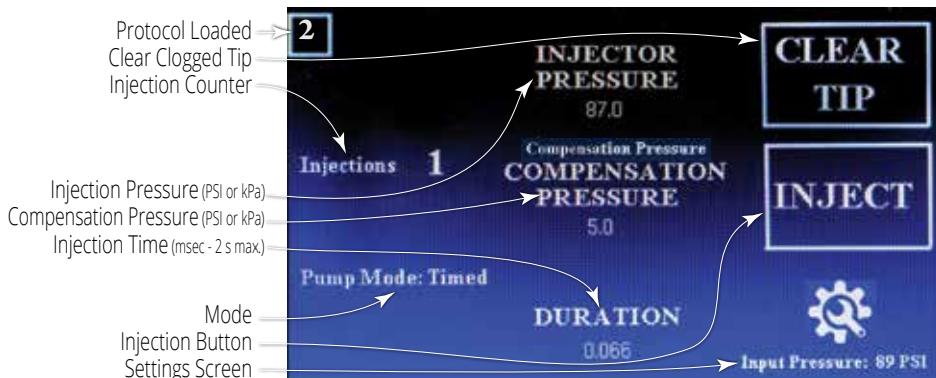


Fig. 4—The Touch Panel Display gives you access to all the parameters and the settings menu.

Protocol Loaded – The number in the box in the upper left corner indicates the protocol with stored parameters that is loaded. You can store and recall up to three of your most used protocols in the **PV850** memory. If the box is empty, no protocol is loaded.

Clearing Clogged Tip – Press Clear Tip to force a 500 ms burst of maximum pressure (87 PSI) through the injection tip. This can be used if the tip becomes clogged. The valves are closed when the compensation pressure is at zero. To exercise the CLEAR function, you can set it at 0.01.

Injection Counter – Every time you depress the foot switch all the way (when the port is enabled), the injection counter increments. To reset the injection counter to zero, touch the injection counter on the display.

Injection Pressure – Touch the parameter and rotate the knob to adjust the pressure used to inject. When you depress the foot switch all the way, this is the pressure that is forced through the injection tip.

Compensation Pressure – Touch the parameter and rotate the knob to adjust the compensation pressure that is used to prevent the back filling of the pipette.

Injection Time – Touch the parameter and rotate the knob to adjust the length of each injection.

Mode – This indicates if the pump mode, either Timed or Manual.

Injection Button (Manual)– Use this button to manually initiate an injection.

Settings Screen – Touch anywhere in the settings area (bottom right corner) of the screen to access the settings menu. This area also shows the status of the warning tones.

Setting Menu

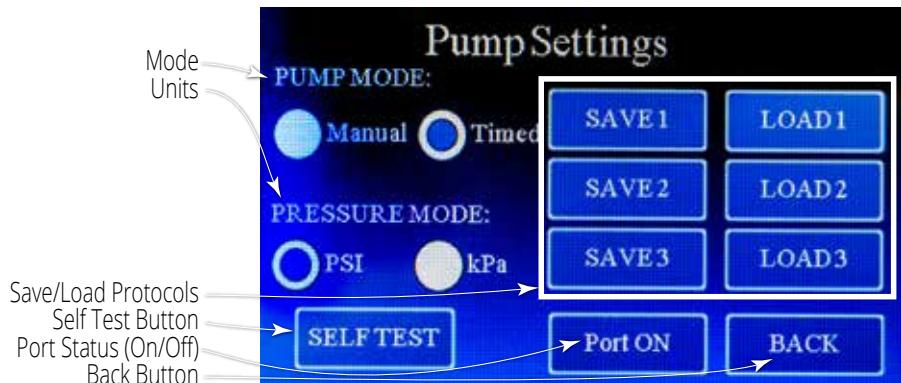


Fig. 5—From the Settings menu, you can save and load parameters, choose the mode, set the units, toggle the MICRO-ePORE™ off or on, silence the tones, and toggle the injection port off or on.

Mode – Choose either the Manual or the Timed checkbox to determine the injection mode. Manual control allows for injection pressure to be applied for as long as the foot switch is fully depressed. Timed injections use the duration set on the main screen.

Units – Select one of the checkboxes to set the display to either PSI or kPa.

Save/Load Protocols – The **PV850** can store your three favorite parameter sets (protocols). On the main screen set the parameters as desired. Then press one of the Save buttons. For example, press **SAVE1** to save the parameters as Protocol 1. To load a set of saved parameters (a protocol), press the appropriate **LOAD** button. The number of the loaded protocol displays in the box in the upper left corner of the main screen.

Self Test Button – The Self Test runs whenever the unit is powered on. To initiate a test manually, press this button. It runs a series of programmed diagnostics and displays results on the screen.

Port Status – Port ON indicates that the injection port is enabled. To disable the port, touch the Port ON button. The button will change to Port OFF. To re-enable the port, press the Port OFF button, and the button will change to Port ON.

Back Button – Touch the Back button to return to the main screen.

Setup

A 1/4" hard tubing (included) can be cut to fit. It is used for connecting an air pump or tank. The soft tubing (included with the Capillary Kit) is used for connecting the micropipette holders to the microinjector's output (**INJECTOR**) ports.

Installing Hard Tubing

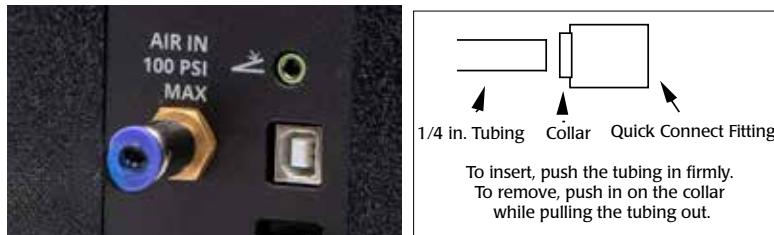


Fig. 6—The air input port is located on the rear panel.

! **CAUTION:** Do not remove tubing holders. These parts are intended to remain on the pump.

To install the hard tubing, firmly insert tubing into the quick-connect fittings on the rear of the instrument. When the tubing is inserted far enough, it engages and cannot be pulled out. To remove the tubing, press in on the surrounding collar while pulling the tubing out. Plastic tubing can be disconnected easily, while metal tubing may require more effort.

Making Connections and Startup

1. With the **PV850** turned off, make the following connections:
 - Connect Power Supply to the rear of the unit and plug it into a wall outlet.
 - Plug the Foot Switch Connector into the Foot Switch Port.



Fig. 7—The Foot Switch Connector is labeled "Rear Panel." Do NOT plug it into the injection port on the front panel.

NOTE: DO NOT connect the capillary kit hose to the **PV850**.

2. Verify that the capillary kit hose is disconnected from the Injection Port. Then, power on the **PV850**. On power up, the following happens:
 - The power up screen appears.
 - The **PV850** performs a self-test.

After the self-test completes, the main display screen appears. See "Touch Panel Display" on page 7. It displays the configuration settings used when it powered off the last time.

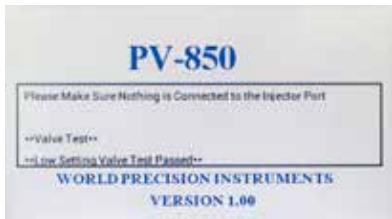
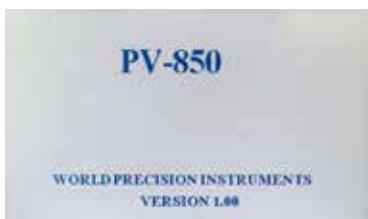


Fig. 8—(Left) The power up screen shows the software revision on your PV850.

Fig. 9—As the self-test runs through the programmed diagnostic sequence, the display shows the results.

3. Press the touchscreen to access the main screen. By default, the injection port is disabled. Tap the warning or the Settings icon to enable the Injection Port.



Fig. 10—The warning indicates that the injection port is disabled by default on startup. To enable the port, press the Port OFF button. The Port OFF message will become the Inject button.

Assembling the Capillary Kit



Fig. 11—The 300753 Capillary Kit must be properly assembled.

1. Slide the bare end of the Adapter Tubing Assembly through the Pipette Handle.
2. Connect it to the barb of the Pipette Holder Body. Be careful so that the barb does not break when attaching or removing the tubing.
3. Slide the Pipette Handle over the barb and carefully screw it onto the Body of the Pipette Holder. Be careful not to cross thread it.
4. Place a gasket of the correct size in the cap. Refer to the table below. Then, insert the blunt end of the micropipette (pulled capillary glass) into the cap through the gasket and into the body. Screw the cap in place. The screw cap and rubber gasket firmly hold the glass micropipette.

| | | | | |
|-----------------------|-------|-------|-----|-------|
| Gasket Color | Green | Black | Red | White |
| Pipette Diameter (mm) | 1.0 | 1.2 | 1.5 | 1.65 |



WARNING: DANGER OF INJURY EXISTS IF THE PIPETTE IS INSECURE. HIGH PRESSURE CAN CAUSE EJECTION AT HIGH VELOCITY.

5. The other end of the small tubing has a quick connector. Connect it with the blue **PV850** Tubing Assembly provided.
6. Verify that the Injection Port is disabled. If not, touch the Settings area of the display to access the Settings menu and touch the Port ON button to set it to Port OFF.
7. Align the easy-connect Injector connector with the injector port on the front of the **PV850**, slide the connector securely into the port and attach it with a slight clockwise twist.



Fig. 12—(Left) Align the connector with the *Injection Port*, slide the connector in position and rotate the connector a little clockwise to lock it into place.

Fig. 13—(Right) The PicoNozzle is connected to the injection port.

Connecting to a Pulse Generator for TTL Control

If you wish to use a computer or pulse generator to send a TTL pulse to trigger an injection, connect the **803130** Y stereo splitter to the foot switch port on the rear of the **PV850**. Connect the CBL102 jack to one of the female ports on the Y stereo splitter. Plug the BNC cable into the appropriate TTL port.

To computer or pulse generator



Fig. 14—Use the CBL102 and the 803130 to configure the system for a TTL trigger.

The **μPump** will inject on the high to low transition of the incoming TTL pulse.



Fig. 15—The injection happens on the high to low transition of the TTL pulse.

OPERATING INSTRUCTIONS

Changing the Injection Parameters

1. Press anywhere in the settings area to access the Settings menu. Press Port Off to turn on the pressure port (PORT ON).



Fig. 16—(Left) Touch the setting area in the bottom right corner to access the Settings menu.

Fig. 17—(Right) Touch the Port OFF button to enable the injection port.

2. On the Settings screen you may choose the Pump Mode as TIMED or MANUAL. In the MANUAL mode, the valve stays open for as long as the Inject button or foot switch is depressed. In the TIMED mode, the range is fixed.
 - MANUAL mode-The pressure valve opens and remains open as long as the Inject button or foot switch is pressed or a 5 V TTL pulse is triggered by an external pulse generator.
 - TIMED mode-An electronic timer controls the duration that the pressure valve stays open, and the Inject button, foot switch or 5V TTL trigger serves only to start the timer. Although the timer control allows you to set sub-millisecond burst intervals, the true burst interval is limited by the mechanical response of the valve, which is about 0-2 seconds, depending on the pressure.

The TTL output connector produces a logic level output (+5 V) corresponding to the time interval during which the eject pressure valve is energized. At all other times the monitor output is low (0 V).

3. To change the parameters, just touch the screen on the parameter you want to change. A box appears around the parameter to highlight it. Adjust the parameter by turning the knob. To change the dial adjustment speed, press the knob on the front of the **PV850**. The snail mode allows movements in 0.01 increments to the compensation pressure only, and only when it is below 3.0 PSI. Both conditions must be met. The running man changes it in increments of 0.

NOTE: When adjusting the COMPENSATION PRESSURE under 3.0 PSI, you may set it in 0.01 PSI increments.

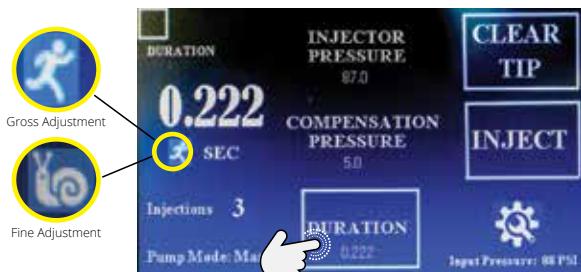


Fig. 18—Touch the parameter in the center column of the screen that you want to adjust and rotate the knob to modify the parameter. Press the knob to toggle between gross and fine tuning adjustments, indicated by the running man and the snail on the screen.

4. Two seconds after a parameter is adjusted, it is saved into the memory. If you move from one parameter to the next before two seconds, the first parameter is automatically saved into the memory as the working parameter. This setting will be recalled on power up.

NOTE: This is different from saving parameter sets (protocols) to load.

5. Activate an injection using one of these methods. You will hear an audible alarm when the injector port is activated.
 - Press the foot switch.
 - Press the Inject button on the touch screen controller.
 - Send a TTL pulse from a pulse generator. See "Connecting to a Pulse Generator for TTL Control" on page 11.

NOTE: An audible "hissing" sound during operation of the unit is NORMAL. This is a result of air ventilation to regulate system functionality.

Resetting the Injection Counter

This area in the left column shows the injection count. Whenever the foot switch is depressed or the Inject button is pressed, the injection counter increments. Reset it to zero by pressing the injection area of the main display touchscreen.

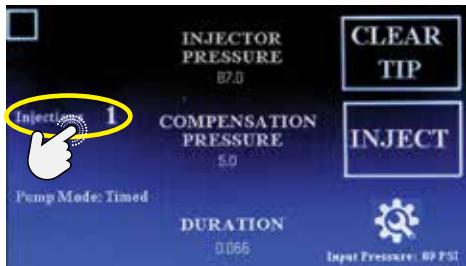


Fig. 19—Press *Injections* to reset the injection counter.

Adjust the PV850 Settings

The setting section (bottom right corner of the main screen) displays the current settings for the **PV850** mode (Manual or Timed).

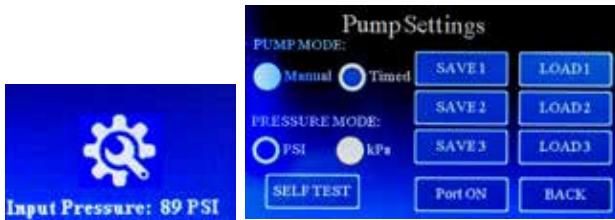


Fig. 20—(Left) The Setting area is located in the bottom right corner of the main screen.

Fig. 21—(Right) Touch the Setting area to display the Settings menu.

1. To modify these settings, press the Settings section on the touchscreen.
2. From there, you can make the following adjustments:
 - Change the **PV850** mode (manual or timed).
 - Change the pressure units displayed on the main screen (PSI or kPa).
3. Press the BACK button to save the settings and return to the main screen.

Saving a Protocol

After you have adjusted the parameters, you can save the set of parameters as a protocol. **PV850** will store up to three (3) protocols.

1. Set the parameters, as desired.
2. Press the Settings section on the touchscreen to access the Settings menu.



Fig. 22—The Settings menu lets you save a protocol or load a saved protocol.

3. To save the existing set of parameters from the main screen, press the SAVE1, SAVE2 or SAVE3 button. The parameters are saved in Protocol 1, Protocol 2 or Protocol 3, depending on which SAVE button you chose.
4. Press the BACK button to return to the main screen.

Loading a Protocol

NOTE: Saved settings will likely require slight calibration each time a new pipette tip is used to ensure your desired injection volume. Tip inner diameter carries variability and, in turn, it affects the programming required for the necessary injected volume.

Saved protocols may be recalled, and this loads the saved parameters for use. When a protocol is loaded, the protocol number (1, 2 or 3) appears in the box located in the upper left corner of the main screen.

1. Press the Settings section on the touchscreen to access the Settings menu.



Fig. 23—The Settings menu lets you save a protocol or load a saved protocol.

2. To bring up a saved protocol with the **PV850** parameters, press the LOAD 1, LOAD2 or LOAD3 button. The unit loads the saved parameters of Protocol 1, Protocol 2 or Protocol 3, depending on which LOAD button you chose.
3. Press the BACK button to return to the main screen.

Changing the Pipette

To change the pipette, turn off the injection PORT to avoid depleting the reservoir. The button indicates the present state of the port.

1. To turn off the Injection Port, press the Settings section on the touchscreen.



Fig. 24—The Settings menu lets you save enable or disable the Injection Port by pressing the Port ON/Port OFF toggle button.

2. Press the Port ON button to disable the Injection Port
3. Press the BACK button to save the settings and return to the main screen. A warning indicates that the Injection Port is disabled.



Fig. 25—When the Injection Port is disabled a notice appears on the main screen.

4. To install the micropipette, see “Assembling the Capillary Kit” on page 10.
5. When the micropipette is properly installed, click on the Settings area to access the Settings menu and press the Port OFF button to enable the Injection Port.

TECHNIQUES IN MICROINJECTION

The **PV850** was designed for demanding tasks like the microinjection of fluid into cells. In this section, we will look at several important things to keep in mind when working with the **PV850**.

Setting the Compensation Pressure

The compensation pressure is used to counterbalance the capillary action of the fluid backfilling into the pipettes. If you insert an empty pipette into fluid, you can see a meniscus rising from the capillary tip.

Set the Compensation Pressure parameter by touching the Compensation Pressure parameter on the main screen and adjusting the knob until the meniscus stops at the desired position in the micropipette. In many applications, colored dye or fluorescent dye is dissolved in the injection fluid. The capillary effect may be observed with the color change at the tip of the pipette. When the fluid flows into the pipette, the color of the tip becomes lighter. If the compensation pressure is higher than the capillary pressure, the fluid oozes out of the pipette. The solution around the pipette will be colored. Adjusting the compensation pressure prevents this from happening.

NOTE: When adjusting the COMPENSATION PRESSURE under 3.0 PSI, you may set it in 0.01 PSI increments.

Understanding how capillary action causes the front filling of the pipette helps you to correctly use the compensation pressure. The flow rate is determined by the capillary action and the tip size. Since the tip size is often determined by the requirement of the application, controlling the compensation pressure becomes the main option to eliminate the uptake of fluid by capillary action.

NOTE: While setting your compensation pressure is important, this paired with using a long-taper capillary is critical to maintaining a successful balance between your sample versus the exterior bath interface.

If you assume the pipette tip is a cylindrical shape, the pressure of capillary action can be described by the LaPlace equation:

$$\gamma = \text{surface tension}$$

$$P = 4\gamma \cos\theta/d \quad \theta = \text{contact angle between the water and glass}$$

$$d = \text{inner diameter of the capillary where the meniscus is located}$$

In most cases, we can assume the contact angle for glass and water is zero (unless the glass surface is treated). From this equation we see that the smaller the inside diameter, the greater the capillary action.

TIP: If the lowest pressure setting is ineffective at preventing the pipette fluid from leaking out, try setting the compensation pressure to zero to see if the gravity and friction are sufficient to counterbalance the front fill.

TIP: The capillary action can also be reduced by adding a hydrophobic fluid (such as silicone oil) behind the hydrophilic saline solution. It can be completely eliminated by silanizing the shank of the pipette (silanization increases the θ in the LaPlace equation to 90°).

Manufacturing Micropipettes

Pulling suitable micropipettes is one of the biggest obstacles to taking full advantage of the **PV850**. Both care and steady hands are required. The volume of fluid ejected is markedly dependent on the micropipette tip size.

The most important of these steps is cleaning the glass. Small amounts of dust or grease can easily clog micron-sized tips. Cleaning with chromic acid solutions before use is commonly performed, but care must be taken to thoroughly rinse the pipettes to remove all traces of the chromic acid, which has some affinity for glass. Some researchers prefer hydrochloric or nitric acid.

Silanization of the glass is also recommended for long-taper tips. With sub-micron tips, capillary action becomes prohibitive, and the hydrophilic surface of the glass greatly limits the flow of fluid through the tip in a controlled manner. Silanization decreases the surface tension and allows the fluid to flow smoothly through the tip. Long-tapered tips with filaments make for easier pipette filling as well.

Calibrating Volume by Measuring Droplet

For ejected volumes greater than 1 nL, visual inspection using a microscope can be an accurate gauge of volume. A single pulse deposits a drop of fluid on the tip of the micropipette. The volume of this drop may be calculated by measuring the radius of the drop and assuming the drop to be spherical. Fig. 26 may be helpful in determining the volume for a given radius. See "Appendix A: Droplet Volume" on page 27 for a comparison of spherical and cubical volumes.

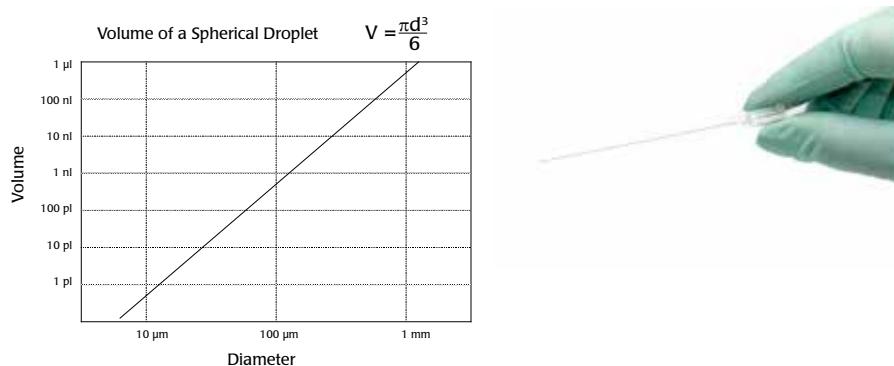


Fig. 26—(Left) The volume of a droplet increases as the diameter increases. See "Appendix A: Droplet Volume" on page 27.

Fig. 27—(Right) WPI's MicroFil is helpful in backfilling glass pipette tips. The MicroFil's tip is thin and very flexible.

For ejected volumes less than 1 nL, visual inspection in air proves to be difficult due to rapid evaporation. The same technique may be used though if the drop is kept

submerged under oil. Droplets may seem to disappear after emergence from the tip. Sometimes this is due to creepage of the aqueous fluid back along the outside shank of the micropipette. This creepage may be decreased by silanizing the outside of the pipette.

TIP: Precise assays of ejected volume may also be obtained through various radioisotopic methods. See the bibliography for further information.

A slight deflection of the micropipette tip may be noticed during the application of the pressure pulse. This deflection may be eliminated by ensuring that the micropipette is firmly seated in the holder and that the holder is firmly held by a manipulator.

Calibrating Rate using a Known Volume

We can calculate the volume of liquid contained in a 1 mm length of a glass pipette, if we know the inner diameter (ID). Then, we can inject that known volume and measure the time it takes to inject it. From this we can calculate the rate of injection. For example, if the volume in 1 mm of glass with 0.58 mm ID (WPI #1B100) is 264 nL, and it takes 30 seconds to inject that volume, then the flow for 1 second is 264 nL/30 s or 8.8 nL/second, and a 1 nL volume then takes 0.113 seconds to inject.

This method is discussed in the following JoVE video: <http://www.jove.com/video/2079/intravenous-microinjections-zebrafish-larvae-to-study-acute-kidney>.

In the Jove video, the capillary tip is 10–20 μm . By following these steps, we are able to calculate the timing needed to deliver a 1 nL injection by counting the time between 1 mm marks as the fluid is injected.

NOTE: The **PV850** timing should not be adjusted to go below 10–15 ms as an absolute minimum, since it takes 6–10 ms for the pressure valves to respond.

1. Fill a small dish with mineral oil and place it under a stereo microscope.
2. Turn on your air and vacuum pumps, and set your compensation pressure and your injection pressure. The proper settings must be determined experimentally. Turn on the **PV850**.

NOTE: The injection pressure must exceed the compensation pressure in order to eject the fluid.

3. Use a pipette to inject a 10 μl sphere of fluid into the mineral oil.
4. Using a fine pointed permanent marker, mark the injecting micropipette at 1 mm increments.
5. Mount your “graduated” micropipette on the **PV850** that is connected to the Injection Port on the **PV850**.
6. Set the mode to Manual. Hold down the foot switch to inject fluid from the micropipette into the oil-filled dish.
7. Record the amount of time it takes in seconds for the meniscus in the

micropipette to travel from one 1 mm mark to the next. It should take between 20 and 30 seconds for each injection. You may need to adjust the injection pressure to achieve this time frame.

8. Repeat steps 6–7 three times
9. Average the three trials to find the average time it takes to inject a 1 mm column of fluid.
10. Calculate the volume of a 1 mm segment of your micropipette using the volume formula for a cylinder:

r = radius of the pipette = ID/2

h = length of the pipette = 1000 μm

$V = \pi r^2 h = \pi ID^2 1000 / 4 = 785 * r^2$

- Thin wall 1 mm glass has a nominal 0.750 μm ID, and a 1 mm length contains 0.4418 μL of fluid (442 nL).
- Standard wall **1B100** glass has a 0.580 μm ID, and a 1 mm length holds 0.2641 μL of fluid (264 nL).

Both those ID's have a $\pm 100 \mu\text{m}$ tolerance, and that ID dimension is vital to making a correct injection of 1 nL.

11. Divide the average found in step 9 by the calculated volume in nL of a 1 mm length of your pipette found in step 10. This establishes the time in milliseconds that it takes to inject a known volume. From there you can calculate how many nanoliters can be injected per second and how many milliseconds it takes to inject a single nanoliter of fluid.

NOTE: This volumetric calibration can also be performed using a stage micrometer, for efficiency.

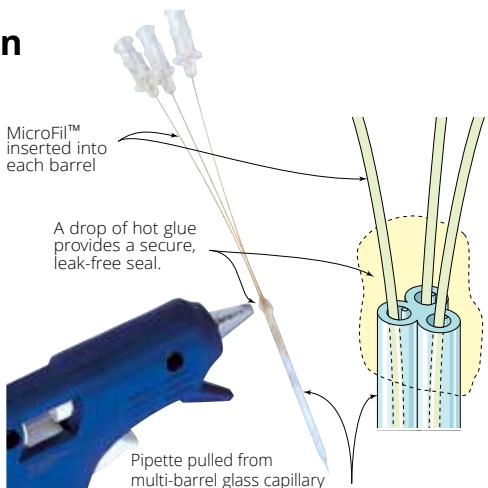
Understanding Injection Ranges with Respect to Tip Size

| Application | Taper Length | Tip Size | Compensation/ Hold Pressure (PSI) | Injection Duration (sec) | Injection Pressure (PSI) |
|--|--------------|-------------------|---|-----------------------------|--|
| Adherent Cell (5-10 μ m Cells) | 7-8 mm | 0.1 – 0.3 μ m | 0.29 – 0.58 | 0.20 | 7.5 – 15 PSI |
| Adherent Cell (20-40 μ m Cells) | 4 – 6 mm | 0.3 – 0.5 μ m | 0.29 – 0.58 | 0.20 | 4.5 – 11.5 PSI |
| Pronuclear & Cytoplasmic Injection | 7 – 9 mm | 0.3 – 0.5 μ m | 0.29 – 0.58 | 0.20 | 4.5 – 15 PSI |
| C. Elegans | 6 – 8 mm | 0.5 – 0.9 μ m | 0.07 – 0.15 | 0.20 | 1.5 – 3.0 PSI |
| Drosophila | 6 – 8 mm | 0.5 – 0.9 μ m | 0.07 – 0.15 | 0.20 | 1.5 – 3.0 PSI |
| Zebrafish | 6 – 8 mm | 0.5 – 0.9 μ m | 0.07 – 0.15 | 0.04 | 65 – 72.5 PSI |
| Xenopus | 10 – 15 mm | 0.5 – 0.9 μ m | 0.07 – 0.15 | 0.2 – 0.5 | 0.75 – 3.0 PSI |
| Sea Urchin Eggs SQUID Mosquito Egg | 7 – 9 mm | 0.3 – 0.5 μ m | 0.15 – 0.29 | 0.2 – 0.5 | 3.0 – 5.75 PSI |
| Small Fish Egg (100-500 μ m) | 8 – 10 mm | 0.3 – 0.5 μ m | 0.15 – 0.29 | 0.40 | 7.5 – 15 PSI / 3.0 – 7.5 PSI (broken back tip) |
| Large Fish Egg (500-1500 μ m) | 8 – 10 mm | 0.3 – 0.5 μ m | 0.15 – 0.29 | 0.40 | 7.5 – 15 PSI / 3.0 – 7.5 PSI (broken back tip) |

Multibarrel Microinjection

For injection with a multi-barrel micropipette, the PolyFil multi-barrel micropipette coupling kit can be purchased from World Precision Instruments. This multi-barrel micropipette coupling kit allows easy and secure coupling of a multi-barrel micropipette to a pressure source. The kits include a five-port manifold which allows use of a single **PV850** to drive up to six micropipette barrels independently.

Fig. 28—*PolyFil multibarrel micropipette coupling kit*



MAINTENANCE

The **PV850** has been designed to yield reliable performance. However, some laboratory conditions may require occasional replacement of the pressure filters. If this is necessary, return the instrument to the factory. If the unit displays a warning indicating that the pump needs service, please return it to the factory.



Fig. 29—Warning errors like this one display when the unit needs to be returned to the factory for routine service.

Cleaning

Do not use alcohol, aromatic hydrocarbons or chlorinated solvents for cleaning. They may adversely react with plastic materials used to manufacture the instrument. The exterior of this instrument may be cleaned periodically to remove dust, grease and other contamination. There is no need to clean the inside. Use a soft cloth dampened with a mild solution of detergent and water. Do not use abrasive cleaners.

ACCESSORIES

| Part Number | Description |
|--------------------|--|
| 75122-110 | Glass gasket green 1.0 mm, pkg. of 10 |
| 75122-210 | Glass gasket black 1.2 mm, pkg. of 10 |
| 75122-310 | Glass gasket red 1.5 mm, pkg. of 10 |
| 75122-410 | Glass gasket white 1.65 mm, pkg. of 10 |
| 75125-6 | Replacement Pipette Holder for 5430-ALL, pkg. of 6 |
| 300753 | PV850 Capillary Kit |
| MICRO-ePORE | Pinpoint Cell Penetrator |

TROUBLESHOOTING

The PV850 continuously monitors the tank pressure to detect malfunctions in the pressure system. Depending on the fault detected, different error messages are displayed. When errors are detected an error screen with the error number and suggested action is displayed. Upon exiting the Error Screen, the system returns to the main screen with the Injection Port disabled. After addressing the failure source, press the yellow banner to re-enable the port.

On power up the **PV850** performs a diagnostic self-test. If there is a failure during the self-test, an error code displays and the unit stops the sequence. It will not progress into the operational mode unless it is powered off and then on, and the self-test completes without error.

| ERROR | Condition | Possible Cause | Action |
|-------|---|---|--|
| 104 | Tank pressure is too low to deliver the desired injection pressure. | Tank depleted due to previous injections or leak. | Wait for pump to charge. Check all pressure connections and make sure the pipette is not broken. |
| 202 | Failed to charge to the maximum pressure during the self-test. | Charging time exceeded. | Ensure nothing is connected to Injection Port. Call WPI technical support if the problem persists. |
| 203 | High setting port valve test failed during the self-test. | Failed to reach the proper Injection Port pressure. | Ensure nothing is connected to Injection Port. Call WPI technical support if problem persists. |
| 204 | Low setting port valve test failed during the self-test. | Failed to reach the proper Injection Port pressure. | Ensure nothing is connected to Injection Port. Call WPI technical support if problem persists. |

NOTE: If you have a problem/issue with that falls outside the definitions of this troubleshooting section, contact the WPI Technical Support team at (941) 371-1003 or technicalsupport@wpiinc.com.

SPECIFICATIONS

This unit conforms to the following specifications:

PRESSURE

| | |
|---|-----------------------------------|
| Time Interval | 0.001 - 2.000 s |
| Time Increment | 0.001 s |
| Injection Pressure Range, Controlled | 0.01 - 87.0 PSI (0.07-599.84 kPa) |
| Injection Pressure Increment | 0.1 PSI (0.1 kPa) |
| Injection Pressure, Uncontrolled | 0 PSI (0 hPa) |
| Compensation Pressure Range, Controlled | 0.01 - 87.0 PSI (0.07-599.84 kPa) |
| Compensation Pressure Increment | 0.1 PSI (0.1 kPa) |
| Compensation Pressure, Uncontrolled | 0 PSI (0 hPa) |

PHYSICAL SPECIFICATIONS

| | |
|---------------------|--|
| Power | 90-264 V, 50/60 Hz |
| Power Consumption | 7.5 W |
| Dimensions | 160 (W) x 217 (D) x 273 (H) mm (6.3 x 8.56 x 10.76") |
| Shipping Weight | 5.5 kg (12 lb.) |
| Ambience | For indoor use only |
| Ambient Temperature | 15°C - 40°C |
| Relative Humidity | 10 - 75%, non-condensing |

* Both Backfilling and Eject Pressures

AMBIENT CONDITIONS

| | |
|----------------------|--|
| Ambience | Only for use indoors |
| Ambient Temperature | 15° C - 40° C |
| Relative Humidity | 10% - 75%, non-condensing |
| Atmospheric Pressure | 795 hPa - 1060 hPa |
| Degree of Pollution | Use up to a height of 2000 m above sea level |
| | 2 (IEC 664) |

STORAGE

| | Air Temp | Relative Humidity | Atmospheric Pressure |
|-----------------------------|---------------|-------------------|----------------------|
| In Transport Packaging | -20 C - 70° C | 10% - 80% | 300 hPa - 1060 hPa |
| Without Transport Packaging | - | - | - |

TRANSPORT

| | Air Temp | Relative Humidity | Atmospheric Pressure |
|-------------------|---------------|-------------------|----------------------|
| General Transport | -25 C - 60° C | 10% - 95% | 30 kPa - 106 kPa |
| Air Freight | -40 C - 55° C | 10% - 95% | 30 kPa - 106 kPa |

APPENDIX A: DROPLET VOLUME

Use the chart below to gauge the volume of a droplet and the table to determine the volume a micropipette can hold.

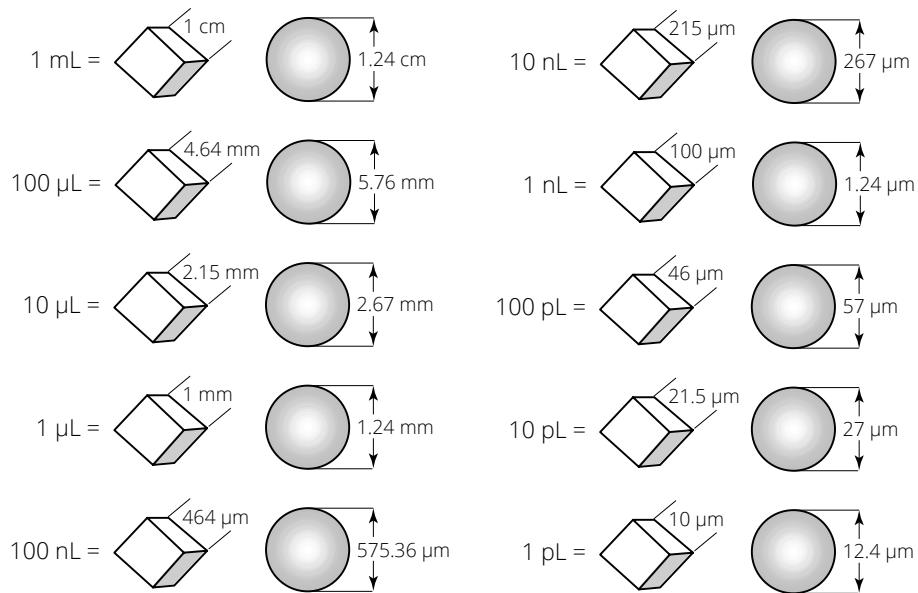


Fig. 30—This graph compares the dimensions of a cube and a sphere with the same internal volume.

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DECLARATION OF CONFORMITY



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DECLARATION OF CONFORMITY CE

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

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

PV850

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

Low Voltage Directive (Safety) 2014/35/EU:

- EN 61010-1:2010+A1:2019

EMC Directive 2014/30/EU:

- EN IEC 61326-1:2021
- EN IEC 61326-2-3:2021
- EN IEC 61000-3-2:2019+A1:2021
- EN IEC 61000-3-3:2013+A2:2021

Issued On: December 12, 2022


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F-QC-006 Rev D

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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