WORLD
PRECISION
INSTRUMENTS

INSTRUCTION MANUAL

MICRO-ePUMP

Microinjector

Serial No._____

www.wpiinc.com

111623

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—MIRO-ePUMP Microinjector includes an internal pressurepump and the integrated MICRO-ePORE Cell Penetrator technology.

INTRODUCTION

Designed to simplify intracellular injection and a variety of other microinjection tasks, the **MICRO-ePUMP** 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 using the foot switch.

The **MICRO-ePUMP** 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.

WPI's **MICRO-ePORE™** Pinpoint Cell Penetrator technology is embedded inside the **MICRO-ePUMP**. When the researcher enables the The **MICRO-ePORE™**, it delivers a highly localized voltage signal to a targeted injection site to facilitate penetration with minimal trauma. The researcher determines the amplitude and frequency of the signal that best suits the application. The signal originates in the control box, and it is transmitted through the electrode interface cable to the microelectrode holder. A silver wire is used to transmit the signal into the electrically conductive substance being injected. A reference electrode is used to place the media at 0.0 V potential with reference to the generated voltage.

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

Features

- Regulated compensation and injection pressure
- Pressure output: 0.3-87 PSI
- Onboard **MICRO-ePORE™**
  - Pinpoint Cell Penetrator for targeted microinjection and increased viability of injected embryos
  - Audio continuity tone indicating active probe
  - Injection counter to indicate total number of injections
  - User-adjustable frequency and voltage through touch screen
- Foot switch to activate voltage for **MICRO-ePORE™** and injection
- Low volume tubing assembly

Benefits

- Internal pressure source
- Intuitive user-interface for injector and **MICRO-ePORE™**
- 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

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 KPA).

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) **MICRO-ePUMP**
(1) Foot switch
(1) **99164** WPI **MICRO-ePORE™** Injection Assembly
(1) **99788** MICRO-ePUMP Ground Cable
(1) AC/DC 24 V Power Adapter
(1) **300744** MICRO-ePUMP Capillary Kit which includes:
- (4) **75122-110** 1.0 mm pipette gaskets (green)
- (4) **75122-210** 1.2 mm pipette gaskets (black)
- (4) **75122-310** 1.5 mm pipette gaskets (red)
- (4) **75122-410** 1.65 mm pipette gaskets (white)
- (1) **75123** Pipette handle
- (2) **802828** Sealing o-rings
- (1) **75125** Pipette holder
- (1) **99862** MICRO-ePUMP tubing assembly
- (1) **99865** MICRO-ePUMP adapter tubing assembly
- (1) **CBL102** 6' Cable, 3.5 mm Mini Phone Plug to BNC
- (1) **803130** Stereo Splitter Cable, 3.5 mm

(1) Input Kit for PicoPumps
- (1) 0.25" NPT Fitting for Nitrogen Tank Regulator

(1) 10' Hard Tubing
(1) Vacuum Fitting
(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.

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 31 of this manual.

INSTRUMENT DESCRIPTION

Front Panel

Fig. 2—The major features of the MICRO-ePUMP 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.

MICRO-ePORE™ Interface Cable Port – If you are using the **MICRO-ePORE™**, connect the Microelectrode Interface Cable (probe cable) to this port. Press the connector into the port. This cable is labeled as Front Panel.

Fig. 3—The Interface Cable Connector is labeled "Front Panel." Do NOT plug it into the foot switch port on the rear panel.

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

Rear Panel

Fig. 4—The foot switch connection, power supply connection and power switch are located on the back panel of the MICRO-ePUMP.

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 down to engage the **MICRO-ePORE™**, and depress it fully to make an injection. (See "Foot Switch" on page 6.)

USB Grounding Port –This port gives you access to ground potential, if it is required. This may be used for connecting with other instruments to ensure a common ground reference. The MICRO-ePUMP is grounded and the probe interface cable is shielded, so use of this port for grounding is optional. A green grounding cable (WPI #**99788**) is included with the system. The interface is also used for factory service.

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.

Foot Switch

The foot switch is a two-step switch. Press the switch half way down to activate the **MICRO-ePORE™**. If the **MICRO-ePORE™** is disabled, just press the switch all the way down. When the switch is depressed all the way, the **MICRO-ePUMP** is activated.

Not depressed *Half way depressed* *Fully depressed*

Touch Panel Display

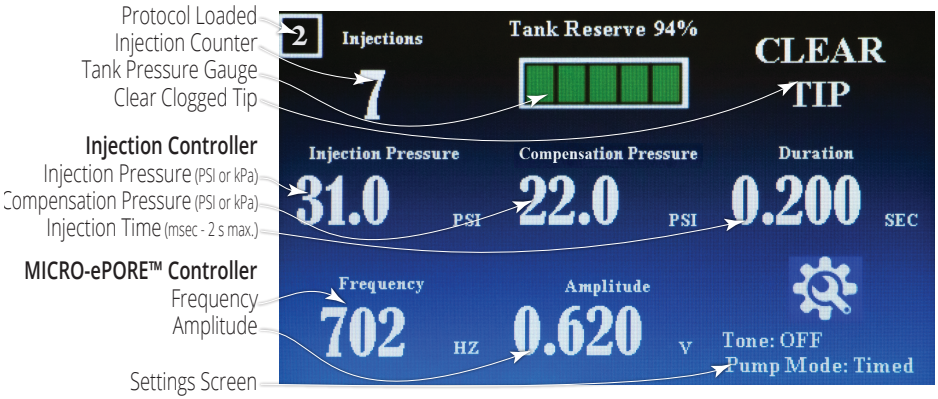


Fig. 5—The Touch Panel Display gives you access to all the parameters and the settings menu.

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 **MICRO-ePUMP** memory. If the box is empty, no protocol is loaded.

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.

Tank Pressure Gauge – The internal pressure tank is pressurized to 100 PSI. When the pressure in the tank falls below 90 PSI (90%), the tank automatically refills. This 10% is considered the tank reserve. The Tank Pressure Gauge shows the status of the tank reserve as the pressure falls to 90%. If you want to manually start the repressurization of the tank, touch the Tank Pressure Gauge. When you power up the **MICRO-ePUMP**, the gauge may register below 90% as the tank fills initially. The display will readout in PSI until the tank reaches 90 PSI. Then, the display will show the percentage of the reserve pressure (0 – 100%).

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.

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 and the pump mode (Timed or Manual).

Injection Controller

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.

MICRO-ePORE™ Controller

Frequency – The WPI **MICRO-ePORE™** Pinpoint Cell Penetrator delivers a highly localized voltage signal to a targeted injection site to facilitate penetration with minimal trauma. The researcher determines the amplitude and frequency of the signal that best suits the application. If you are using the **MICRO-ePORE™** techcnology, touch the parameter and rotate the knob to adjust the frequency of the signal delivered. To engage the **MICRO-ePORE™**, depress the foot switch half way down. When you press the foot switch all the way down, the **MICRO-ePORE™** disengages and the injection is made. To disable the **MICRO-ePORE™**, see "Setting Menu" on page 7.

Amplitude – If you are using the **MICRO-ePORE™** techcnology, touch the parameter and rotate the knob to adjust the amplitude of the signal delivered when you depress the foot switch half way down.

Setting Menu

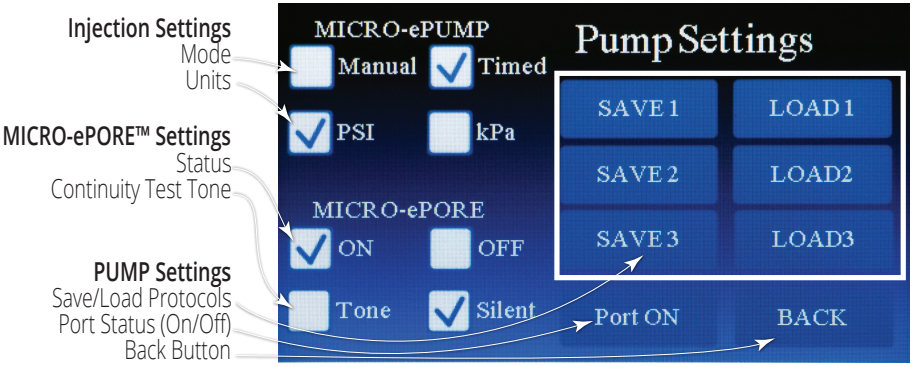


Fig. 6—The Settings menu lets you save/load parameters, choose the mode, set the units, toggle the MICRO-ePORE™ off/on, silence the tones, and toggle the injection port off/on.

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.

Status – Select the ON checkbox to enable the **MICRO-ePORE™** or select the OFF checkbox to disable the **MICRO-ePORE™**. When the **MICRO-ePORE™** is disabled, the bottom section of the main display is blank.

Continuity Test Tone – By default, tones are disabled when you start the **MICRO-ePUMP**. If tones are enabled, a tone sounds when the circuit between the probe tip and the reference electrode is not complete. Without a complete circuit, the **MICRO-ePORE™** will not be able to apply current. Every time you depress the foot switch half way (when the **MICRO-ePORE™** is enabled, a continuity test is conducted. A good tone sounds if the circuit is complete. Otherwise a warning tone sounds. To silence the tone while you correct a setup error, select the Silent check box. Select the Tone checkbox to enable the tones.

Save/Load Protocols – The **MICRO-ePUMP** 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.

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

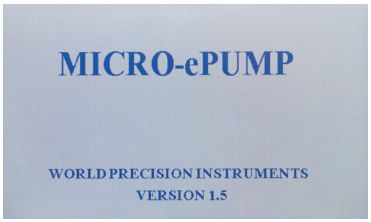
1. With the **MICRO-ePUMP** turned off, make the following connections:
   - Connect Power Supply to the rear of the unit and plug it into a wall outlet.
   - Plug the Foot Switch Connector into the Foot Switch Port.

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 **MICRO-ePUMP**.

2. Verify that the capillary kit hose is disconnected from the Injection Port. Then, power on the **MICRO-ePUMP**. On power up, the following happens:
   - The power up screen appears.
   - The **MICRO-ePUMP** performs a self-test.
   - The operating pressure begins to build.

   After the self-test completes, the main display screen appears. See "Touch Panel Display" on page 6. It displays the configuration settings used when it powered off the last time.



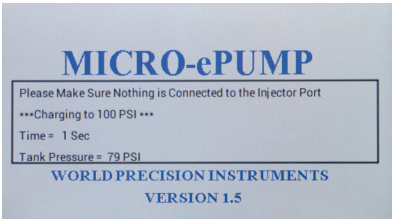


Fig. 8—(Left) The power up screen shows the software revision on your MICRO-ePUMP.
Fig. 9—As the self-test runs through the programmed diagnostic sequence, the display shows the results.

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.

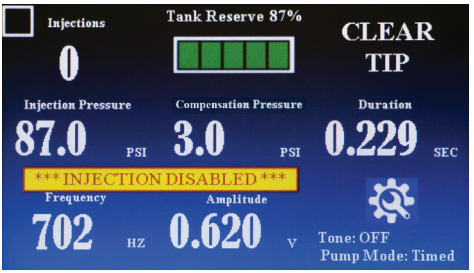


Fig. 10—The warning indicates that the injection port is disabled by default on startup.

Assembling the Capillary Kit

Fig. 11—The 300744 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 **MICRO-ePUMP** 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 **MICRO-ePUMP**, slide the connector securely into the port and attach it with a slight clockwise twist.

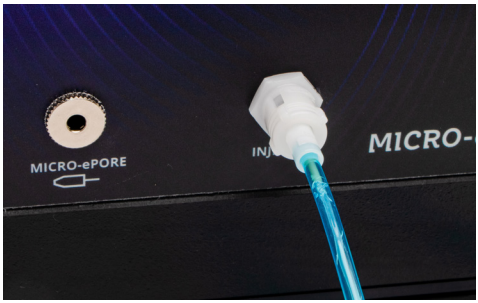


Fig. 12—(Left) Align the connector with the Injection Port, slide the connector in position and rotate the connector a little clockwise to lock it into place.
Fig. 13—(Right) The PicoNozzle is connected to the injection port.

Setting up the MICRO-ePORE™

WARNING: GLASS PIPETTE SHOULD BE SECURED TIGHTLY BEFORE MAKING INJECTIONS.

1. The pipette holder has a silver wire that must be inserted into the glass micropipette. The wire must be long enough to make contact with the substance to be injected. This wire is intentionally left longer than most pipettes to allow for different lengths. Trim the wire to the preferred length.

Fig. 14—This pipette holder is designed for use with the WPI MICRO-ePUMP.

2. The Microelectrode Interface Cable has two 2 mm male pin connectors.

Fig. 15—The Microelectrode Interface Cable has two connectors, one for the reference electrode and one for the microelectrode holder.

- The red pin connects to the 2 mm socket on the microelectrode holder. Insert the red pin into the socket on the microelectrode holder.

Fig. 16—The red pin of the microelectrode interface cable connects to the microelectrode holder.

- The black pin of the microelectrode interface cable connects to the 2 mm socket on one of the reference wires. This is necessary to complete the electrical connection to solution. For the well-style electrode, insert the black pin into the socket of the reference electrode (Fig. 17). For the probe-style electrode, the black pin of the microelectrode interface cable connects to the back side of the reference electrode block. The probe-style electrode pin plugs into the front of the block (Fig. 18).

Fig. 17—(Left) The well-style electrode is properly connected. [Ring reference electrode – OD: 12.7 mm (0.5"), Thickness: 0.4 mm (0.16")]
Fig. 18—(Right) The probe-style electrode is properly connected.

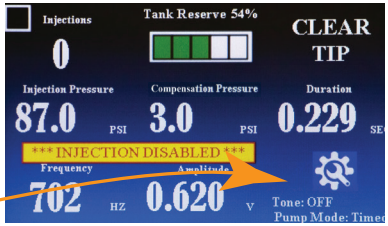
3. A. **Well-style electrode**–If you use the well-style electrode, the gold ring must be secured to your slide using vacuum grease to create an impermeable temporary seal. Place the liquid medium with your embryos inside the gold ring.

   B. **Probe-style electrode**–If you are using the probe-style electrode, place the block near the injection site. Then, bend the bare reference wire as needed to make contact with the liquid medium in order to complete the circuit.

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).



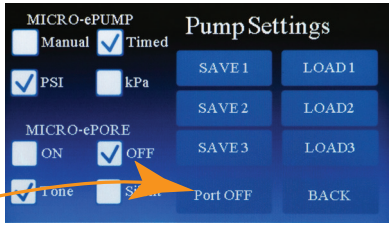


Fig. 19—(Left) Touch the setting area in the bottom right corner to access the Settings menu.
Fig. 20—(Right) Touch the Port OFF button to enable the injection port.

2. 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 **MICRO-ePUMP**. 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 0.1

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

Fig. 21—(Left) Touch the parameter to adjust and rotate the knob to modify the parameter.
Fig. 22—(Right) Press the knob to toggle between gross and fine tuning adjustments.

3. 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.

Changing the MICRO-ePORE™ Parameters

1. When the **MICRO-ePORE™** is disabled, the home screen does not show the **MICRO-ePORE™** parameters in the bottom half of the screen. To enable the **MICRO-ePORE™**, touch the Settings area of the main screen to go to Settings menu.

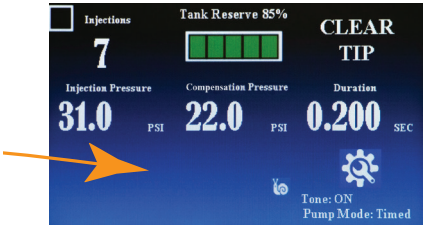


Fig. 23—The MICRO-ePORE™ is disabled in this configuration. No parameters show in the bottom half of the screen.

2. Tap a parameter and turn the knob to change the parameter.

Fig. 24—Touch the Frequency parameter or the Amplitude parameter and rotate the knob.

3. 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 to load.

Improving Cell Penetration

When using the MICRO-ePORE™ correctly, you should be able to perceive an improvement in the penetration of tissue. The first time you use the MICRO-ePORE™ you may need to adjust the voltage applied to optimize it for your particular application. WPI recommends increasing the voltage 10 mV at a time until the desired result is achieved.

CAUTION: Unlike earlier versions (REVISION E and earlier) of the MICRO-ePORE the MICRO-ePUMP does not have the continuity function engaged before injections. This ensures no voltage is applied at the end of the pipette prior to an injection. The continuity function will be activated for 400 ms by the footswitch or by manual activation on the screen, preceding the injection.

WPI recommends that you activate the voltage prior to contacting the cell membrane (Fig. A). Once the MICRO-ePORE is activated, enter the injection micropipette (Fig. B) and make your injection. This ensures that the continuity check finished shortly before you touch the cell, and the penetration is achieved with the programmed voltage and frequency rather than the continuity function voltage.

WARNING: DO NOT ACTIVATE THE MICRO-EPORE WHEN IN CONTACT WITH THE CELL MEMBRANE (FIG. C), BECAUSE THE SYSTEM WILL NOT FIND CONTINUITY WITH THE REFERENCE INSIDE THE MEDIA AND WILL ELEVATE THE VOLTAGE TO MAXIMUM VOLTAGE (3 V), POSSIBLY DAMAGING THE CELL.

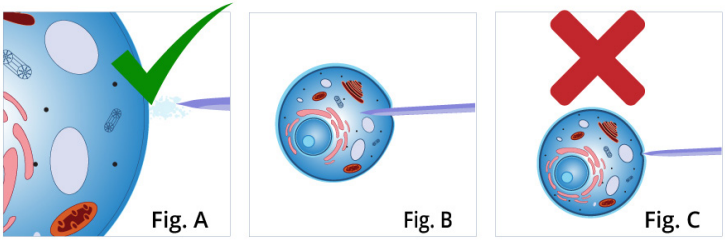


Fig. 25—Activate the voltage (A) before penetration (B). Do NOT activate the MICRO-ePORE when the pipette is in contact with the cell membrane (C).

Your protocol should follow a sequence like this.

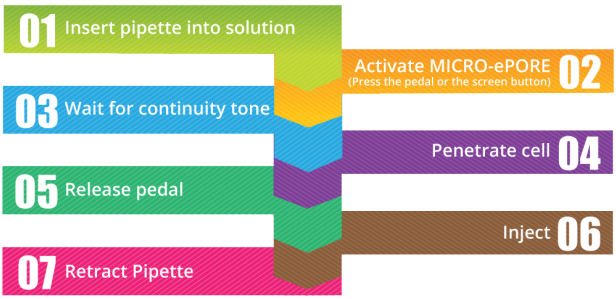
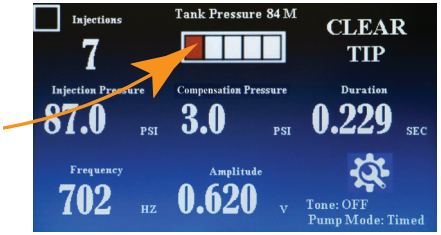


Fig. 26—To protect your cells when making an injection, follow this procedure.

Refilling the Air Tank

1. The internal pump reservoir automatically replenishes itself when it fall below 90% of its full capacity.
2. To reset the pump manually, press the gauge meter on the touchscreen, and the reservoirs starts refilling.



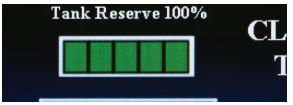
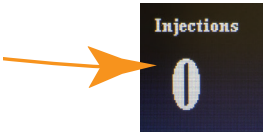


Fig. 27—(Left) Touch the gauge to start refilling the reservoir.
Fig. 28—(Right) When the tank is fully charged at 100 PSI, the tank reserve gauge is green.

Resetting the Injection Counter

This area in the upper left corner shows the injection count. Whenever the foot switch is fully depressed, the injection counter increments. Reset it to zero by pressing on the Injections area of the main display touchscreen.



Adjust the MICRO-ePUMP Settings

The setting section (bottom right corner of the main screen) displays the current settings:

- **MICRO-ePORE™** status (Off or On)
- **MICRO-ePUMP** mode (Manual or Timed)

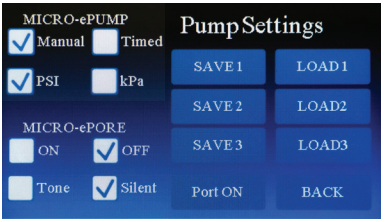


Fig. 29—(Left) The Setting area is located in the bottom right corner of the main screen.
Fig. 30—(Right) Touch the Setting area to display the Settings menu.

1. To modify these settings, press the Settings section on the touchscreen.
2. From there, you can make the following adjustments:
   - Disable or enable the **MICRO-ePORE™**.
   - Disable or enable the **MICRO-ePORE™** continuity test tone.
   - Change the **MICRO-ePUMP** 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. **MICRO-ePUMP** 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.

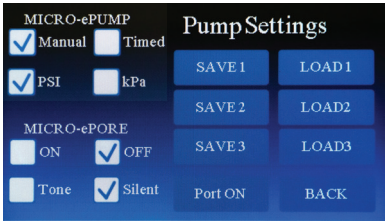


Fig. 31—The Settings menu lets you save a protocol or load a saved protocol.

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

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.

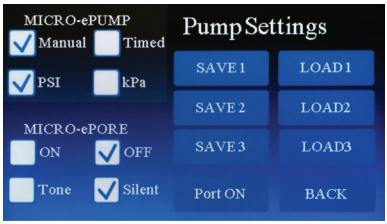


Fig. 32—The Settings menu lets you save a protocol or load a saved protocol.

2. To bring up a saved protocol with the **MICRO-ePUMP** 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.

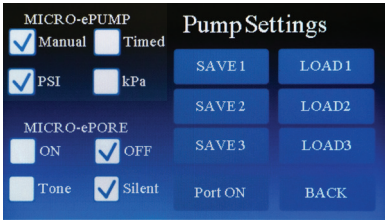


Fig. 33—The Settings menu lets you save enable or disable the Injection Port by pressing the Port ON/Port OFF toggle button which is located to the left of the BACK button.

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.

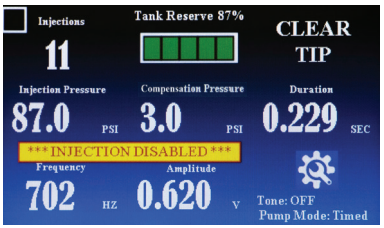


Fig. 34—When the Injection Port is disabled a notice appears on the main screen.

4. To install the micropipette, see "Assembling the Capillary Kit" on page 9.
5. When the micropipette is properly installed, click on the Settings area (the gear icon in the bottom right corner) to access the Settings menu and press the Port OFF button to enable the Injection Port.

TECHNIQUES IN MICROINJECTION

The **MICRO-ePUMP** 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 **MICRO-ePUMP**.

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: 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 0.5 µm ID tip to the 0.5 mm shank.

The pressure at 0.5 µm 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. 10 PSI can counterbalance a meniscus at the section of tip where the inner diameter is 4 µm.

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 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 90º).

Manufacturing Micropipettes

Pulling suitable micropipettes is one of the biggest obstacles to taking full advantage of the **MICRO-ePUMP**. 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 1 µm, 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 1 µm 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.

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. 35 may be helpful in determining the volume for a given radius. See "Appendix A: Droplet Volume" on page 25 for a comparison of spherical and cubical volumes.

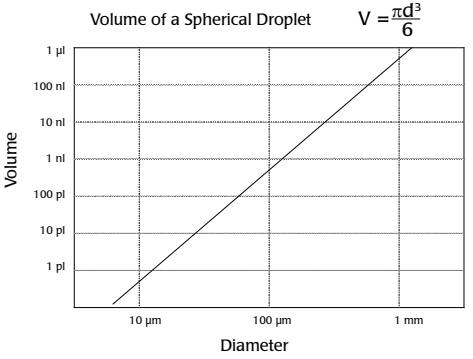


Fig. 35—(Left) The volume of a droplet increases as the diameter increases. See "Appendix A: Droplet Volume" on page 25.
Fig. 36—(Right) WPI's MicroFil is helpful in backfilling glass pipette tips. The MicroFil's tip is thin and very flexible.

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

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

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 **MICRO-ePUMP** 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 **MICRO-ePUMP**.

   NOTE: The injection pressure must exceed the compensation pressure in order to eject the fluid.
3. Use a pipetter 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 **MICRO-ePUMP** that is connected to the Injection Port on the **MICRO-ePUMP**.
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 ±100 µm 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.

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 **MICRO-ePUMP** to drive up to six micropipette barrels independently.

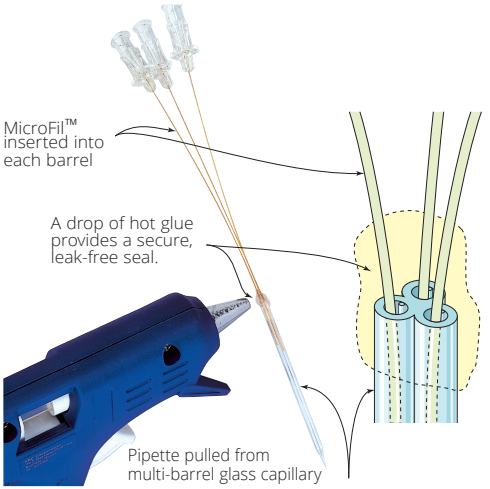


Fig. 37—PolyFil multibarrel micropipette coupling kit

MAINTENANCE

The **MICRO-ePUMP** 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. 38—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
300683	Microelectrode Holder for the MICRO-ePORE™
300744	MICRO-ePUMP Capillary Kit

TROUBLESHOOTING

The MICRO-ePUMP 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 **MICRO-ePUMP** 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
101	A leak was detected while the instrument was idle.	Faulty pressure connection or broken pipette or tubing.	Check all pressure connections and make sure the pipette is not broken.
102	Low pumping pressure was detected.	Faulty pressure connection or broken pipette or tubing.	Check all pressure connections and make sure the pipette is not broken.
		Pump failure.	If the problem persists with nothing connected to the Injection Port connector, call WPI technical support.
103	Pumping time to fully charged pressure exceeded 5 minutes.	Faulty pressure connection or broken pipette or tubing.	Check all pressure connections and make sure the pipette is not broken.
		Pump failure.	If the problem persists with nothing connected to the Injection Port connector, call WPI technical support.
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.
200	Drain valve self-test failed	Drain Valve of connections defective.	Call WPI technical support.
201	Failed to charge tank during self-test at low pressure.	Charging time exceeded.	Ensure nothing is connected to the Injection Port. Call WPI technical support if the problem persists.
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	0.07 - 87.0 PSI (5 - 6000 hPa)
Injection Pressure Increment	0.1 PSI (0.1 kPa)
Injection Pressure, Uncontrolled	0 PSI (0 hPa)
Compensation Pressure Range, Controlled	0.07 - 87.0 PSI (5 - 6000 hPa)
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	48 W
Dimensions	160 (W) x 217 (D) x 273 (H) mm (6.3 x 8.56 x 10.76″)
Shipping Weight	8.6 kg (19 lb.)
Ambience	For indoor use only
Ambient Temperature	15ºC - 40ºC
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 Use up to a height of 2000 m above sea level
Degree of Pollution	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.

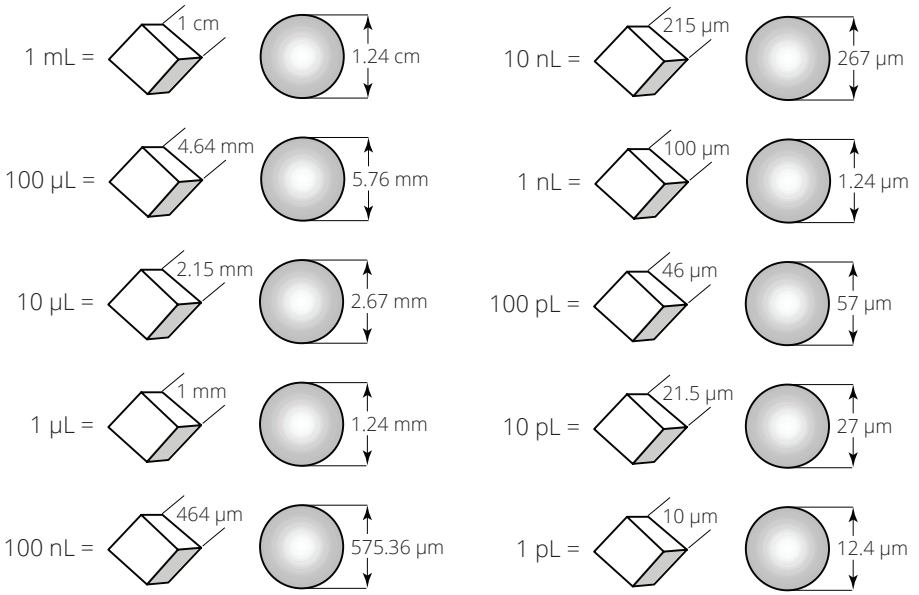


Fig. 39—This graph compares the dimensions of a cube and a sphere with the same internal volume.

Volume of a Micropipette

OD	ID	Approx. Volume/inch
1.0 mm	0.58 mm	6.7 µL/in
1.2 mm	0.68 mm	9.2 µL/in
1.0 mm	0.75 mm	11.2 µL/in
1.5 mm	0.84 mm	14.1 µL/in
1.2 mm	0.90 mm	16.2 µL/in
1.5 mm	1.12 mm	25 µL/in
2.0 mm	1.12 mm	25 µL/in

BIBLIOGRAPHY

R.E. McCaman, D.G. McKenna and J.K. Ono, A Pressure System for Intracellular and Extracellular Ejections of Picoliter Volumes , Brain Research 136 141-147 (1977)

T.W. Stone, Microintophoresis and Pressure Ejection (1985)

Y. K llstrom and S. Lindstr m, A Simple Device for Pressure Injections of Horseradish Peroxidase into Small Central Neurons , Brain Research 156 102-105 (1978)

R.E. Hammer, et al. Production of Transgenic Rabbits, Sheep and Pigs by Microinjection , Nature 315 680-683 (1985)

R.J. Griesbach, Advances in the Microinjection of Higher Plant Cells BioTechniques 3 (5) 348-350 (1985)

Morikawa, H. and Y. Yamada, Capillary microinjection into protoplasts and intranuclear localization of injected materials Pl. Cell Physiol. 26 229-236 (1985)

Ansorge W. Improved system for capillary microinjection into living cells Exper Cell Res 140 31-37 (1982)

Munoz, J.L., F. Deyhimi, and J.A. Coles, Silanization of glass in the making of ion-sensitive microelectrodes , J. Neuroscience Methods 8 231-247 (1983)

Deyhimi, F. and J.A. Coles, Rapid silylation of a glass surface: Choice of reagent and effect of experimental parameters on hydrophobicity , Helv. Chim. Acta 65 (6) 1752-1759 (1982)

Capecchi, M.R., High efficiency transformation by direct microinjection of DNA into cultured mammalian cells , Cell 22 479-488 (1980)

Diacumakos, F.G., Methods for micromanipulation of human somatic cells in culture Methods in Cell Biology, edited by D.M. Prescott, pp. 287-311. Academic Press, New York (1973)

Gordon, J.W., G.A. Scangos, D.J. Plotkin, J.A. Barbosa and F.H. Ruddle, Genetic transformation of mouse embryos by microinjection of purified DNA , Proc. Natl. Acad. Sci. USA 77 7380-7384 (1980)

Graessmann, A., M. Graessman and C. Mueller, Microinjection of early SV 40 DNA fragments and T antigen Methods Enzymol. 65 816-825 (1980)

Kopchik, J.J., G. Ju, A.M. Skalka and D.W. Stacy, Biological activity of cloned retroviral DNA in microinjected cells , Proc. Natl. Acad. Sci. USA 78 4383-4387 (1981)

A. Crossway, H. Hauptli, C.M. Houck, J.M. Irvine, J.V. Oakes and L.A. Perani, Micromanipulation techniques in plant biotechnology , BioTechniques 4 320-334 (1986)

INDEX

DECLARATION OF CONFORMITY

WORLD PRECISION INSTRUMENTS, LLC.
Telephone: (941) 371-1003 Fax: (941) 377-5428
e-mail wpi@wpiinc.com

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):

MICRO-ePUMP

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

Cory Boyes / Director of Design and Development

Europe Representative
Mr Andrew Waldes
Managing Director
World Precision Instruments Germany GmbH,
Pfingstweide 16, 61169 Friedberg, Germany

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.*

USA
175 Sarasota Center Blvd., Sarasota FL 34240-9258
Tel: 941-371-1003 • Fax: 941-377-5428 • E-mail: sales@wpiinc.com

UK
1 Hunting Gate, Hitchin, Hertfordshire SG4 0TJ
Tel: 44 (0)1462 424700 • Fax: 44 (0)1462 424701 • E-mail: wpiuk@wpi-europe.com

Germany
Pfingstweide 16, D-61169 Friedberg (Hessen), Germany
Tel: +49 (0)6031 67708-0 • Fax: +49 (0)6031 67708-80 • E-mail: wpide@wpi-europe.com

China & Hong Kong
WPI Shanghai Trading Co., Ltd. Rm 2608
Tongmao Mansion, No 357, Songlin Road, Pudong New District, Shanghai, PR China
Tel: +86 21 6888 5517 • Toll Free: 400 688 5517 • E-mail:chinasales@china.wpiinc.com

Internet
www.wpiinc.com • www.wpi-europe.com • www.wpiinc.net