



WORLD
PRECISION
INSTRUMENTS

TECH SUPPORT BULLETIN

MICRO-ePUMP, μ Pump, PV850

Best Practices Before Transfection

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When setting up your New Generation Microinjector (PV850, μ PUMP, or MICRO-ePUMP), it is important to follow these steps to ensure proper functionality of your system for success in your application.

AIR TEST

Air test your system to inspect your tip integrity. (THIS SHOULD TAKE PLACE PRIOR TO TIP SUBMERSION INTO SOLUTION OR SAMPLE LOADING.)

It is important to check for tip damage or 'overcutting' before you load it with your sample OR position your injector into a solution.

1. Backfill your tip with colored sterile test solution with similar viscosity to your sample to be injected during experiment (or any sterile colored solution of your choosing) and ensure no air bubbles are present.
 - Ensure there are no air bubbles present within your sample. Inspect the very end of the taper of the glass pipette tip, to the meniscus of your sample. If air bubbles are present, the sample must be redistributed into a new tip, or carefully removed using your sterile MicroFil™.
 - **NOTE:** If bubbles are present, the sample will not dispense upon initiation of injection, because a bubble resists the injection pressure applied, and the CLEAR TIP function cannot be executed.



Fig. 1—Inspect the pipette for bubbles, which are seen here at the tip.

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. (rear of glass + screw cap + gasket).



Fig. 2—Slide the loaded tip into the screw cap and through the appropriate size gasket.

NOTE: Ensure your gasket size is appropriate for the outer diameter (OD) of your glass pipette tip. If using a WPI pre-pulled glass pipette tip (1.0 mm OD), the corresponding gasket is green.

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

3. Place the prepared tip and capillary holder steadily into a micromanipulator, within range of your field of view under your microscope.

NOTE: LEAVE THE TIP SET UP SUSPENDED IN AIR.

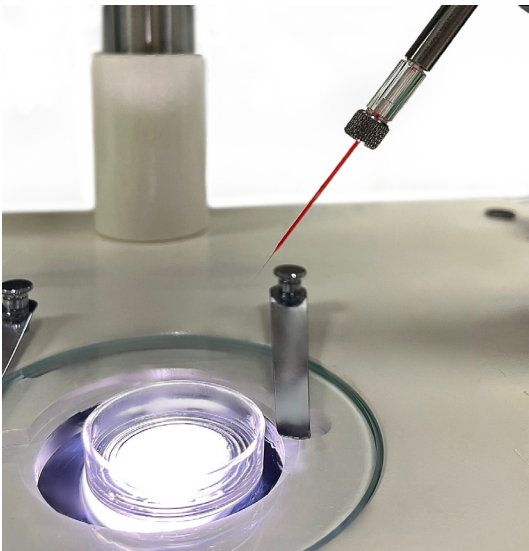


Fig. 3—The setup is shown under a microscope with the pipette suspended in the air.

4. Under the microscope, focus on your capillary tip. Then, on the digital display of your pump, set your injection pressure to about 3 PSI, and the compensation pressure at about 0.9 PSI. To do this:
- **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.

5. Select the yellow INJECTION DISABLED banner to turn your PORT ON.

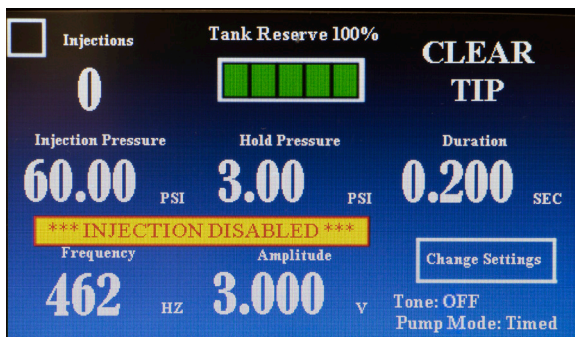


Fig. 4—Press the yellow banner (*Injection Disabled*) to toggle the port on.

NOTE: An immediate and total dispensing of your test solution in air indicates a BROKEN TIP. Replace your glass pipette tip and repeat these steps. (You should only see a small, controlled bead leaving your glass tip, and only after the injection is initiated with the port on.)

CALIBRATION

Next calibrate your injection and compensation pressure, and set your injection duration using the test solution.

1. Using mineral oil in a small petri dish (FluoroDish), and a micrometer corresponding to the scale of the desired droplet volume to be injected, bring your loaded glass pipette tip with your colored test solution in view of the dish under a microscope. The micrometer used with your microscope could be either a glass stage micrometer that slides under your petri dish, or a microscope lens reticle, an etched ruler that fits into your ocular or is integrated into the ocular.
2. With the PORT OFF, lower your tip into the solution until it is submerged. Focus on the tip using your microscope.
3. Using the previous settings (injection pressure is set to about 3 PSI, and the compensation pressure is about 0.9 PSI), turn your port on. Verify there is no colored 'trail' dispensed from the tip.

If you observe slow leaking, adjust the compensation pressure. Usually, lowering the value should do the trick, but some toggling will be required until you can achieve proper suspension of the tip contents under submersion.

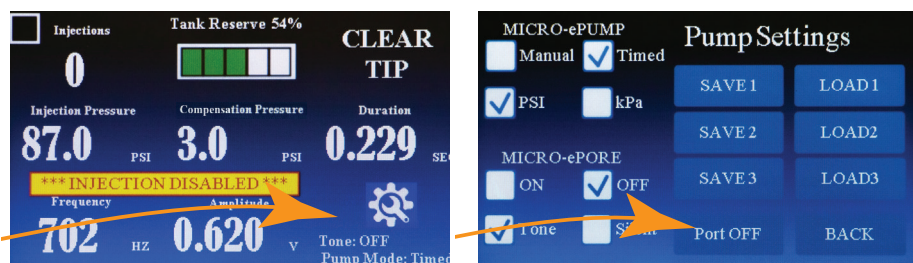


Fig. 5—(Left) Touch the setting area in the bottom right corner to access the Settings menu.

Fig. 6—(Right) Touch the PORT OFF/PORT ON toggle button to enable the injection port.

4. Once suspension is achieved, initiate an injection by pressing the foot switch, and measure your bead size. Be sure to save these settings for your experiment by pressing 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.

- If no fluid dispenses, slightly increase your injection pressure.

NOTE: Mineral oil often creates a barrier where you may experience some 'resistance.' If you still are unable to dispense a droplet, press the CLEAR TIP button.

- If you still cannot see any solution injected into the oil, you likely have a bubble creating a clog. Turn your PORT OFF by pressing the PORT ON/PORT OFF toggle button. Withdraw the tip from the mineral oil and inspect your glass pipette tip for bubbles. Re-attempt the air test to verify proper solution dispensing prior to re-attempting injection in mineral oil.
5. Turn the port off and remove your tip from the mineral oil.
 6. Use a new petri dish, and fill it with the aqueous environment you will be working in during your experiment. (For example, saltwater for zebrafish injection.)
 7. Lower your glass pipette tip into the petri dish until the tip is submerged.
 8. Press the Setting area in the lower right corner on your pump's digital display, and VERY CAREFULLY keep your finger over the PORT OFF/PORT ON toggle button. DO NOT ENGAGE THE PORT until your eyes are focused on the tip under your microscope.

If you observe immediate leaking when the PORT ON is selected, quickly turn the PORT OFF. You will need to adjust your injection and compensation pressures. Toggle the PORT OFF/PORT ON button after adjusting these parameters until the proper sample suspension is achieved.

NOTE: Salt water may require a slight adjustment to the settings you saved during your mineral oil test, since its properties require more 'barrier-resistance' between your sample and the solution.

REMOVE TEST SOLUTION/PREP SAMPLE LOADING

1. Once you have finalized your desired injection parameters, you will need to remove the sterile test solution before loading of your desired sample. Simply press the CLEAR TIP button until the test solution is completely emptied from your tip.

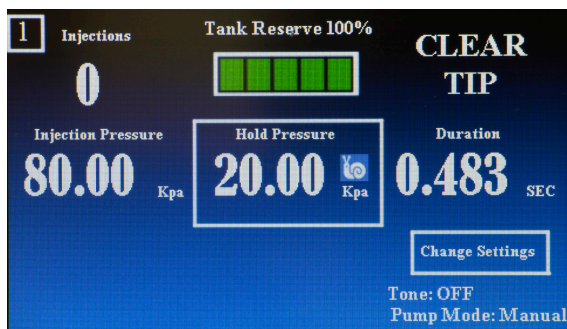


Fig. 7—The Clear Tip button is located in the upper right corner of the screen.

2. Pipette your sample into your glass tip, again ensuring no bubbles are present, and load it into your capillary holder.

...You should now be ready to transfect your cells!

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