

Model PLI-90A

Pico-Injector

User's Manual

Model PLI-90A Pico-Injector User's Manual 64-1738
Model PLI-90A Plus Pico-Injector User's Manual 64-1739



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Warranty and Repair Information

Serial Numbers

All inquiries concerning our product should refer to the serial number of the unit. Serial numbers are located on the rear of the chassis.

Calibrations

All electrical apparatus is calibrated at rated voltage and frequency.

Warranty

Harvard Apparatus warrants this instrument for a period of two years from date of purchase. At its option, Harvard Apparatus will repair or replace the unit if it is found to be defective as to workmanship or material.

This warranty does not extend to damage resulting from misuse, neglect or abuse, normal wear and tear, or accident.

This warranty extends only to the original customer purchaser.

IN NO EVENT SHALL HARVARD APPARATUS BE LIABLE FOR INCIDENTAL OR CONSEQUENTIAL DAMAGES. Some states do not allow exclusion or limitation of incidental or consequential damages so the above limitation or exclusion may not apply to you. **THERE ARE NO IMPLIED WARRANTIES OF MERCHANTABILITY, OR FITNESS FOR A PARTICULAR USE, OR OF ANY OTHER NATURE.** Some states do not allow this limitation on an implied warranty, so the above limitation may not apply to you.

If a defect arises within the two-year warranty period, promptly contact Harvard Apparatus, Inc. 84 October Hill Road, Building 7, Holliston, Massachusetts 01746-1371 using our toll free number 1-800-272-2775. Goods will not be accepted for return unless an RMA (returned materials authorization) number has been issued by our customer service department. The customer is responsible for shipping charges. Please allow a reasonable period of time for completion of repairs, replacement and return. If the unit is replaced, the replacement unit is covered only for the remainder of the original warranty period dating from the purchase of the original device.

This warranty gives you specific rights, and you may also have other rights which vary from state to state.

Repair Facilities and Parts

Harvard Apparatus stocks replacement and repair parts. When ordering, please describe parts as completely as possible, preferably using our part numbers. If practical, enclose a sample or drawing. We offer a complete reconditioning service.

CAUTION

This apparatus is not registered with the FDA and is not for clinical use on human patients.



CAUTION: Not for clinical use on human patients.

Please read the following safety precautions to ensure proper use of your pico injector. To avoid potential hazards and product damage, use this product only as instructed in this manual. If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

To Prevent Hazard or Injury:

USE PROPER POWER SUPPLY

The product is supplied with an approved power supply and line cord.

USE PROPER LINE CORD

Use only the line cord shipped with the product and make sure line cord is certified for country of use.

GROUND PRODUCT

This product is grounded through the grounding conductor of the power cord. To avoid electric shock, use only approved line cord with the product and insure it is connected to earth ground.

MAKE PROPER CONNECTIONS

Make sure all connections are made properly and securely.

ORIENT EQUIPMENT PROPERLY

Do not position the equipment such that it is difficult to reach the disconnecting device.

OBSERVE ALL TERMINAL RATINGS

Review the operating manual to learn the ratings on all connections.

AVOID EXPOSED CIRCUITRY

Do not touch any electric circuitry inside the product.

DO NOT OPERATE WITH SUSPECTED FAILURES

If damage is suspected on or to the product do not operate the product. Contact qualified service personnel to perform inspection.

OBSERVE ALL WARNING LABELS ON PRODUCT

Read all labels on product to ensure proper usage.



CAUTION
Refer to Manual



*Protective Ground
Terminal*

CAUTION

This product is not registered with the FDA and is not for clinical use on human or veterinary patients. It is intended for research use only.

CAUTION
FOR RESEARCH USE ONLY
NOT FOR CLINICAL
USE ON PATIENTS

Gas Usage Warning

**WARNING: Pico-Injector uses gas even when off.**

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.

Plan accordingly. Notice, that the BALANCE is on continuously once an output hose is attached to the front panel: without a micropipette attached, even this gas usage can be significant.

The PLI-90A Pico-Injector allows small liquid volumes to be delivered precisely through micropipettes by applying a regulated pressure for a digitally set period of time^{1,2}. The pressure is applied pneumatically (compressed gas) to deliver volumes from microliters to femtoliters from the same instrument.

This digital injection pressure is enhanced by two auxiliary pressures:

Balance:

A (lower) balance pressure applied to the delivery pipette between injections prevents clogging caused by pipette movement as well as dilution of the injected material by capillary action.

Clear:

Momentary application of high pressure can be used to clear clogged pipettes.

Triggering of injection is accomplished two ways: panel push button or optional foot switch. The duration of injection can be determined by an internal clock set with a digital switch or the depression of an optional foot switch.

This manual assumes the reader has no previous experience with microinjection, either extracellular or intracellular. Those planning on larger volume delivery (100 pL and up) can ignore the BALANCE feature. Some reference will be made to a less precise technique for smaller volume delivery to highlight the precision determining features of this instrument. This technique^{3,4} involves pressurizing the gas in a macro-syringe attached to a delivery micropipette by advancing the piston of the syringe with a micrometer screw. Ejection takes place continuously from the pipette; the volume delivered depends both on the unregulated over pressure in the syringe and the poorly known time the pipette is left inside the cell. This continuous ejection technique supposedly reduces the frequency of pipette clogging.

Reliable microinjection also requires skill in making and using micropipettes. Authorities on microinjection^{5,6} state that the most important single factor in microinjection is the micropipette. The Pico-Injector with its reproducibility simplifies the search for the optimum micropipette.

Specifications

Specifications

Input Gas Pressure	75-105 psi (517 to 720 kPa)
Injection Pressure	0.2 to 60 psi (413 kPa), regulated, multi-turn control
Balance Pressure	0.1 to 3.5 psi (68.9 kPa) regulated, multi-turn control, other ranges available on request
Clearing Pressure	Input gas pressure (unregulated)
Injection Time	0.01 to 99.99 seconds
Injection Time Accuracy	±0.01% (Crystal Time Base)
Pressure Display Digital	3.5 digits
Duration Mode	Internally timed or externally gated
Time Trigger Mode	Front panel, foot switch, or external TTL pulse (BNC)
Pressure Units	PSI / kPa switch selectable
Pressure Monitor Output	BNC, front panel, 10 mV/psi or 1 mV/kPa
Pressure Readout	Inject, balance, clear, output port
Pressure Display Resolution	3½ Digits, 0.1 psi or 1 kPa
Injection Count Display	0 to 9999 Injections
Power Input	External 9VDC @ 400mA (min) Power Supply universal input 90 to 264 VAC
Weight	5.44 kg (12 lbs)
Dimensions (H x W x D)	89 x 432 x 250 mm (3.5 x 17 x 9.8 in)
Warranty	Two years, parts & labor

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 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 movement can be produced by an inexpensive mechanically linked micromanipulator for large cells such as frog oocytes. More commonly, a hydraulic one for fine, vibration-free movement is mounted on a coarse mechanical manipulator. Suitable equipment is available from Harvard Apparatus, Inc.

Required supplies include compressed gas and microcapillaries. Compressed air is suitable for oxygen insensitive injection material. Nitrogen is a satisfactory inert gas for the general case. A pressure of 105 psi is sufficient: a regulator will be needed if supplied from a bottle of compressed gas.

Optional equipment for microinjection 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 an micro-incubator (to hold the cells at incubation temperatures during microinjection). Suitable equipment is available from Harvard Apparatus, Inc.

Accessories are also available to enhance your Pico-Injector (see Price List).

Micropipettes are made with a pipette puller from a microcapillary (1-2 mm. in 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.

Two useful distinguishing parameters of the micropipette are the inside diameter of its tip and the angle of taper to the tip. The smaller this angle, the longer the tapered region⁸ 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 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 it will tolerate. The smaller the tip, the more likely it is to clog.

Please reference page 16 for more information.

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 microscope⁶, most injections are done from the side or from above the cells. Four 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).
4. 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.

Rear Panel Connectors and Switches



Refer to Figure 2 above for the location of controls and connectors. Numbers below refer to the order of listing below.

1. Foot Switch Inputs (Connectors)

An optional footswitch (PLI-FS) can be connected to any one of these connectors. When connected to INJECT, pushing the footswitch starts injection. When connected to GATE, injection pressure continues as long as the footswitch is depressed.

2. Pressure Input Connector

This connector is the input for the compressed gas. See Preliminaries Section for recommendations on the gas. A maximum of 105 psi. can be safely applied; 105 psi. is optimal. At lower pressure gas will be used at a slower rate. Avoid input pressures less than 105 psi.

3. Input Filter

The PICO-INJECTOR is supplied with an input filter. It traps particles larger than 0.1 micron as well as the liquid often present in a building's compressed air lines. Drain by pushing the button on the underside of its transparent case.

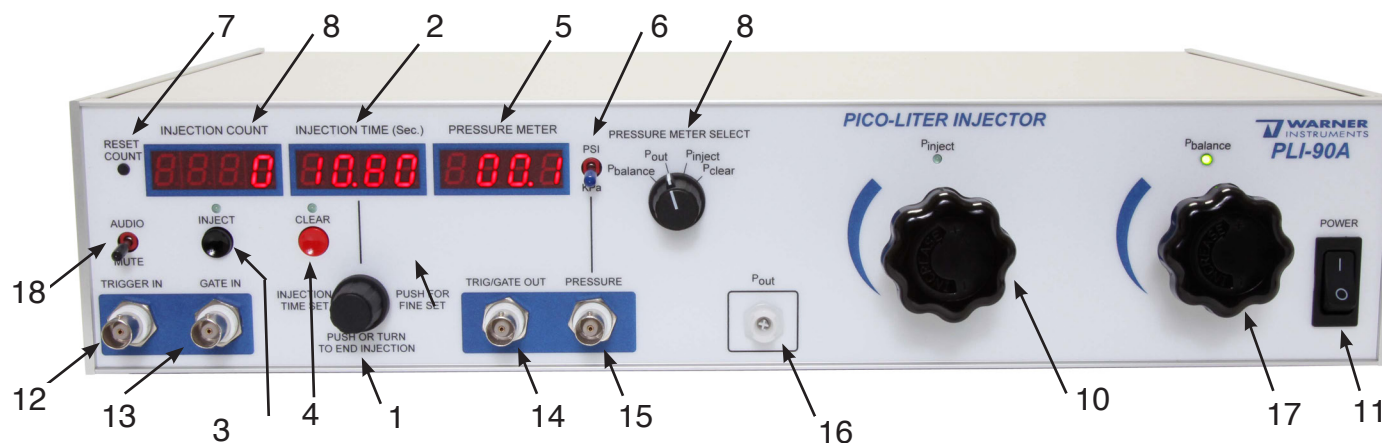
4. Filter Window

Allows for easy viewing of filter to determine when it needs to be drained.

5. Power Input Connector

Jack for universal AC input 9 volt 6 watt DC output power supply.

Front Panel Controls and Connectors



Refer to front panel photo above for the location of controls and connectors. Numbers refer to the order of listing below.

1. INJECT 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.

2. INJECT TIME Display

Displays injection time in seconds.

3. INJECT PUSHBUTTON

Push to initiate a single injection.

4. CLEAR pushbutton

Push this briefly to deliver a half second pressure surge to clear a clogged pipette. The pressure applied is the supply pressure. If the button is left pushed in for longer than a half second, the clearing surge is extended. The button remains lit for the duration of the clear.

5. PRESSURE display

This three digit display gives the “gauge” pressure selected by the PRESSURE METER SOURCE switch. A negative (-) sign indicates suction.

6. PRESSURE UNITS 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 in kilopascals (kPa.) 1 psi = 6.89 kPa.

7. RESET pushbutton

This button resets the injection count display to zero.

8. INJECTION COUNT display

This four digit display (0-9999) gives the total number of injections triggered manually by the panel pushbutton or footswitch or electrically since the last reset.

9. PRESSURE METER SOURCE switch

This switch selects various places inside the PICO-INJECTOR for reading the pressure. It does not change the pressure applied to the output. P_{INJ} is the pressure at the output during injection. P_{BAL} is the pressure applied to the pipette when injection is not taking place. P_{CLR} is the pressure externally supplied to the input on the rear panel and used the CLEAR mode. P_{OUT} is the pressure currently applied to the output port.

10. P_{INJ} regulator

This seven turn control is used to set the injection pressure over the range from about 0.4 to 60.0 psi (about 2.8 - 413 kPa). Clockwise rotation increases the pressure. Locking nut should be loose to avoid damaging the shaft's threads if changing pressure constantly.

11. POWER rocker switch

Controls power to the unit.

12. TRIG IN connector

This BNC connector is for electrically initiating injection. A positive TTL level pulse is required (baseline 0.0 V, trigger 5 V, lasting at least 50 microseconds).

13. GATE IN connector

This BNC connector is for external timing of the duration of injection. A positive TTL level pulse (see 13.) is required with the duration of injection determined by the duration of the applied pulse.

14. TRIG/GATE OUT connector

This TTL output (see 13.) can be used to trigger or synchronize other electrical instruments. Its duration is that of the injection.

15. PRESSURE MONITOR out

This BNC connector delivers an electrical level whose amplitude gives the actual pressure applied to the meter. The conversion factor is 10 mV /psi or 1mV/KPa.

16. P_{OUT} connector

This connector is attached to the injection pipette using the supplied output hose (PLI-OH). Without the hose attached the port is closed. In use, the injection, balance, fill, and clear pressures are delivered here.

17. P_{BAL} regulator

This regulator sets the balance pressure over the range from 0.1 to 10 psi. (about .7-70 kPa.). Clockwise rotation increases the pressure.

18. AUDIO / MUTE switch

Controls injection sound.

General Considerations

It is much easier to reliably inject large volumes than small ones. For “large” volumes, the balance pressure capability is not needed. Pipettes seldom clog so the clear capability is also 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 pLiter range. (For convenient visualization and approximate geometric measurement, 1 fLiter is a cube 1 micron on a side or a sphere 1.24 microns in diameter, 1 pLiter is a cube 10 microns on a side or a sphere 12.4 microns in diameter, while 1 nLiter 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 is often of “small” volumes (but not for frog oocytes). A more quantitative way to distinguish between “large” and “small” is given in the balance section below.

Interconnections and Initial Setup

Connect the gas input hose (PLI-IH) to the rear panel input connector of the Pico-Injector. Connect the other end (with optional input hose adapter, if needed) to the gas supply. Turn on the POWER switch and verify the input gas pressure with the digital meter by setting the PRESSURE METER SOURCE switch to P_{clear} . Practice with the inject and balance pressure controls by first turning the METER SELECT switch and then adjusting each in turn. With non zero values of inject and balance pressure set the PRESSURE METER SOURCE switch to P_{out} .

NOTE: The PRESSURE METER SELECT switch must be set to P_{out} to read changes in pressure on the display or monitor output. Set the inject time to five seconds and push the panel INJECT switch to see the temporary change in pressure from balance to inject and back. A buzzer will sound during injection. If this is not wanted, turn off the switch on the rear panel.

One output hose (PLI-OH) is supplied with the Pico-Injector. These are designed for any of the three optional pipette holders (PLI-PH1, PLI-PH1A, PLI-PPH) described in the price list.

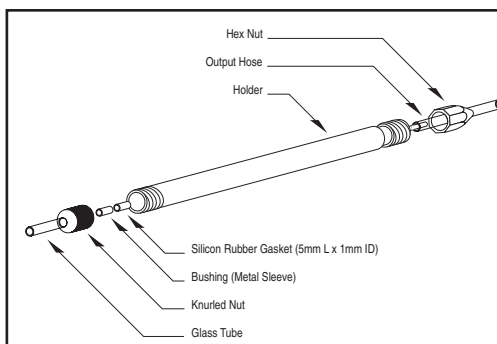


Fig. 3. PLI-PH1/PH1A 1.0 mm OD Glass Pipette Assembly

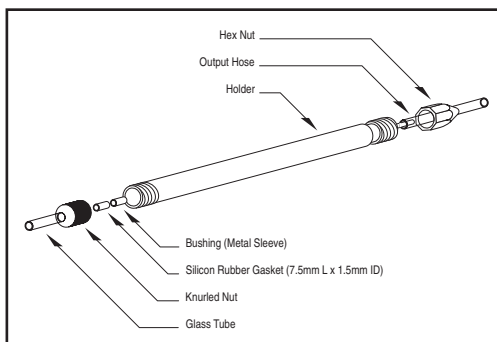


Fig. 4. PLI-PH1/PH1A 1.5 mm OD Glass Pipette Assembly

Connect the PLI-OH hose to the chosen holder(s) as follows:

PLI-PH1 & PLI-PH1A:

Unscrew the end of the holder with a 2 mm. diameter hole. This end hex piece should be placed over the tube end. Thread the hex piece on the holder and tighten firmly. The 1 mm OD capillary is inserted in the opposite end of the holder through its "o" ring and its fitting is then tightened.

PLI-PPH: Unscrew the end of the holder with the 2 mm. diameter hole and place it over the output hose (PLI-OH). Reattach this fitting securely with the hose inserted as far as it will go. The 1.5 mm. OD capillary is similarly attached to the other end of the holder through its "O" ring. Attach the output hose to the front panel. Tighten them securely so that the valve within this port is open, allowing pressure to be controlled in the hose and connecting micropipette.

Balance

The inflow into the pipette (caused by capillary forces) prior to or in-between injections should be a small percentage of that being injected. As the volume desired gets smaller, the relative inflow gets larger even faster due to the smaller tip size being used. To avoid this problem, set the balance pressure before placing the pipette in the cell's external medium. Ten percent of the injection pressure is a good starting value. Exact balance is difficult to determine: often the fastest way to handle this is to set the balance high enough that slight outflow is observed. Balance is assessed by watching the movement of the liquid meniscus in the pipette. The outflow left is still small compared to the continuous streaming used in the continuous ejection technique.

If a filling fiber is used, the capillary inflow is larger so a higher balance pressure is needed. In general, however, when a balance pressure is being used, such a fiber should not be used: sometimes the injection material will run out spontaneously or the pressure needed to balance will abruptly change with time. (These may be due to particles lodging on the fiber). If desired, a rough estimate of the volume coming in can be made. Focus the microscope on the liquid meniscus in the loaded pipette while its tip is still in air (with the balance pressure set to zero). Use an eyepiece reticle to measure the meniscus movement when liquid is then raised to cover the tip. The inflow volume is approximately the difference between the volume of two cones. The estimate is more precise for smaller initial volumes in the pipette.

Clear

To clear a clogged micropipette, push the CLEAR button momentarily once the tip has been removed from a cell. Watch the tip region while this is done to see the brief movement of liquid denoting that the tip is clear. Repeat if needed. The clearing pressure pulse has a preset duration of 500 msec. to avoid excessive release if the clog clears immediately. Repeat as needed.

Footswitches

Optional footswitches can be used for inject. These switches are plugged into the rear panel: each is wired in parallel with the corresponding front panel switch. See the instructions for each panel switch to see how they operate. When a footswitch is used with the GATE connector, the duration of injection is manually determined by the duration of pressing the footswitch.

Injection Volume Adjustment

Adjust injection volume by changing either injection pressure, injection duration, or the micropipette (inside) tip diameter or taper. The dependence on (net) injection pressure, duration and pipette taper angle is linear; the dependence on tip diameter is cubic.

Volume Calibration Chart

Formula:

Volume in Nanoliters = .17952(*) x (tip I.D. in micron)³ x (Pressure in psi) x (Time in sec)

Example:

Volume = .17952 x (5)³ X (10) X (1) Nanoliters = 224.40 Nanoliters

Pressure (p.s.i.)	Time (sec.)	Pipette Tip I.D. (µm)	Femtoliters	Picoliter	Nanoliter	Microliter	Milliliter
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

(*) Constant includes unit conversion factors, and effects of water like viscosity and needle taper for a cone half angle of 10 (medium taper).

Universal Switching Power Supply

The PLI-90A is equipped with a universal AC input 9 volt 6 watt DC output power supply with interchangeable input blades.

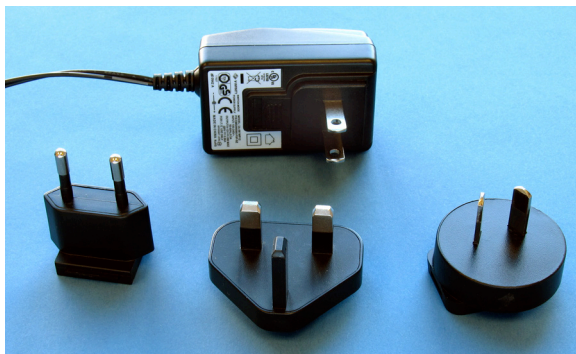


Figure 1. Wall adapter with interchangeable input blades

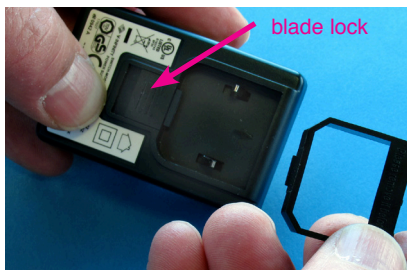


Figure 2. Remove contact protector

1. Slide the blade lock in the direction of the arrow with one hand and lift out the contact protector with your other hand.

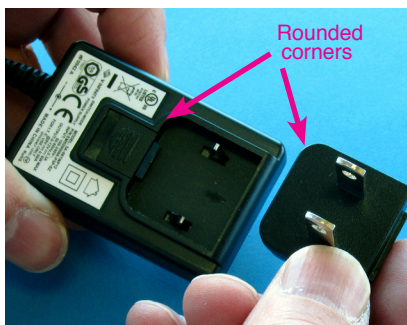


Figure 3. Install input blade

2. Align the rounded corners of the input blade being installed with the rounded corners on the adapter.
3. Lower the square corners of the blade into the adapter.
4. Pull back on the blade lock and lower the rounded corners into adapter, release the blade lock.



Figure 4. Complete assembly

1. McCaman, R.E. et.al. "A pressure system for intracellular and extracellular ejections of microliter volumes" *Brain Research* 142, 141 (1977).
2. Palmer, M.R. et al., "Physical and physiological characteristics of micropressure ejection ... from ... pipettes". *Neuropharmacology* 19, 931-938 (1980).
3. Hiramoto, Y. *Exp. Cell Res.* 27, 416-426 (1962). Pneumatic, micrometer syringe, mercury. See Kiehart, D.P. *Methods Cell Biol.* 25, 13ff (1982) for a careful explanation.
4. Graessmann, A. *Exp. Cell Res.* 60, 373-382 (1970).
5. Mario Capecchi (private communication).
6. Dennis Stacey (private communication).
7. 10 micron pipettes would be suitable for frog oocytes, but there is little need for the sizes between 1.5 and 10 microns.
8. Pipettes will have more than one taper angle if the pulling force and/ or heat change during pulling. (Two stage pullers give two angles. for example.) Delivery rate depends on the angle nearest the tip.
9. Stephens, D.L. et al, "Easy to use equipment for the accurate microinjection of nanoliter volumes into ... oocytes" *Anal. Biochem.* 114, 299-309 (1981).
10. Brinster, R. L., et al, *Proc. Natl. Acad. Sci.* 82, 4438-4442 (1985), DNA into mouse oocytes.
11. Webster, D. R., et al *J. Cell Biol.* 105, 265-276 (1987), tubulin into human fibroblasts and hamster ovary cells.
12. Palmer, M. R. et al, in *Electrophysiological Techniques in Pharmacology*, pages 169-187 (1986), Alan R. Liss, Inc. Various materials onto mammalian neurons.
13. mRNA into *Xenopus* oocytes- see footnote 9.
14. Stacey, D. W. et al, *Exp. Cell Res.* 171, 232-242 (1987), ras protein into tumor cells.
15. Pasti, G., et al, *Nature* 324, 375-377 (1986), protein kinase C into Swiss 3T3 cells.
16. Silver, R.B. *Proc. Nat. Acad. Sci.* 83:4302-4306 (1986) antibodies into sand dollar embryo
17. de Laat, A.M. and Blaas, J. *Pl. Sci.* 50:161-169 (1987) cytoplasmic organelles into plant protoplasts

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.

Setting, Pressures and Time Pulses

A. Balance Pressure

Set the balance pressure while viewing the pipette under magnification. This method will help the user to stabilize the solution within the pipette easily.

B. 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.

Notes

Notes

Declaration of Conformity

Manufacturer: *Harvard Apparatus Inc.
84 October Hill Rd.
Holliston, Massachusetts 01746-1388 U.S.A.
Phone: (508) 893-8999*

We herewith declare that the following product:

Product Name: *Pico Injector*

Model Numbers: *PLI-100A, PLI-90A, PLI-10*

To which this declaration relates, is in conformity with the applicable EC Directives, harmonized standards, and other normative requirements:

Application of

Council Directive(s): *2006/95/EC Low Voltage Directive
2004/108/EC Electromagnetic Compatibility Directive*

Standard(s) to which conformity is declared:

Safety: *IEC 61010-1:2010 (3rd Edition)
UL 61010-1:2012
CSA C22.2 No. 61010-1-12*

Emissions/Immunity: *EN 61326-1:2013
EN 61000-4-2:2009
EN 61000-4-3:2006+A1:2008+A2:2010
EN 61000-4-4:2004+A1:2010
EN 61000-4-5:2006
EN 61000-4-6:2--9
EN 61000-4-11:2004
EN 61000-3-2:2006+A1:2009+A2:2009
EN 61000-3-3:2008
EN 55011:2009+A1:2010 Class A
FCC 47FR 15B:2014 Class A*

EMC and Safety compliance were evaluated by Intertek/ETL Semko

Reference test report file numbers: *101853174 Box-002, Box-003a*

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

Place: *United States of America*
Date: *09 APRIL 2015*



(Signature)

Mark Davis

(Full Name)

Director, Global MPE

(Position)



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