INSTRUCTION MANUAL

PV820/PV830

Pneumatic Picopump

www.wpiinc.com
080824

CONTENTS

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—PV820 Pneumatic PicoPump (top) and PV830 Pneumatic PicoPump with vacuum regulation (bottom)

INTRODUCTION

Designed to simplify intracellular injection and a variety of other microinjection tasks, **PicoPumps** use carefully regulated air pressures for securing cells and injecting them with fluid. Injected volumes range from picoliters to nanoliters. Separate ports supply positive and negative pressure—positive pressure for high-pressure ejection, and suction for supporting the cell or for filling the pipette from the tip. A second pressure port maintains a low positive "holding" pressure to the injecting pipette between injection pulses, to prevent fluid uptake through capillary action.

Timing, ejection pressure, holding pressure, and suction are adjusted independently by control knobs and indicator gauges on the front panel. Injection pressure is controlled by a 20-turn regulator on the front panel. A built-in timing circuit allows precise control of the amount of time that the injection pressure is applied to the output port. Time intervals can range from 10 seconds down to 10ms or less, depending on the eject pressure setting. The injection pressure interval can be triggered manually on the front panel, by foot switch or by a computer controlled TTL pulse. A 5V monitor output provides a logic-level pulse for your computer or other monitoring device.

The **PicoPumps** are 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. Vacuum may also be applied on a separate channel to hold cells or small objects and to load pipettes from the tip. Therefore, cells may be held by vacuum and simultaneously injected using pressure.

PV820

Like the **PV830**, the **PV820** offers separate regulated hold and ejection pressures with a precision timing circuit that switches from eject pressure to hold pressure automatically. Although regulated vacuum is not provided in this model, suction can be provided by connecting a pre-regulated vacuum source to the vacuum port on the rear panel. Suction is then available through the pressure ports.

PV830

On the **PV830**, eject pressure, hold pressure and vacuum are all available, controlled by separate regulators on the front panel. Eject pressure supplies a high-pressure pulse for injecting fluid. Hold pressure, which is not sufficient to cause fluid ejection, is used to prevent front filling of the pipette by capillary action when not injecting. Pressure in the injection pipette is automatically switched between Eject and Hold pressure by a precision timing circuit that controls a solenoid valve. Vacuum is regulated the same way, by a 20-turn knob on the front panel. The vacuum port may be switched from regulated vacuum to atmosphere by using a switch on the front panel. Vacuum may also be routed to the eject port.

Notes and Warnings

⚠ **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 120PSI (1000KPA).**

⚠ **WARNING: USE DRY AIR, NITROGEN OR OTHER INERT GASES ONLY. DO NOT USE OXYGEN.**

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

Parts List

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

(1) **PicoPump** (**PV820** or **PV830**)

(1) **3316** Startup Kit, which includes:

- (1) 0.25″ female NPT fitting for nitrogen tank regulators (Fig. 3.)
- (1) PV830 vacuum connector, silicone (Fig. 4.)
- (1) 5′ length of hard pressure tubing, $\frac{1}{4}$″ OD. (Cut it in half to make pressure and vacuum lines.)

(1) **5430-ALL PicoNozzle Kit** (Fig. 5) which includes:

- (2) **PicoNozzle** tip assemblies (Handle diameter is 6.25 x 100mm.)
- 10′ tubing
- (4) 1.0mm pipette gaskets (green)
- (4) 1.2mm pipette gaskets (black)
- (4) 1.5mm pipette gaskets (red)
- (4) 1.65mm pipette gaskets (white)

(1) Instruction Manual


Fig. 2—(Left) The PV830 Startup kit includes tubing and several fittings.
Fig. 3—(Right) The 1/4″ female, NPT, quick-connect, threaded fitting is for a nitrogen regulator. Depress the plastic collar to remove tubing.

Fig. 4—Use the silicone suction connector to join hard tubing to a vacuum pump.

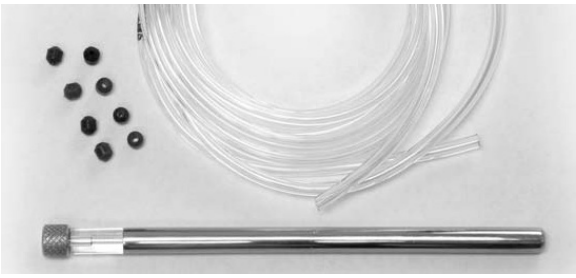


Fig. 5—Each PicoPump is supplied with a 5430-ALL PicoNozzle (Version 2) kit.

Legacy PicoNozzle Kits

NOTE: The legacy Version 1 **PicoNozzle Kits** are still available and sold separately and also work well with the **PicoPumps**.

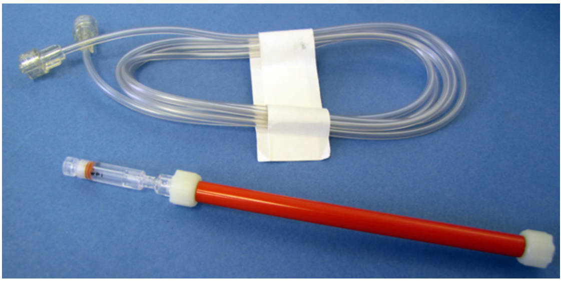


Fig. 6—PicoNozzle Version 1 Kits (#5430-10, 5430-12, 5430-15, 5430-20).

Version 1 **PicoNozzle** kits each contains:
(1) **MPH6S** microelectrode holder
(1) Handle for the **MPH6S** (4″ hollow tube with male Luer fitting at both ends-handle diameter is 6.25 x 100mm.)
(1) 5′ tubing (0.060″ ID, 0.120″ OD, male locking Luer fitting on one end and a female locking Luer fitting at the other end, rated for 200 PSI and 86 durometer shore A)

The microelectrode holder is equipped with a female luer to attach to one end of the 4˝ male/male luer lock adapter. The 5´ tubing also has a female luer at one end to attach to the opposite end of the 4˝ luer lock adapter. The male luer fitting of the 5´ tubing must be cut off in order to attach it to the barbed pressure port of the **PicoPump**.

When using a **MPH6S** handle, a firmer hold on the glass can be achieved by using two gaskets in the micropipette holder. For a list of replacement parts (micropipette holders and gaskets), see “Accessories” on page 18.

NOTE: Capillary holders can be obtained from WPI which contain Ag/AgCl half-cells. These holders (**MPH6S, MPH6R**) can be easily mounted on amplifier headstage probes so that potential and/or current, as well as pressure, can be measured or dispensed through the capillary tip.

To mount the micropipette, pulled capillary glass may be inserted in the holder. A screw cap allows the glass micropipette to be firmly held by a rubber gasket. The luer fittings make changing micropipettes easy by allowing quick removal of the pipette holder from the 4″ luer lock adapter. Test to make sure the micropipette is firmly held by pulling on it.

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 27 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 100 mm (four inches) of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 27 of this manual.

INSTRUMENT DESCRIPTION

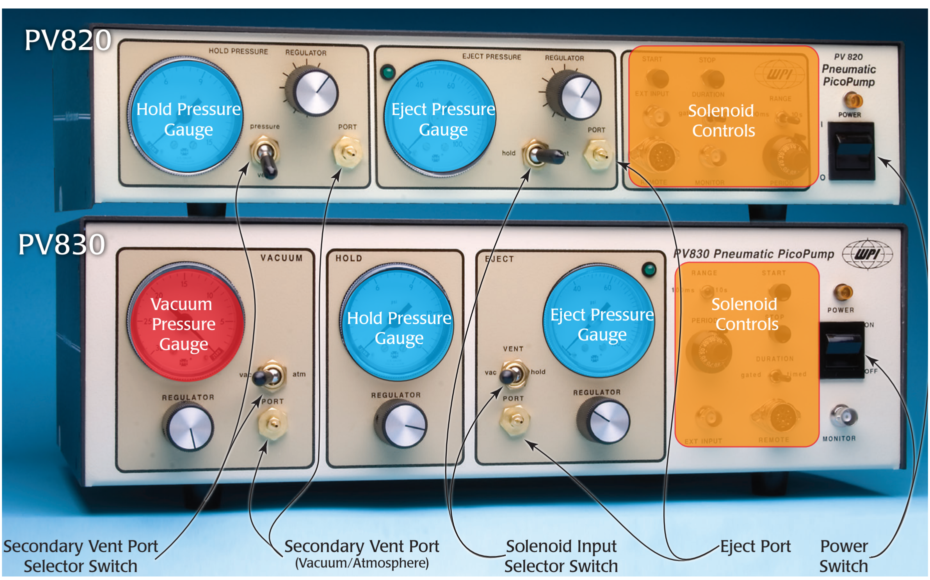


Fig. 7—The major features of the PicoPumps are labeled in this diagram.

Regulators/Gauges– The **PV820** has a hold pressure regulator and an eject pressure regulator. In addition to those two, the **PV830** also has a vacuum regulator. Each **precision** regulator is connected with a measuring gauge for monitoring the pressure that flows through the regulator.

Adjust the pressure by turning the **Regulator** knobs located near the pressure gauges. Turn the knob clockwise to increase the pressure, and counter-clockwise to decrease it. Because the regulating mechanism is self-venting, pressure is automatically released when the regulator setting is decreased. Although the pressure gauges cannot be read with high accuracy (especially at pressure settings below 2PSI), reproducible pressures may be obtained, because the pressure regulators have a 20-turn resolution.

The pressure regulating mechanism functions by continuously bleeding a small amount of gas. This bleed rate increases as the difference between the input pressure and the output pressure increases. Loss of gas may be minimized by decreasing the difference between the input and output pressures, but some decrease in regulation may be noticed if the input pressure is not at least 10% greater than the output pressure.

CAUTION: Be careful NOT apply too much pressure when turning the knobs fully counterclockwise to minimum pressure. They cannot be adjusted to 0.0 PSI. Over-tightening the knobs damages the regulators.

CAUTION: The regulators leak a small amount of gas as a part of their normal operation. To conserve tank contents, make sure to turn off the main gas supply pressure when the **PicoPump** is not in use.

Vacuum is adjusted by turning the vacuum **Regulator** knob located next to the vacuum gauge. Turning the knob clockwise increases the vacuum (lower pressure), and counter-clockwise decreases the vacuum. Because the regulating mechanism is self-venting, vacuum is automatically released when the regulator setting is decreased. Although the vacuum gauge cannot be read accurately at very low vacuum settings (below 2 in. Hg), reproducible settings may still be obtained by means of the 20-turn dial regulator knob.

Solenoid Controls–When the solenoid valve is engaged, the eject pressure is routed to the **Eject** port. The solenoid may be engaged by pressing the optional foot switch, pressing the **Start** button or by an external TTL control. When the solenoid valve is inactive, the hold pressure or vacuum is routed through the **Eject** port, depending on the setting of the **Solenoid Input Selector Switch**. The solenoid controls are used to set the timing and trigger the eject pressure bursts from the **Eject** port. The solenoid controls are discussed later. See "Typical Setups" on page 7.

Solenoid Input Selector Switch–This switch determines what the solenoid routes to the **Eject** port when it is inactive, hold pressure or vacuum.

- When this switch is set to **Hold**, the hold pressure is routed through the **Eject** port when the solenoid is inactive.

- When this switch is set to **Vent** on the **PV820** or **Vac** on the **PV830**, the vacuum is routed through the **Eject** port when the solenoid is inactive. The **Vac**/**Vent** setting allows the pressure ejection channel to have either atmospheric pressure or a vacuum (depending on the position of the **Secondary Vent Port Selector Switch**) on the line when the eject pressure is not being applied.

Eject Port–The output of the solenoid is routed through this port, either eject pressure (when the solenoid is active), or hold pressure or vacuum (when the solenoid is inactive).

Secondary Vent Port–On the **PV820**, this is the **Hold Pressure** port, and on the **PV830**, it is the **Vacuum** port. Either vacuum or atmospheric air is routed through this port, as determined by the setting of the **Secondary Vent Port Selector Switch**. The operation of this port is **independent of the solenoid-controlled** pressure **line.**

Secondary Vent Port Selector Switch–When this switch is set to **Vac**, the external vacuum source is routed through the **Secondary Vent Port**. On the **PV820**, the vacuum must be pre-regulated by an external regulator. On the **PV830** the vacuum runs through a regulator. When the switch is set to **Pressure** on the **PV820** or **ATM** on the **PV830**, the port is open to the atmosphere and not connected with any vacuum or pressure source.

Power Switch– Switching the **Power** on (|) provides power for the solenoid and timing circuit. An amber light above the switch indicates when power is on.

Typical Setups

Most of the time, you will use one of two setups on the **PicoPump**:

Purpose	2nd Vent Port Selector Switch*	Solenoid Input Selector Switch**	Result
Hold a cell or reverse fill a pipette	**Vac**	**Vent** (**PV820**) **Vac** (**PV830**)	Vacuum applied at the **Eject** port until solenoid is engaged
Normal operations	**Vac**	**Hold**	Vacuum available at the **Secondary Vent Port** Positive hold pressure applied at the **Eject** port until solenoid is engaged.

*On the **PV820**, this is the **Hold Pressure** port, and on the **PV830**, it is the **Vacuum** port.

** On the **PV820**, this switch is not labeled (hold/vent), and on the **PV830**, it is the **Vent** switch (vac/hold).

Solenoid Controls

You control the duration of the eject pressure burst using the solenoid controls on the right side of the instrument panel. Once the timing is setup, you may initiate the pressure burst in one of three ways:

- Push the **Start** button.
- Apply +5V at the **External Trigger Input Connector**.
- Depress an optional foot switch (WPI #**3260**).

NOTE: A green lamp next to the **Eject** pressure gauge illuminates for as long as the pressure solenoid is open (active/energized).

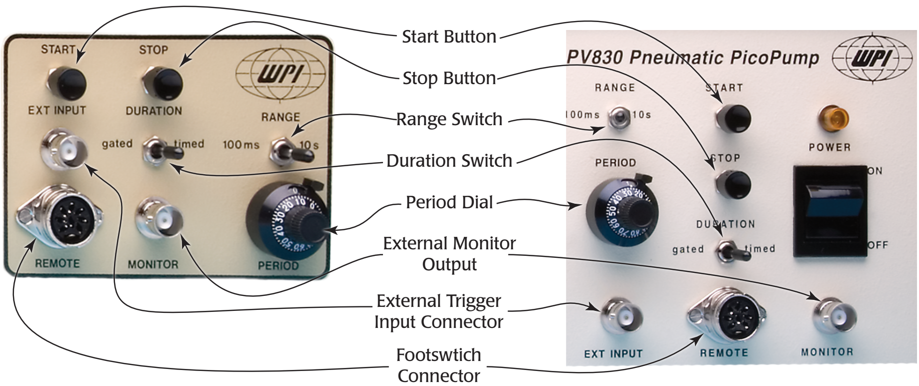


Fig. 8—The solenoid controls the timing, duration and start/stop of the eject pressure.

Start/Stop Buttons–Press the **Start** button to initiate a timed sequence (timed mode) or to manually eject pressure (gated mode). Press the **Stop** button to manually abort a timed sequence.

Range Switch/Period Dial–The duration of the solenoid open time, in the timed mode, is determined by the 10-turn **Period** dial and the setting of the **Range** switch. In the 100ms range, the solenoid times may be set from 0.1–100ms (every turn of the dial is 10ms). In the 10s range, pulses may be set from 10ms to 10s (every turn of the dial is 1.0s). Although the timer control allows setting sub-millisecond burst intervals, the true burst interval is limited by the mechanical response of the solenoid valve, which is approximately 3-10ms, depending upon the pressure. The minimum time interval is limited by the speed of the solenoid, which varies from approximately 10ms at 0 PSI to 3ms at 100 PSI.

Duration Switch–Use this switch to select the gated or timed mode.

- Gated: The pressure solenoid opens and remains open as long as the **Start** button is depressed, a 5V TTL trigger is applied to the **EXT Input** or the foot switch is depressed.
- Timed: An electronic timer controls the duration that the pressure solenoid stays open, and the **Start** button, 5V TTL trigger or foot switch serves only to start the timer.

External Monitor Output–The **Monitor** output BNC connector produces a logic-level output (+5V) corresponding to the time interval during which the **Eject** pressure solenoid is energized. At all other times the monitor output is low (0V).

External Trigger Input Connector–If desired, you may connect an external pulse generator here to supply a +5V to trigger a timed pressure burst sequence or gate the solenoid.

Foot switch Connector–To use the optional foot switch (WPI #**3260**), plug it into the **Remote** connector. Press the foot switch to initiate a timed sequence (timed mode) or to manually eject pressure (gated mode). The foot switch is sold separately.

Rear Panel

A polarized, 3-conductor, connector is used for line (mains) power input to the instrument. A removable cordset, terminated with a grounded 3-prong connector, is standard. An alternate cordset may be supplied when local circumstances dictate different mains voltages and connections. Supply voltage ranges from 100-240V.

A fuse holder contains a protective fuse in series with the high side (brown or black wire) of the mains. The holder accepts 5x20mm fuses of the type indicated on the rear panel (120V, 0.5A or 230V, 0.25A).

Setup

NOTE: For flow diagrams/schematics, see “Appendix A: Flow Diagrams” on page 21.

The 1/4” hard tubing (included) can be cut to fit. It is used for connecting an air pump or tank and a vacuum pump (if it is required). See Fig. 9. The soft tubing (included with the **PicoNozzle Kit**) is used for connecting the micropippette holders to the **PicoPump** ports.

Installing Hard Tubing

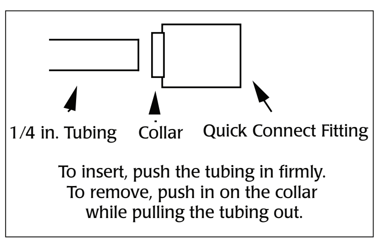


Fig. 9—The PicoPump Pressure and Vacuum ports are located on the rear panel.

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. An example of a common setup is shown in Fig. 10.

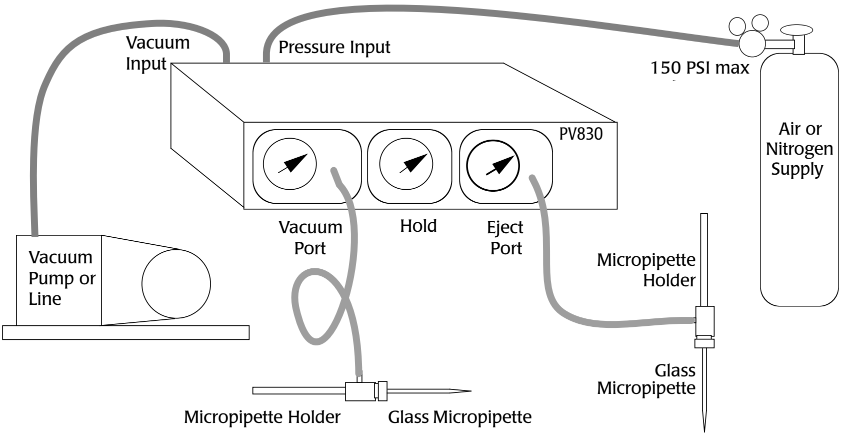


Fig. 10—This diagram show an experimental setup of the PV830. The PV820 setup is identical.

Vacuum Input–Connect the **Vacuum Input** (located at the back of the instrument) to a suitable source of vacuum, such as a vacuum pump (WPI #**LM-500865**) or aspirator, using 0.25″ OD hard tubing and the silicone connector (supplied, Fig. 4). Vacuum may be anywhere in the range of 0–30 in. Hg. The vacuum line does not need to be connected when only positive pressure is needed.

Pressure Input–Connect the **Pressure Input** (located at the back of the instrument) to a suitable source of pressure, such as a compressed gas tank or an air line, using 0.25″ OD tubing supplied. Pressure may be anywhere in the range of 0–120 PSI. Connect this 0.25″ OD tubing to the quick connect fitting. A 0.25″ female NPT fitting is supplied for connecting to a nitrogen regulator on a N_2 tank. Recommended gases are dry air, nitrogen or argon.

CAUTION: Never use corrosive gases. PV800 series instruments are not designed for use with oxygen.

CAUTION: If an air pressure line containing oil or water vapor is used, an external filter is recommended to prevent excessive contamination of the internal pneumatic components.

CAUTION: The precision regulators used in this instrument continuously vent a small amount of supply pressure as a part of their normal function. To prevent waste of gas, always turn off the main supply pressure when the **PicoPump** is not in use.

Assembling the PicoNozzles

The **PicoPump** comes with a **PicoNozzle Kit** version 2 (#**5430-ALL**) which includes two **PicoNozzles** and tubing to connect the holders to the pressure and vacuum ports. (Fig. 5 on page 4) Use one **PicoNozzle** for pressure, and the other for vacuum.

NOTE: If you prefer the original **PicoNozzles**, they may be purchased as an option for use with the **PicoPumps**. See "Legacy PicoNozzle Kits" on page 4.

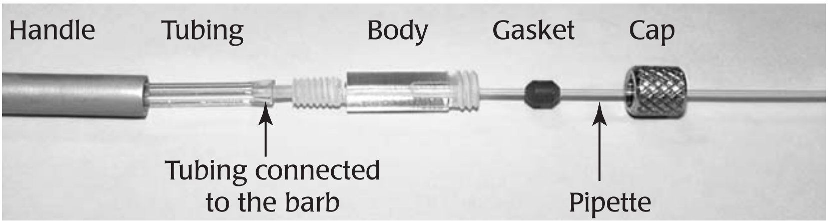


Fig. 11—The 5430-ALL (version 2) must be properly assembled.

1. Slide the superthane tubing through the handle and connect it to the barb of the body. Be careful so that the barb does not break when attaching or removing the tubing. Then, slide the handle over the body and screw it in place.
2. 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

3. Connect the other end of the small tubing to the appropriate port on the front of the **PicoPump**.

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

NOTE: Long lengths of tubing tend to decrease the time response of the system due to a loading effect of the increased volume. For fastest possible response, keep the tubing length (and diameter) as small as possible.

OPERATING INSTRUCTIONS

CAUTION: Be careful NOT apply too much pressure when turning the knobs fully counterclockwise to minimum pressure. They cannot be adjusted to 0.0 PSI. Over-tightening the knobs damages the regulators.

CAUTION: The regulators leak a small amount of gas as a part of their normal operation. To conserve tank contents, make sure to turn off the main gas supply pressure when the **PicoPump** is not in use.

Techniques In Microinjection

The **PicoPump** 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 **PicoPump**.

Setting the Hold Pressure

The **Hold** 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.

1. Set the **Solenoid Input Selector Switch** to the **Hold** position.
2. Set the **Hold** pressure by adjusting the regulator setting until the meniscus stops at the desired position. 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 hold pressure is higher than the capillary pressure, the fluid oozes out of the pipette. The solution around the pipette will be colored. Adjusting the **Hold** pressure prevents this from happening.

Understanding how capillary action causes the front filling of the pipette helps you to correctly use the **Hold** 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 hold pressure becomes the main option to eliminate the uptake of fluid by capillary action.

NOTE: The pressure of capillary action is determined by the inner diameter of the glass capillary where the meniscus of air/liquid interface is located. It has nothing to do with the pipette tip size.

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

$$P=4\gamma \cos\theta/d$$

γ = surface tension

θ = contact angle between the water and glass

d = 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. The capillary pressure can vary a thousand times when the meniscus is moved from a 0.5µm ID tip to the 0.5mm shank.

The pressure at 0.5µm tip is about 80 PSI (in aqueous solution) while at the shank will be only 0.08 PSI. Using one regulator to counterbalance the pressure in such a large dynamic range is not practical. The hold regulator in this instrument is optimized to work in the 0.2–10 PSI range. 10 PSI can counterbalance a meniscus at the section of tip where the inner diameter is 4µm.

In practice, this is the highest pressure ever needed. On the lower pressure end, it becomes difficult to exactly counterbalance the capillary pressure when the meniscus is at the shank of the pipette. However, a 0.1–0.2 PSI pressure imbalance will not cause a significant problem if the tip is small enough. The gravity of the fluid and the flow resistance caused by friction from the glass wall will both help to stop the solution flow at this pressure level.

TIP: If the lowest pressure setting is ineffective at preventing the pipette fluid from leaking out, try to switch off the hold pressure to see if the gravity and friction are sufficient to counterbalance the front fill. In addition, resetting the regulator could allow the regulator to perform much better in this range (see the "Troubleshooting The Hold Pressure" on page 19).

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 90º).

Micropipette Manufacture

Pulling suitable micropipettes is one of the biggest obstacles to taking full advantage of the **PicoPump**. Both care and steady hands are required.

The volume of fluid ejected is markedly dependent on the micropipette tip size. When using micron-sized tips a reduction in tip-size of a few percent may give an order of magnitude difference in the flow rate. With tip sizes less than 1µm, pressure ejection becomes increasingly difficult and special steps must be taken.

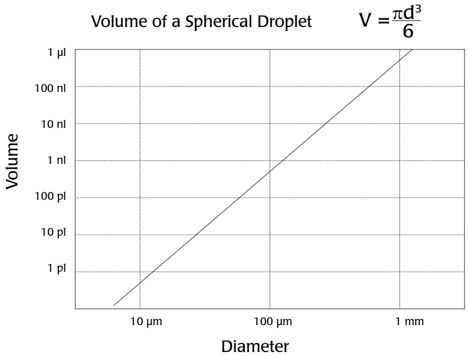
The most important of these steps is cleaning the glass. Small amounts of dust or grease can easily clog micron-sized tips. Cleaning with chromic acid solutions before pulling the electrode is commonly performed, but care must be taken to thoroughly rinse the pipettes to remove all traces of the chromic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

Silanization of the glass is also recommended for small tips. With 1µm and smaller tips, capillary action becomes prohibitively large, and the hydrophilic surface of the glass greatly limits the flow of fluid through the tip. Silanization decreases the surface tension and allows the fluid to flow smoothly through the tip. For similar reasons, we don't recommend use of a capillary with an internal filament. Some of the many papers on the art of silanization are listed in the bibliography.

When using the vacuum line to hold and manipulate individual cells, a large tip (about 10–20% of cell size) is recommended. To prevent damage to the cell, this tip should be fire-polished.

Volume Calibration by Measuring Droplet

For ejected volumes greater than 1nL, 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. 12 may be helpful in determining the volume for a given radius. See "Appendix B: Droplet Volume" on page 23 for a comparison of spherical and cubical volumes.



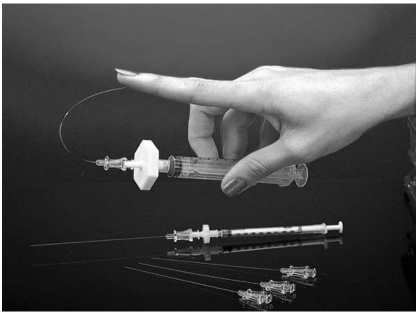


Fig. 12—(Left) The volume of a droplet increases as the diameter increases. See "Appendix B: Droplet Volume" on page 23.
Fig. 13—(Right) WPI's MicroFil is helpful in backfilling glass pipette tips. Syringe filters (available separately) help prevent clogged micropipette tips.

The following table is useful for converting between different units of volume.

Cubic Measure	$1cm^3$	$1mm^3$	$(100\mu m)^3$	$(10\mu m)^3$	$1\mu m^3$
Volume	1mL	1µL	1nL	1pL (10^{-12}L)	1fL (10^{-15}L)

For ejected volumes less than 1nL, 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. If you choose to silanize only the outside of the pipette, the **PicoPump** may be used to applying air flow through the pipette during the silanization.

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.

Rate Calibration using a Known Volume

We can calculate the volume of liquid contained in a 1mm 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 1mm of glass with 0.58 mm ID (WPI #**1B100**) is 264nL, and it takes 30 seconds to inject that volume, then the flow for 1 second is 264nL/30s or 8.8nL/second, and a 1nL 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 10–20µm. By following these steps, we are able to calculate the timing needed to deliver a 1nl injection by counting the time between 1mm marks as the fluid is injected.

NOTE: The **PicoPump** timing should not be adjusted to go below 10–15ms as an absolute minimum, since it takes 6–10ms for the pressure relay 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 hold pressure and your eject pressure. The proper settings must be determined experimentally. Turn on the **PicoPump**.

   NOTE: The eject pressure must exceed the hold pressure in order to eject the fluid.
3. Use a pipettor to inject a 10µl sphere of fluid into the mineral oil.
4. Using a fine pointed permanent marker, mark the injecting micropipette at 1mm increments.
5. Mount your "graduated" micropipette on the **PicoNozzle** that is connected to the **Eject** port on the **PicoPump**.
6. Submerge the tip of the pipette in the fluid droplet you placed in the mineral oil. Front fill the injecting pipette using the **Vac** (**PV830**)/**Vent** (**PV820**) setting on the **Solenoid Input Selection Switch**. When the pipette is full, set the **Solenoid Input Selection Switch** to **Hold**.
7. Set the **Duration** switch to Gated. Hold down the foot switch to eject fluid from the micropipette into the oil-filled dish.
8. Record the amount of time it takes in seconds for the meniscus in the micropipette to travel from one 1mm mark to the next. It should take between 20 and 30 seconds for each injection. You may need to adjust the eject pressure to achieve this time frame.
9. Repeat steps 7–8 three times
10. Average the three trials to find the average time it takes to inject a 1mm column of fluid.
11. Calculate the volume of a 1mm segment of your micropipette using the volume formula for a cylinder:

    r= radius of the pipette = ID/2
    h= length of the pipette = 1000µm
    $V= \pi r^2 h = \pi ID^2 1000/4 = 785 * r^2$

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

Both those ID's have a ±100µm tolerance, and that ID dimension is vital to making a correct injection of 1nL.

12. Divide the average found in step 10 by the calculated volume in nL of a 1mm length of your pipette found in step 11. 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.

Multibarrel Microinjection

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

Multi-barrel micropipette coupling kit

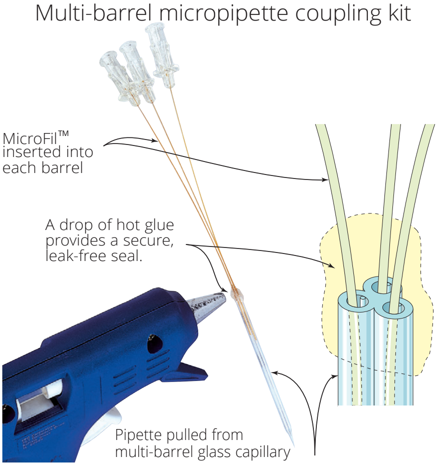


Fig. 14—PolyFil multibarrel micropipette coupling kit

MAINTENANCE

The **PicoPump** has been designed to yield reliable performance. However, some laboratory conditions may require occasional replacement of the pressure and vacuum filters. If this is necessary, return the instrument to the factory.

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.

Fuses

The correct replacement fuse for 110 V operation is 0.5 A, fast blow. Use a 0.25 A, fast 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. A (Fig. 15).

1. Turn the main power switch off (I).
2. Unplug the power cord from the power cord socket on the back of the unit (Fig. 15).

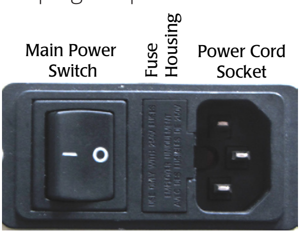


Fig. 15—Unplug the power cord to access the fuse housing release.

3. Insert a small flat blade screwdriver under the lip on the right side of the fuse housing cover (Fig. 16).

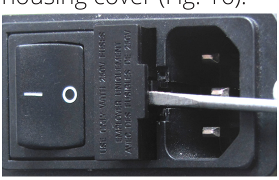


Fig. 16—Insert the screw driver under the fuse housing lip and pry the housing open.

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. 17).

Fig. 17—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.

ACCESSORIES

Part Number	Description
3260	Foot Switch
2932	Rack Mount Kit, 31 2-in. high (PV800 & PV820)
2933	Rack Mount Kit, 51 4-in. high (PV830)
5430-10	PicoNozzle 1 Kit (MPH6S for 1.0 mm pipette & 5-ft tubing assembly)
5430-12	PicoNozzle 1 Kit (MPH6S for 1.2 mm pipette & 5-ft tubing assembly)
5430-15	PicoNozzle 1 Kit (MPH6S for 1.5 mm pipette & 5-ft tubing assembly)
5430-20	PicoNozzle 1 Kit (MPH6S for 2.0 mm pipette & 5-ft tubing assembly)
5430-ALL	PicoNozzle 2 Kit
5440	PolyFil Multi-Barrel Micropipette Coupling Kit
MPH6S	Micropipette Holder (specify 1.0, 1.2, 1.5 or 2.0 mm)
MPH6R	Micropipette Holder (specify 1.0, 1.2, 1.5 or 2.0 mm)
3316	Replacement Input Kit
LM-500865	Vacuum Pump

Replacement Parts

Part Number	Description
75122-110	PicoNozzle gasket green 1.0mm, pkg. of 10
75122-210	PicoNozzle gasket black 1.2mm, pkg. of 10
75122-310	PicoNozzle gasket red 1.5mm, pkg. of 10
75122-410	PicoNozzle gasket white 1.65mm, pkg. of 10

Legacy Replacement Parts

Part Number	Description
MPH6S-10	Legacy Micropipette holder, 1.0mm
MPH6S-12	Legacy Micropipette holder, 1.2mm
MPH6S-15	Legacy Micropipette holder, 1.5mm
MPH6S-20	Legacy Micropipette holder, 2.0mm
G01-100	Legacy gaskets, 1.0mm, qty. 100
G02-100	Legacy gaskets, 1.2mm, qty. 100
G03-100	Legacy gaskets, 1.5mm, qty. 100
G04-100	Legacy gaskets, 2.0mm, qty. 100

TROUBLESHOOTING THE HOLD PRESSURE

If the **Hold** regulator can't reduce the pressure low enough to keep the liquid from coming out of the pipette tip, DO NOT try to shut off the pressure by forcing the regulator knob counterclockwise. The regulator is designed so that the flow can't be shut off completely. It should, however, be able to reduce the pressure to below 0.2 PSI, which is low enough for most applications. If the regulator can control the pressure above 0.5 PSI, but not below 0.5 PSI, a simple reset will fix the problem.

This problem is caused by a mis-alignment of the o-ring. However, the problem can be corrected by applying a high pressure into the output port to re-position the o-ring. Use the following procedure to introduce a high pressure:

1. Remove the 1/4" hard pressure tubing from the back of the pump.
2. Use pieces of tubing with 1/4" OD to make a small 1/16" ID drop down extension.
3. Position the extension inside the end of the 1/4" hard pressure tubing.
4. Place the other end of the extension on the **Eject** port
5. Then, apply an injection at 20 PSI for 10 seconds.
6. Once the O-ring re-seats, it will remain there even when the output pressure again increases, as long as there is no high flow output. When there is no restriction at the output and the pressure is adjusted too high, the diaphragm has to move a substantial distance to allow the valve to open more to compensate the for pressure loss. This also causes the O-ring to move. Therefore, if low-pressure regulation is very important for the application, you should not set the **Hold** regulator at the high-pressure position when there is no restriction at the output. There are two ways to achieve this:
   - Always reduce the regulator pressure to minimum before assembly and disassembly of the injection pipette.
   - Turn off the **Hold** pressure valve before assembly and disassembly of the injection pipette.

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:

	PV820	PV830
PRESSURE		
Pressure Input	0 to 120 psi	0 to 120 psi
Pressure Output	0.3 to 90 psi *	0.3 to 90 psi
Pulse Width (10-Turn Dial)	10 ms to 10 s in Timed Mode	10 ms to 10 s in Timed Mode
Regulator Accuracy	0.1% (20-turn dial) *	0.1% (20-turn dial) *
Regulator Repeatability	0.05 psi *	0.05 psi *
Gauge Accuracy	3% at full scale *	3% at full scale *
Input Connector	Quick Connect (¼ in. OD Tubing)	Quick Connect (¼ in. OD Tubing)
Output Connector	Barbed (1/16-in. ID Tubing)	Barbed (1/16-in. ID Tubing)
Control	Solenoid	Solenoid
VACUUM		
Vacuum Input	0 to 30.0 in. Hg	0 to 30.0 in. Hg
Vacuum Output	Unregulated	0.2 to 29.9 in. Hg
Lowest Regulated Vacuum	Unregulated	3″ water (0.7kPa)
Regulator Accuracy	Unregulated	0.1% (20-turn dial)
Regulator Repeatability	Unregulated	0.03 in. Hg (0.1kPa)
Gauge Accuracy	None	3% at full scale
Input Connector	Quick Connect (¼ in. OD Tubing	Quick Connect (¼ in. OD Tubing)
Output Connector	Barbed (1/16 in. ID Tubing)	Barbed (1/16 in. ID Tubing)
Control	Manual	Manual
Vent	Atmosphere	Atmosphere
CONNECTIONS INCLUDED		
Input Kit	10-ft nylon tubing (¼-in. OD, 1000 PSI), one ½-in female NPT adapter	
Output Kit	Two PicoNozzle assemblies, each consisting of one MPH6S pipette holder, 60-in. of PVC tubing (200 PSI), and a luer-fitted aluminum handle	
PHYSICAL SPECIFICATIONS		
Fuse (Older models)	120 V: 0.5 A, fast, 0.25x1.25″ USA 230 V: 0.25 A, fast, 0.25x1.25″ USA	120 V: 0.5 A, fast, 0.25x1.25″ USA 230 V: 0.25 A, fast, 0.25x1.25″ USA
Fuse (2019 models)	120V: 0.5A, fast, 5 x 20mm metric 230V: 0.25A, fast 5 x 20mm metric	120V: 0.5A, fast, 5 x 20mm metric 230V: 0.25A, fast 5 x 20mm metric
Power	95-135V or 220-240V, 50/60Hz	95-135V or 220-240V, 50/60Hz
Dimensions	17x3.5x9.5 in. (43x9x24 cm)	17x5.25x9.5 in. (43x13x24 cm)
Shipping Weight	11 lb (5 kg)	14 lb (6.3 kg)

** Both Hold and Eject Pressures*

APPENDIX A: FLOW DIAGRAMS

The following diagrams depict how air and vacuum are routed through the **PV820** and **PV830** pumps.

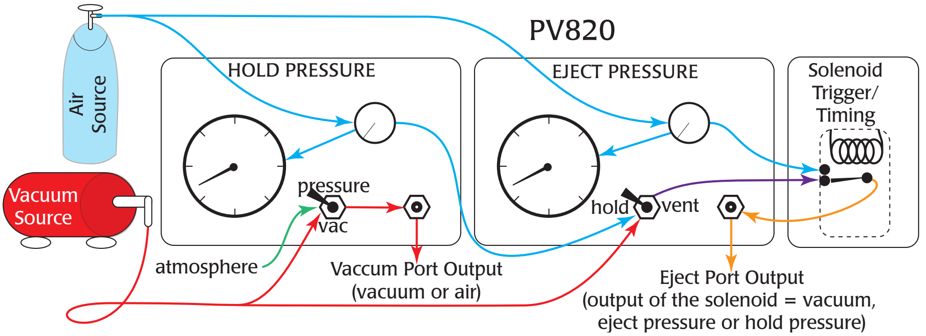


Fig. 18—Air and vacuum flow through the PV820 based on the switch settings, and the air pressure (for both the hold and eject) is regulated.

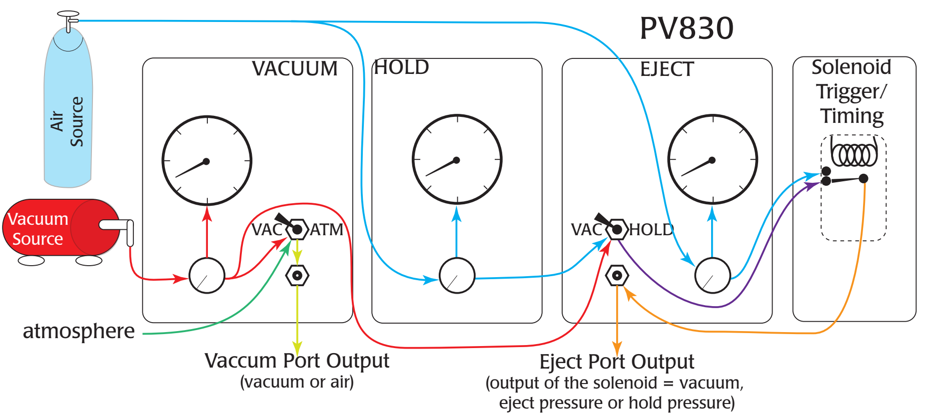


Fig. 19—Air flows through the PV830 based on the switch settings, and the air pressure (for both hold and eject) and vacuum are regulated.

Schematics

PV820 Block Diagram

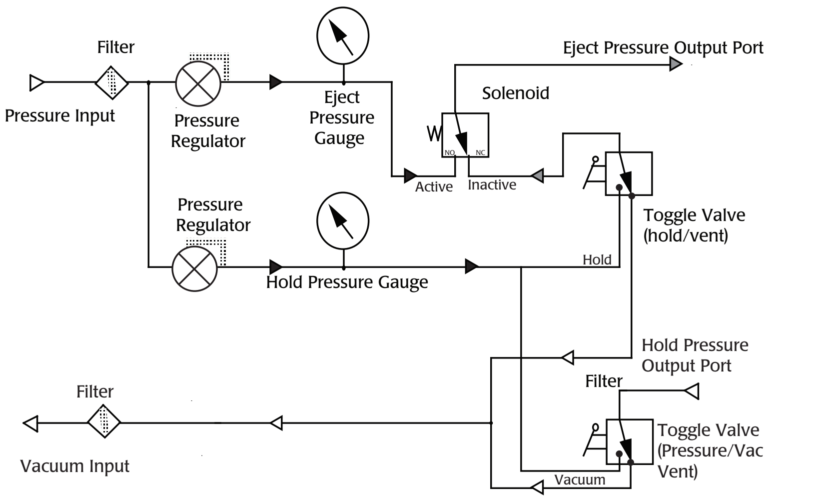


Fig. 20—The schematic diagram shows the inner workings of the PV820 PicoPump.

PV830 Block Diagram

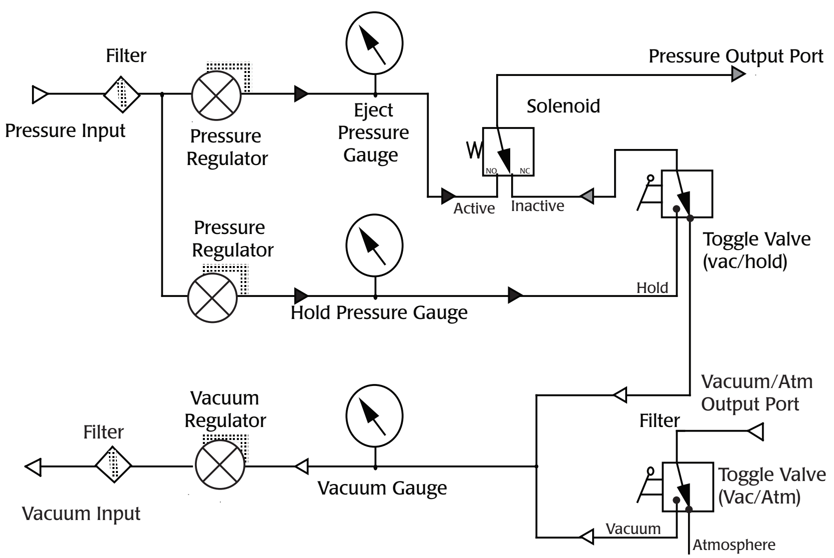


Fig. 21—The pneumatic block diagram of the PV830 PicoPump is shown.

APPENDIX B: DROPLET VOLUME

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

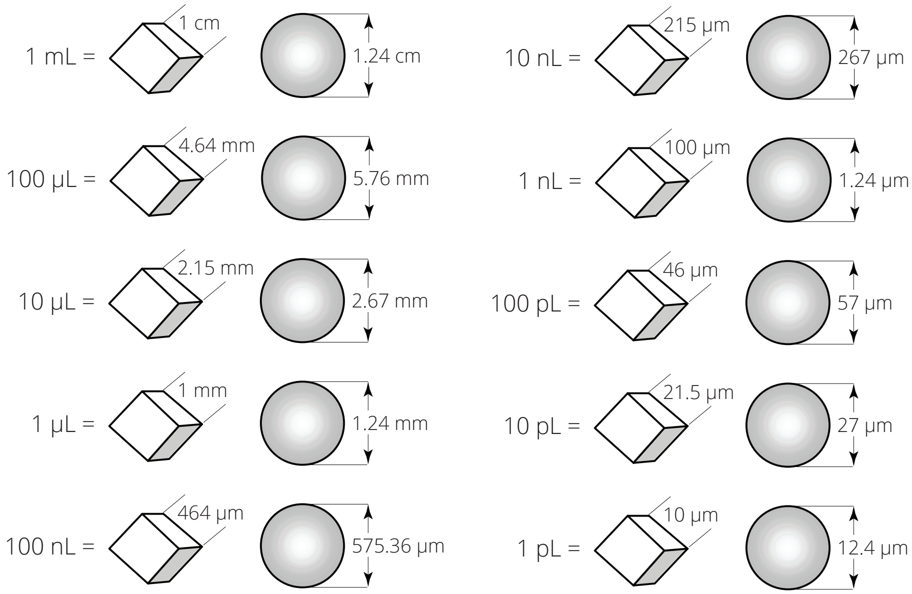


Fig. 22—This graph compares the dimensions of a cube and a sphere with the same internal volume.

Volume of a Micropipette

Outside Diameter	Inside Diameter	Approximate Volume per Inch
1.0 mm (1B100)	0.58 mm	6.7 µl / inch (264 ηl /mm)
1.0 mm (TW100)	0.75 mm	11.2 µl / inch (442 ηl /mm)
1.2 mm (504949)	0.53 mm	5.6 ul /inch (220 ηl /mm)
1.2 mm (1B120)	0.68 mm	9.2 µl / inch (363 ηl /mm)
1.2 mm (TW120)	0.90 mm	16.2 µl / inch (636 ηl /mm)
1.5 mm (1B150)	0.84 mm	14.1 µl / inch (554 ηl /mm)
1.5 mm (TW150)	1.12 mm	25µl / inch (985 ηl /mm)
2.0 mm (1B200)	1.12 mm	25µl / inch (985 ηl /mm)

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DECLARATION OF CONFORMITY

WORLD PRECISION INSTRUMENTS, LLC.
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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 **manufacture/distributor** of the apparatus listed, declare under sole responsibility that the product(s):
PV820 / PV830

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

Safety:
EN 61010-1:2010
EMC:
EN61326-2-3:2013, EN 61326-1:2013
EN 61000-3-2:2014, EN 61000-3-3:2013

And therefore conform(s) with the protection requirements of Council Directive 89/336/EEC relating to electromagnetic compatibility and Council Directive 73/23/EEC relating to safety requirements:

Issued on: **July 20, 2018**

Quality Department Manager

F-QC-006 Rev B

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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