

# Instructions for the Tritech Research Xenopus MICROINJECTOR™ System Front-Fill Model

## **WARNINGS!!!!**

NITROGEN GAS IS COMPLETELY ODORLESS AND NOT DETECTABLE BY HUMAN SENSES! WHEN RELEASED IN SUFFICIENT QUANTITIES IT CAN DISPLACE THE OXYGEN-CONTAINING AIR AND SUFFOCATION CAN RESULT WITHOUT WARNING; THEREFORE, NITROGEN SHOULD BE USED WITH ADEQUATE VENTILATION AND CARE SHOULD BE TAKEN TO TURN OFF TANK SOURCE WHEN NOT IN USE! MAKE SURE THAT NEEDLES AND TUBING ARE SECURELY ATTACHED, BECAUSE IF NOT, THEY MAY BE PROJECTED RAPIDLY. THE MICROINJECTOR™ SYSTEM IS FOR RESEARCH USE ONLY AND MUST BE USED WITH CAUTION. IF SET UP AND USED CORRECTLY, THE MICROINJECTOR CAN PROVIDE AN ACCURATE MEANS FOR INJECTING SUBSTANCES INTO CELLS; HOWEVER TRITECH RESEARCH, INC. DOES NOT WARRANT THAT THE SYSTEM OR DOCUMENTATION IS ERROR-FREE OR 100% RELIABLE. IN NO EVENT SHALL TRITECH RESEARCH BE LIABLE FOR DIRECT, INDIRECT, SPECIAL, INCIDENTAL, OR CONSEQUENTIAL DAMAGES. IF ANY PART OR STATEMENT IN THE WARRANTY OR DISCLAIMER IS UNLAWFUL, THE REMAINING PARTS SHALL CONTINUE TO BE IN FULL EFFECT. IF YOU DO NOT AGREE TO FOLLOW THE WARNINGS AND ACCEPT THE DISCLAIMERS, PLEASE RETURN YOUR UNIT TO TRITECH RESEARCH FOR A FULL REFUND.

## **SETTING UP**

Congratulations on your purchase. If you follow the instructions, the microINJECTOR™ should provide years of happy, successful injections. Obtain a steel gas cylinder containing gaseous nitrogen (N<sub>2</sub>) under high pressure (inexpensive industrial grade or medical grade at about 3000 psi is adequate). **Make sure you obtain a cylinder with a CGA-580 output fitting unless you specified a different fitting when placing your order.**

To connect the high-pressure regulator, insert nipple from the regulator's fitting into the tank's fitting, and screw the collar from the regulator's fitting into the tank fitting. Tighten the fitting securely with a wrench (this fitting must withstand the 3000 psi in the tank). **Make sure tank is secure and can not fall over!!!**

## **THE UNIT HAS BEEN SHIPPED TO YOU WITH SMALL PIECES OF TUBING INSTALLED IN THE HIGH-PRESSURE REGULATOR'S QUICK-CONNECT FITTINGS AS A DEMONSTRATION**

For ease of use, the gas connections for the high-pressure regulator output and microINJECTOR input have been fitted with a quick-connect system.

To remove the tubing from the connectors, press the orange or red plastic around the connection interface while simultaneously pulling on the tubing firmly.

To connect the gas lines to the connectors, grasp the tubing approximately one inch from the end and insert it into the connector. You will feel the tubing move approximately 1/8"-1/4" inside the connector. To ensure that the connection is tight, firmly tug on the tubing. If the tubing does not pull out of the connector, the connection should be airtight.

- 1) Prior to set-up, situate the microscopes, micro-manipulators, and microinjection stations as you would during use. Centrally locate the nitrogen tank, and secure it.
- 2) Cut tubing to length, making sure that the cut ends are clean and square. Leave enough slack in the tubing so that the gas connections are not strained.
- 3) Completely back out the high and low-pressure regulators (turn the knobs counter-clockwise all the way).
- 4) Attach the high-pressure regulator to the nitrogen tank. (**SEE INSTRUCTIONS ABOVE**)
- 5) Attach tubes from high-pressure regulator to microinjection stations.  
NOTE: IF YOU ARE USING FEWER STATIONS THAN THE NUMBER OF QUICK-CONNECT PORTS ON THE HIGH PRESSURE REGULATOR, INSERT A SHORT PIECE OF TUBING, BENT OVER AND TAPED TO ACT AS A PLUG, IN THE UNUSED PORTS.
- 6) Test system for gas leaks by turning on the nitrogen and turning the high-pressure regulator knob clockwise until the regulated pressure reads 90 psi. Walk over to each microinjection station and listen carefully for leaks. Next, turn each of the low-pressure regulators, clockwise, until the pressure gauge reads 40 psi. Carefully listen for gas leaks. (Prior to shipment, each system was tested under similar pressure, and was found to be free of leaks. Contact Trittech Research if you discover any.)

## **USE**

Make sure all gas connections are securely attached, the vacuum control valves are closed, and the high-pressure regulator is "off" ("backed out") by turning the regulator control counter clockwise until it is loose.

Open the tank valve by turning it counterclockwise. The inlet gauge will show the pressure in the tank.

Attach injection needle to Needle Holder. Plug injection pedal into its micro jack, and make sure the unit is plugged in to a 115 VAC outlet. You are now ready to load the needle and inject.

### **Custom Front Fill Module:**

Your system has a special vacuum option that allows needles to be filled by suction through the tip. Here are instructions for its use:

Located on the right side of your Xenopus microINJECTOR System is a flow control valve. This system works with the Bernulli principle. When the valve is closed, the system provides positive pressure to the needle. When the flow control valve is opened, it creates negative pressure in the needle.

When ready to front-load the needle using the custom front fill module enclosed within your Xenopus microInjection System, set the low-pressure regulator for approximately 40 psi and slowly open the vacuum control / purge valve (silver) by turning it counter-clockwise. As nitrogen escapes through the exhaust port, a vacuum is created, which is available at the needle holder when you press down the foot pedal. Close the vacuum control/purge valve when vacuum is not being used, to conserve nitrogen, and to begin microinjection. The pulse length control knob controls the amount of liquid being sucked up by the needle every time the foot pedal is depressed. However, both the material being sucked up, and the individual differences of each needle affect the amount of liquid sucked up each time. Be sure to calibrate your system each time the needle is changed.

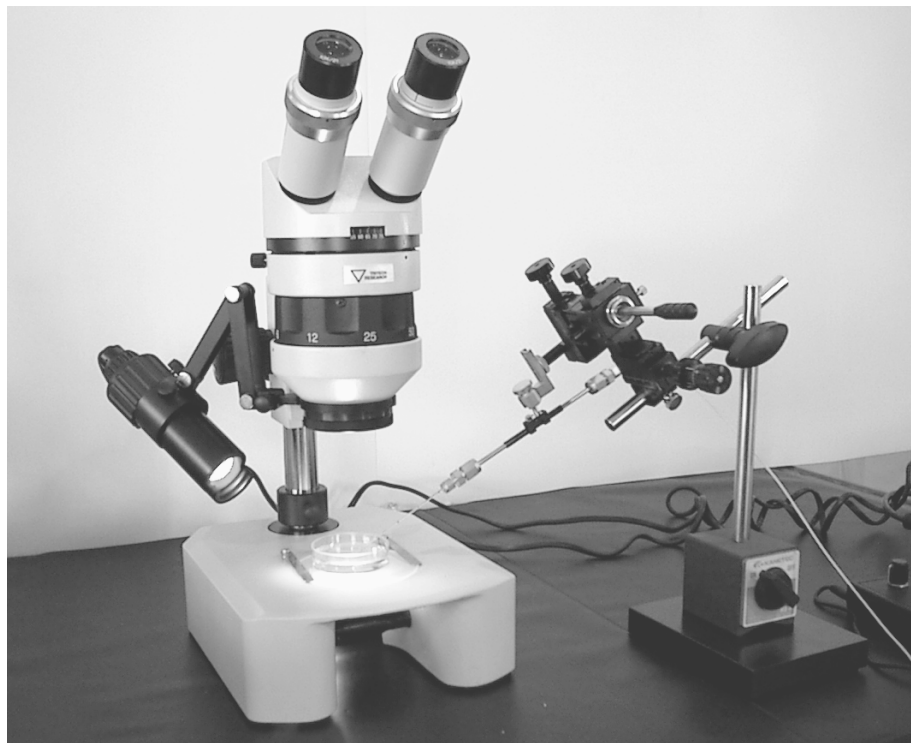
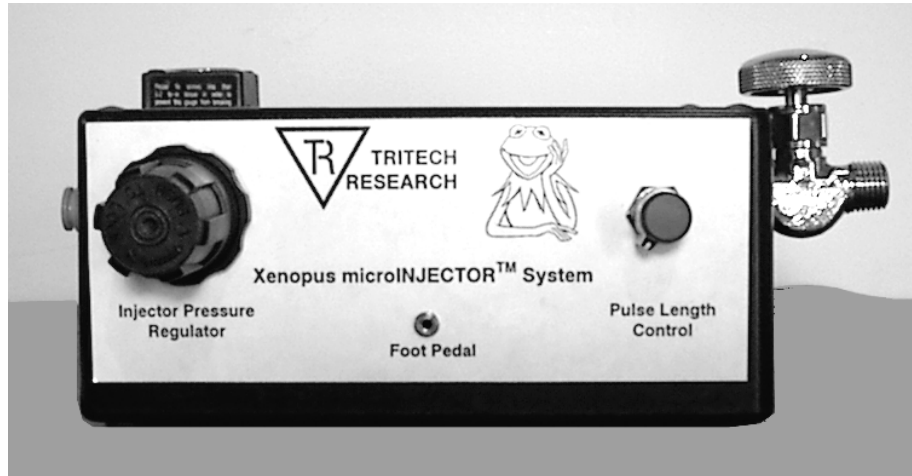
Set the output pressure of the high-pressure regulator to approximately 90 psi. When ready to inject using the microInjection stations, turn the knob clockwise on the low-pressure regulator until the pressure gauge reads 20 psi. (Use this as a starting point, you may want to raise or lower this pressure, depending on your application and needle tip size)

Each of the Trittech Research Xenopus MicroINJECTOR Systems is equipped with a Pulse Length Control Module (PCM). The electronic PCM can be set from ~0.1 to ~15 secs of injection by turning the blue control knob (clockwise for longer times). For timed injection, push and hold injection foot

pedal. For manual control, set for 15 secs (fully clockwise) and release injection pedal early. Increase needle pressure as needed.

When finished injecting, you must turn off the tank and “bleed the line” as follows:

- **Do NOT back out the regulator control.**
- Shut off the tank valve by turning it tightly clockwise.
- **Do NOT bleed the regulator through the injection tubing, pressure is too high.**
- Open the vacuum valve until both regulator gauges read zero. If the regulator control has been decreased, the gauges will not read zero; in that case, slowly turn the regulator control clockwise until all gas is released.
- Close the vacuum control valve. Back out the regulator controls.



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