Warner Instruments

Pico-liter Injector

Model PLI-10

The **PLI-10** Pico-liter Injector is designed to provide controlled delivery of picoliter to nanoliter volumes of solution through a glass micropipette. The **PLI-10** is ideal for applications such as neuronal tract-tracing, delivery of pharmacological agents in vitro, and *Xenopus* oocyte injection.

Features for the **PLI-10** include

- ✓ Low cost
- ✓ Femtoliter to microliter injections
- ✓ Digital readouts for injection pressure, time, and count
- ✓ Reliable optically encoded circuit for injection time set
- ✓ Easy to use
- ✓ Same high quality pressure regulator as PLI-90 and PLI-100

THIS EQUIPMENT IS NOT DESIGNED NOR INTENDED FOR USE ON HUMAN SUBJECTS

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NOMENCLATURE

Text conventions

This manual refers to instrument controls at three functional levels; control blocks, specific controls within a block, and settings of specific controls. To minimize the potential for confusion, we have employed several text conventions which are specified below. Since our goal is to provide clarity rather than complexity, we welcome any feedback you may wish to provide.

- Warner Instrument product numbers are presented using **bold type**.
- References to specific controls within a block are specified using SMALL CAPS.
- Finally, references to individual control settings are specified in *italic type*.
- Special comments and warnings are presented in highlighted text.

Any other formatting should be apparent from context.

GENERAL

Instrument description

The **PLI-10** is specifically designed for solution delivery to extracellular locations in tissues and for intracellular *Xenopus* oocyte injection. These 'large volume' injections (10 pl to greater than 100 pl) are common for many applications that do not require multiple pressure settings. As such, the **PLI-10** is an effective, low-cost alternative to our more elaborate pressure ejection systems (e.g., the **PLI-90** and **PLI-100**).

Pressure to the micropipette is controlled precisely through a high-quality multi-turn regulator and is reported digitally for reproducibility. Injection time is set using an optically encoded circuit which permits fine and coarse settings from a single dial knob. The system trigger timer can be controlled by the front panel push button, a foot switch, or an external trigger (TTL pulse to TRIGGER INPUT BNC).

FRONT AND BACK PANEL CONTROLS AND CONNECTIONS

Front panel

A. **RESET COUNT pushbutton:** Resets the injection count display to zero.

B. **INJECTION COUNT display:** Records and displays the total number of injections triggered (0 to 9999 injections).

C. **INJECT LED:** LED indicator remains lit for duration of injection period.

D. **INJECTION TIME (s):** Displays injection time period in seconds (0 to 99.99 s)

E. **INJECTION PRESSURE:** Displays injection pressure in either PSI or kPa (maximum 60 PSI; 413 kPa).

F. **PRESSURE UNIT toggle switch:** Toggle switch controls injection pressure display unit of measure. Injection pressure can be displayed in either pound-force per square inch (PSI) or kilopascals (kPa).

G. **PRESSURE OUTPUT (P_{out}) connector:** Pressurized gas output line. Output tubing line connects P_{out} to injection pipette holder.

H. **MANUAL INJECTION TRIGGER:** Press-button trigger delivers regulated pressure for the digitally set period of time at the desired pressure.

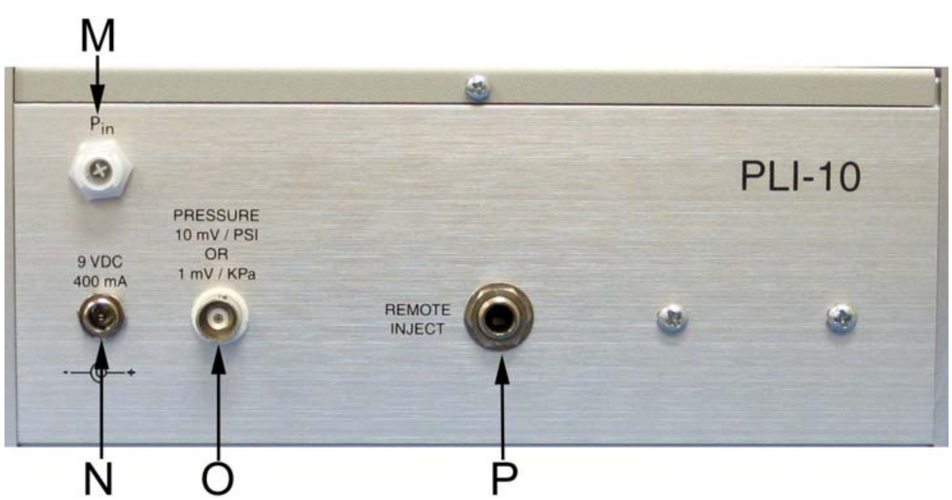
I. **EXTERNAL INJECTION TRIGGER INPUT BNC:** This input is for electrically initiating injection from an external trigger source. A positive TTL pulse of is required (baseline 0 V, trigger 5 V, lasting >10 ms).

J. **INJECTION TIME SETTING DIAL:** This dial controls the injection time setting. Turning the dial changes the injection time in seconds; pressing the dial in and turning changes the injection time in fractions of a second.

K. **INJECTION PRESSURE REGULATOR:** This seven turn control sets the injection pressure over the range from 0.2 to 60 PSI (1.4 to 413 kPa).

L. **POWER switch:** This switch turns the instrument on and off.

Rear panel



M. **PRESSURE INPUT (P_{in}) connector:** This connector is the input for the compressed gas. 75 PSI is the recommended input pressure; maximum input pressure is 105 PSI.

N. **POWER INPUT connector:** This connector is the input for the external power supply (9V DC @ 400 mA; universal input voltage 90-264 VAC).

O. **PRESSURE MONITOR OUTPUT BNC:** This connector outputs the injection pressure (P_{out}) as an analog signal that can be directed to an external recording device. (10 mV/PSI or 1 mV/kPa).

P. **REMOTE INJECT foot switch input connector:** An optional foot switch (PLI-FS) can be connected to the instrument. Pushing the foot switch triggers an injection for the preset time and pressure values displayed on the instrument front panel.

SETUP AND OPERATION

The **PLI-10** is provided with input and output hoses for gas handling and a foot switch for triggering injections. Additional requirements include an acrylic pipette holder (see ordering information below) and glass micropipettes. A regulated air/gas supply and a micromanipulator to hold the injection pipette are needed to create a complete microinjection system. The setup and operation of the **PLI-10** is straightforward.

Setup

1. Connect the instrument to an electrical outlet using the 9V DC power supply input (Item N).
2. Attach one end of the input hose to a regulated air/gas supply and use the quick-clip connector to connect the input hose to P_{in} (Item M) on the back of the instrument.
3. Connect the output hose to the instrument P_{out} (Item G) using the quick-clip connector. Attach the other end of the output hose to the pipette holder. Position and secure the pipette holder into a micromanipulator.
4. Turn the unit on (Item L). The three LED displays (Items B, D, and E) will illuminate.
5. Press the RESET COUNT button (Item A) to reset the injection count display (Item B) to *zero*, if necessary.
6. Turn the TIME SET DIAL (Item J) to the desired injection time (displayed in Item D). Pressing the TIME SET DIAL *in* and turning will allow fine adjustment of injection time in tenths of seconds.
7. Turn the INJECTION PRESSURE set knob (Item K) to set the desired injection pressure (displayed in Item E). Pressure can be set and monitored in units of PSI or kPa, set by the TOGGLE SWITCH (Item F). If the desired pressure is unknown, it can be determined by observing a loaded micropipette under a microscope. Gradually increase injection pressure setting until solution is flowing from the micropipette tip.
8. Optional remote triggering of an injection can be achieved using a footswitch or TTL pulse. Insert the footswitch plug into the REMOTE INJECT jack (Item P). Pressing the footswitch triggers an injection. Similarly, an analog 5V TTL input to the BNC TRIGGER (Item I) can be used to trigger an injection from an external device (such as a digital acquisition system).
9. Optional monitoring/recording of injection pressure settings can be achieved by the analog PRESSURE signal output (Item O). This output is calibrated to 10 mV/PSI or 1 mV/kPa.

Operation

10. The assembled system is ready to use. Turn on the air/gas supply at the source, and slowly increase the outflow regulator to the desired pressure setting.

    NOTE: The input pressure should be at least 7 PSI higher than the desired working/injection pressure for the pneumatic components to operate properly.

11. Turn the instrument on, and set the injection pressure and injection time to the desired values. The P_{OUT} tubing line should remain unattached to the control unit at this time to prevent any latent pressure from building up in the micropipette.
12. Backfill the micropipette with a desired volume of the compound to be injected. Microfill-style syringe tips can be used to fill the micropipette and avoid solution contaminating the outside of the micropipette.
13. Insert micropipette into the pipette holder and secure it into place by tightening the acrylic screw.
14. Attach the P_{OUT} tubing line to the connector on the control unit.
15. Position the micropipette to the location where the injection bolus is to be delivered. This often requires a micromanipulator.
16. Trigger an injection by pressing the inject button (Item H). Alternatively, an external trigger or footswitch can be used to trigger an injection.
17. After injection is complete, the micropipette should be disposed of in an appropriate “sharps container”. For tract-tracing studies, using a fresh micropipette for each injection is advised. A single pipette can be used to serially inject multiple oocytes with the same compound if the micropipette tip remains undamaged.
18. Upon completion of the experiment, remove the pipette from the holder and power the unit *off*. The air/gas input should also be turned *off* at the source.

ADDITIONAL INFORMATION

Note: The following material is excerpted from the user’s manuals for the PLI-90 and PLI-100. These manuals are available for download on our website for your reference.

Microinjection also involves other skills, several other instruments and accessories, and various supplies. The purpose of this section is to give an overview of these techniques for those new to microinjection. Some guidance on equipment selection is also supplied.

Required Auxiliary Equipment for Microinjection

The required equipment includes a pipette puller and micromanipulator(s). Ideally, the puller should be capable of making pipettes with tip diameters in the 0.2 to 1.57 micron range with a short enough taper length for both mechanical strength and low flow resistance. If most injections are to be extracellular, then a puller suitable for extracellular patch clamp pipettes is satisfactory. For intracellular injections, some magnetic pullers may be suitable. Alternatively, a two stage gravity puller with variable weights can be satisfactory over the entire range. The required three dimensional movements of the injection pipette can be produced by an inexpensive mechanically linked micromanipulator for large cells such as frog oocytes.

Required Supplies

These are compressed gas and glass capillary tubes. Compressed air is suitable for oxygen-insensitive injection material. Nitrogen is a satisfactory inert gas for the general case. An input pressure of 105 psi is sufficient: a regulator will be needed if supplied from a bottle of compressed gas.

Optional Equipment for Microinjection

This includes a microforge (to bend a micropipette or to polish a pipette tip for holding a cell), a microgrinder (to bevel the pipette tip to increase the delivery rate without additional cell damage), and a microincubator (to hold the cells at incubation temperature during microinjection).

Micropipettes

The micropipettes are made by pulling a glass capillary (1-2 mm. outer diameter) by heating some 3-10 mm. of its length with a concentric heater while applying a force (gravitational or magnetic) to pull both ends of the capillary apart. Two micropipettes are produced per capillary.

Distinguishing Parameters

Two useful distinguishing parameters of the micropipette are the inside diameter of the tip and the angle of taper to the tip. For a single-stage puller, a smaller taper angle will yield a longer tapered region. The larger the tip, the more material is delivered for the same applied pressure and time. Just a 10% decrease in diameter decreases the delivery rate by over 30%. A 10% decrease in taper angle (longer taper) would decrease the delivery rate about 10%. The extreme sensitivity of delivery rate on tip diameter makes it important to have a reproducible pipette puller. If you use published tip sizes as a starting point, distinguish between the relevant inside diameter and the more visible outside diameter. (The ratio of the two is the same at the tip as for the original capillary glass.)

Choosing the Right Pipette

Choosing a pipette size and shape for intracellular injection is difficult. Larger tips deliver more material but increase the risk of cell damage caused by leakage around the pipette while in the cell or later by incomplete sealing. The smaller the cell, the smaller the pipette tip that is required. The smaller the tip, the more likely it is to clog.

For reference, intracellular electrophysiologists routinely record for an hour or so from cells of 10 micron diameter with pipette tips of 0.1 micron inside diameter. Larger tips can therefore be used for brief injection in such cells. For nuclear injections, a smaller taper angle is needed to avoid leakage further up the shank of the pipette at the plasma membrane.

Although even intracellular injection can be done from below with an upright microscope, most injections are done from the side or from above the cells. Different strategies have been used to suitably fix the cell in position for successful intracellular injection:

1. for suspended cells, a second, larger pipette is used to hold the cell. This pipette's tip is first polished with a microforge (done by placing the pipette within 5 microns of a hot filament for a few seconds).With its axis horizontal, it is moved to hold the cell with applied suction. The injection pipette is also straight and is inserted horizontally from the opposite side. This geometry avoids damage to the cell membrane caused by shearing forces. The optics are straightforward because the pipettes remain in focus as they are advanced.

2. for cells that can be or are attached to a surface in a closely packed layer, straight injection pipettes can also be used. In this case, the pipette axis slopes slightly down from the horizontal. The tendency of the cells to slide when the pipette enters is resisted by the extracellular environment or attachment to the culture surface. The microscope should be focused on the cell's surface. The pipette tip then comes into focus just before injection. If the cell is nearly spherical (the hardest case), the pipette should again enter the cell membrane at right angles to avoid shearing. Non-spherical cells (for example, cultured fibroblasts) have a more robust cytoskeletal structure so the pipette can be pushed in even if not perpendicular to the membrane surface.

3. for less firmly attached cells, the injection pipette can be bent near the tip after pulling. The pipette's main axis slopes slightly down from the horizontal. The angle of bend should allow the tip to point straight down. With an inverted microscope, the tip is viewed through the cell as it is lowered for injection. The microscope is focused on the cell's top surface and the tip comes into focus just before insertion. Again, shearing forces are avoided. Suitable bends can be made with a microforge: a simple way to do this is to move the pipette near a hot filament at the position of desired bend. The tip will spontaneously bend away.

In all of the above techniques, a three dimensional micromanipulator controls the movement of the injection pipette. If this (straight) pipette is instead attached to a condenser mount (inverted scope), then a one dimensional manipulator can be used. The remaining two directions of manipulation are done with stage micrometers moving the vertical injection pipette over each cell in turn. If the vibrations transmitted with the condenser mounting are manageable, then this approach gives the fastest rate of cell injection.

General Considerations

It is much easier to reliably inject large volumes than small ones. For "large" volumes, a balance pressure capability is not needed. The dividing line between large and small is not rigid: it depends on how quantitative a delivery is required. That volume line would typically be in the 10-100 picollier range. (For convenient visualization and approximate geometric measurement, 1 femtoliter is a cube 1 micron on a side or a sphere 1.24 microns in diameter, 1 picoliter is a cube 10 microns on a side or a sphere 12.4 microns in diameter, while 1 nanoliter is a cube 100 microns on a side or a sphere 124 microns in diameter. Because volume goes as the cube of linear dimensions, such geometric volumes are imprecise, but often useful). Extracellular delivery is nearly always "large". Intracellular injection is often of "small" volumes (but not for frog oocytes).

Filling of the injection pipette can be done from the back end (barrel) of the pipette using a syringe with a thin hypodermic needle or "microfil" tip inserted so its tip is down in the tapered section. If the capillary has a filling fiber attached to the inner wall the pipette tip will then fill by capillary action without air bubbles, at least for larger tips. Variations on this procedure involve filling a smaller capillary first and inserting it for the back fill.

Injection Pressure and Time Pulse

Setting the initial injection pressure low prevents the loss of solution. To easily obtain the desired pressure setting, set the time pulse on (1) one second with the injection pressure set at its minimum. Trigger the time pulse while viewing under magnification.

Increase the injection pressure until the solution within the pipette begins to flow out the tip opening. The pressure shown on the LCD can now be used as the initial injection pressure setting. Adjust the injection pressure and timing to obtain the desired injection.

Gas Usage Warning

To provide finely controllable output pressure, the gas regulators are of the "bleeding" type. Such regulators use gas even in the absence of ejections. The Pico-Injector thus uses gas even when off. To eliminate this consumption and as a good safety practice, turn off the gas supply at the source when the Pico-Injector is not in use.

Hose Connections

The input and output hoses should be attached to their respective connectors. If each connector's needle valve, located in the micro-injector body, is not fully opened, the airflow will be restricted or blocked. To prevent this from happening, check each connector for tightness by turning clockwise. This will ensure needle valve depression.

Volume Calibration Chart

Formula: *Volume in nanoliters = .17952 x tip ID³ (in µm) x PSI x time (in sec)*

Example: *Volume = .17952 x (5)³ x (10) x (1) = 224.4 nanoliters*

Pressure (p.s.i.)	Time (sec.)	Pipette Tip I.D. (µm)	Femtoliters	Picoliters	Nanoliters	Microliters	Milliliters
1	1	0.1	179.52	0.18	-	-	-
10	1	0.1	1795.20	1.80	-	-	-
20	1	0.1	3590.40	3.59	-	-	-
30	1	0.1	5385.60	5.39	-	-	-
40	1	0.1	7180.80	7.18	-	-	-
50	1	0.1	8976.00	8.98	-	-	-
60	1	0.1	10771.20	10.77	-	-	-
1	1	1	-	179.52	0.18	-	-
10	1	1	-	1795.20	1.80	-	-
20	1	1	-	3590.40	3.59	-	-
30	1	1	-	5385.60	5.39	-	-
40	1	1	-	7180.80	7.18	-	-
50	1	1	-	8976.00	8.98	-	-
60	1	1	-	10771.20	10.77	-	-
1	1	5	-	-	22.44	0.02	-
10	1	5	-	-	224.40	0.22	-
20	1	5	-	-	448.80	0.45	-
30	1	5	-	-	673.20	0.67	-
40	1	5	-	-	897.60	0.90	-
50	1	5	-	-	1122.00	1.12	-
60	1	5	-	-	1346.40	1.35	-
1	1	10	-	-	179.52	0.18	-
10	1	10	-	-	1795.20	1.80	-
20	1	10	-	-	3590.20	3.59	-
30	1	10	-	-	5385.60	5.39	-
40	1	10	-	-	7180.80	7.18	-
50	1	10	-	-	8976.00	8.98	-
60	1	10	-	-	10771.20	10.77	-
1	1	75	-	-	-	75.74	0.08
10	1	75	-	-	-	757.35	0.76
20	1	75	-	-	-	1514.70	1.51
30	1	75	-	-	-	2272.05	2.27
40	1	75	-	-	-	3029.40	3.03
50	1	75	-	-	-	3786.75	3.79
60	1	75	-	-	-	4544.10	4.54

APPENDIX

Specifications

Input Gas Pressure:	75 PSI Recommended, 105 PSI Maximum (≥7 PSI above injection pressure)
Injection Pressure:	0.2 to 60 PSI (413 KPa) regulated
Injection Time:	0.01 to 99.99 Seconds
Injection Time Accuracy:	±0.01% (Crystal Time Base)
Pressure Display:	3½ Digits, 0.1 PSI or 1 kPa Resolution
Injection Count Display:	0 to 9999 Injections
Trigger Mode:	Front Panel, Footswitch, TTL (Gate In)
Pressure Monitor:	Output BNC, Rear Panel, 10 mV/PSI or 1 mV/KPa
Power Input:	External 9VDC @ 400mA (min)
Power Supply	universal input voltage 90 to 264 VAC
Weight:	2.3 kg
Dimensions::	89 x 215 x 175 mm (H x W x D)
Warranty:	One year, parts & labor

Ordering Information

Order number	Model number	Description
64-1707	PLI-10	Pico-liter injector (includes input hose, output hose, and footswitch)
64-1626	AO161.0	Acrylic pipette holder for 1.0 mm pipettes
64-1627	AO161.2	Acrylic pipette holder for 1.2 mm pipettes
64-1628	LPLI-PPH	Acrylic pipette holder for 1.5mm pipettes
64-1629	AO162.0	Acrylic pipette holder for 2.0mm pipettes
65-0008	PLI-IHN	Gas Input Hose
65-0010	PLI-OHN	Gas Output Hose

Warranty and Service

Warranty

The **PLI-10** Pico-liter Injector is covered by our Warranty to be free from defects in materials and workmanship for a period of one year from the date of shipment. If a failure occurs within this period, we will either repair or replace the faulty component(s). This warranty does not cover failure or damage caused by physical abuse.

In the event that repairs are necessary, shipping charges to the factory are the customer's responsibility. Return charges will be paid by Warner Instruments.

Service

We recommend that all questions regarding service be referred to our Technical Support Department.

- Normal business hours are 8:30 AM to 5:00 PM (EST), Monday through Friday.
- We are located at 1125 Dixwell Avenue, Hamden, CT 06514.
- We can be reached by phone at (800) 599-4203 or (203) 776-0664. Our fax number is (203) 776-1278.
- We can be emailed at support@warneronline.com or through the contact section of our website at http://www.warneronline.com.

IMPORTANT - CUSTOMERS OUTSIDE OF THE U.S.: Please be sure to contact us before return shipping any goods. We will provide instructions so that the shipment will not be delayed or subject to unnecessary expense in clearing U.S. Customs.

Certifications

Declaration of Conformity

CE MARKING (EMC)

Application of Council Directive: 89/336/EEC

Standards To Which Conformity Is Declared:	EN55022 Class A EN61000-3-2 EN61000-3-3 EN50082-1:1992 EN61000-4-2 EN61000-4-3 ENV50204 EN610000-4-4 EN610000-4-8 EN610000-4-11
Manufacturer's Name:	Warner Instruments, LLC
Manufacturer's Address:	1125 Dixwell Avenue Hamden, CT 06514 Tel: (203) 776-0664
Equipment Description:	Pico-liter injector
Equipment Class:	ITE-Class A
Model Numbers:	PLI-10

I the undersigned, hereby declare that the equipment specified above, conforms to the above Directive(s) and Standard(s).

Place: Hamden, Connecticut
USA
Signature:

Full Name: Ralph Abate

Position: General Manager

Declaration of Conformity

CE MARKING (LVD)

Application of Council Directive: 73/23/EEC

Standards To Which Conformity Is Declared:	EN61010-1:2001
Manufacturer's Name:	Warner Instruments, LLC
Manufacturer's Address:	1125 Dixwell Avenue Hamden, CT 06514 Tel: (203) 776-0664
Equipment Description:	Pico-liter injector Safety requirements for electrical equipment for laboratory use
Equipment Class:	Class I
Model Numbers:	PLI-10

I the undersigned, hereby declare that the equipment specified above, conforms to the above Directive(s) and Standard(s).

Place: Hamden, Connecticut USA

Signature:

Full Name: Ralph Abate

Position: General Manager

WEEE/RoHS Compliance Statement

EU Directives WEEE and RoHS

To Our Valued Customers:

Harvard Apparatus is committed to being a good corporate citizen. As part of that commitment, we strive to maintain an environmentally conscious manufacturing operation. The European Union (EU) has enacted two Directives, the first on product recycling (Waste Electrical and Electronic Equipment, WEEE) and the second limiting the use of certain substances (Restriction on the use of Hazardous Substances, RoHS). Over time, these Directives will be implemented in the national laws of each EU Member State.

Once the final national regulations have been put into place, recycling will be offered for those Harvard Apparatus products which are within the scope of the WEEE Directive. Products falling under the scope of the WEEE Directive available for sale after August 13, 2005 will be identified with a "wheelie bin" symbol.

Two Categories of products covered by the WEEE Directive are currently exempt from the RoHS Directive – Category 8, medical devices (with the exception of implanted or infected products) and Category 9, monitoring and control instruments. Most of Harvard Apparatus' products fall into either Category 8 or 9 and are currently exempt from the RoHS Directive. Harvard Apparatus will continue to monitor the application of the RoHS Directive to its products and will comply with any changes as they apply.

- **Do Not Dispose Product with Municipal Waste.**
- **Special Collection/Disposal Required.**