



Nanoliter 2000

Microprocessor controlled nanoliter injector

www.wpiinc.com

INSTRUCTION MANUAL

Serial No. _____

081203



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DECLARATION OF CONFORMITY

We: World Precision Instruments, Inc.
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as the manufacturers of the apparatus listed, declare under sole responsibility that the product(s):


Title: Nanoject II
Model Number 3-xxx-206-A and 3-xxx-205-A, inc. 12V Power Adapter PSD121A
(WPI Model Numbers B203XVB and B203XVZ)

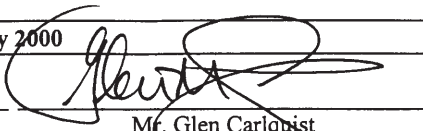
to which this declaration relates is/are in conformity with the following standards or other normative documents:

- Safety:** EN 61010-1:1993 (IEC 1010-1:1990)
- EMC:** EN 50081-1:1992
EN 50082-1:1992

and therefore conform(s) with the protection requirements of Council Directive 89/336/EEC relating to electromagnetic compatibility and Council Directive 73/23/EEC relating to safety requirements.

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WARRANTY

WPI (World Precision Instruments, Inc.) 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 10 days after receipt of shipment. Claims for lost shipments must be made within 30 days of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim settles. 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.
- WPI cannot be held responsible for items damaged in shipment en route to us. Please enclose merchandise in its original shipping container to avoid damage from handling. We recommend that you insure merchandise when shipping. The customer is responsible for paying shipping expenses including adequate insurance on all items returned.
- Do not return any goods to WPI without obtaining prior approval and instructions (RMA#) from our returns department. Goods returned unauthorized or by collect freight may be refused. The RMA# must be clearly displayed on the outside of the box, or the package will not be accepted. Please contact the RMA department for a request form.
- Goods returned for repair must be reasonably clean and free of hazardous materials.
- A handling fee is charged for goods returned for exchange or credit. This fee may add up to 25% of the sale price depending on the condition of the item. Goods ordered in error are also subject to the handling fee.
- Equipment which was built as a special order cannot be returned.

** Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.*

General Comments

1. The wire plungers on all injectors have a point on one end. This wire can be reversed if a blunt end is desired. The point should allow bubbles in the oil to escape when backfilling tips.
2. The preferred tip size for *Xenopus* injection seems to be 20 microns. Some users report using 30 micron tips with no increase in mortality.
3. Injections of 25 nL or less generally require smaller tips, normally 10 microns.
4. A broken tip seems preferable for oocyte injection. Sharp polished tips tend to "skip".
5. Virtually any oil appears suitable for backfilling.
6. A reported technique positions a drop of mercury between the oil and the sample in the micropipet. This apparently acts as a brake allowing better control of injection rate.
7. An excellent reference to all procedures involving the *Xenopus* oocyte is: Kay, B. and H. Peng, "Xenopus laevis: Practical Uses in Cell and Molecular Biology". *Methods in Cell Biology*, Vol. 36, Academic Press.

Introduction

The introduction of the **Nanoliter 2000** offers a volume ranging down to 2.3 nL and up to 69 nL with a higher torque motor than a Nanoliter Injector. This new motor is quieter and smoother in operation further reducing tip movement. The development of a new collet configuration will hold the micropipette more securely and thereby reduce air infiltration and oil leakage. All are factors that enhance the precise delivery of sample.

By reducing air infiltration into the micropipette and by enabling the researcher to inject at a slower speed, smaller pipette tips will perform better than previous units. Although previous models, as well as the Nanoliter 2000, are excellent for *Xenopus* injections, the smaller tips and smaller injection volume may allow the user to perform injections into other specimens that previously could not be performed with older models.



Micromanipulator not included

Positive displacement technology and the use of precision micropipettes, eliminates the need for tedious calibration when the viscosity of the samples change. Contaminants in the sample do not hinder or change the injection volume as with some other injectors.

System Components

- a. Injector head with cord
- b. Control box
- c. Power supply with cord
- d. Capillary glass; 3 vials of 100 pieces per vial
 - 1. 3.5" capillaries, 100 pieces
 - 2. 7.0" capillaries, 100 pieces
- e. Replacement O-ring kit, standard
- f. Allen wrench for replacing wire plunger
- g. MF34G MicroFil™ non-metallic filling needle
- h. 1TIP10XV119 pre-pulled pipette

Cleaning Recommendations

The injector can be cleaned by removing the collet, O-rings, spacer, etc., and wiping them with alcohol. Do not soak in liquid or autoclave Nanoliter 2000. The control box may be cleaned with a clean, dry cloth.

Servicing the Unit

There are only two common service functions that the user will perform: replacement of O-rings and replacement of the wire plunger.

Replacement of O-rings

After a period of time or heavy use, some leakage of oil during injection might be observed. To correct this, the O-rings must be replaced. Two O-ring kits are provided. Refer to **Figure 1** or **Figure 2** for proper installation. This step is critical for proper operation. Note the white spacer in **Figure 1** must be properly oriented.

Replacement of wire plunger

To change the wire plunger, remove the collet and all collet components. Hold the motor housing and unscrew the aluminum barrel. Once free, pull off. You will see a brass fitting with two Allen screws. Unscrew these with the wrench provided. Remove old plunger and place new one in fitting. Tighten the screws, making sure wire is seated fully and straight. Replace aluminum barrel and collet.

Plunger in “HOME” Position

When you plug in the Nanoliter 2000 it will assume it is in the “home” position. When in this position, the tip of the wire plunger should be slightly recessed from the end of the collet. This prevents accidental damage to the plunger when not in use. To reset the plunger to this position, push FILL to retract or EMPTY to extend the plunger and simultaneously hold the INJECT button. When in the home position, release both buttons and the unit is reset.

When unplugging the unit, make sure the plunger is in the home position. Upon restarting the unit, it will start in this position.

Optional Accessories

An optional foot switch is available that plugs into the control box that enables you to inject using the foot switch.

Injection

Injection volumes are determined by the position of the DIP switches. DIP switches #1- #4 control the volume (see **Figure 3**); DIP switch #5 controls the injection rate and the fill rate.

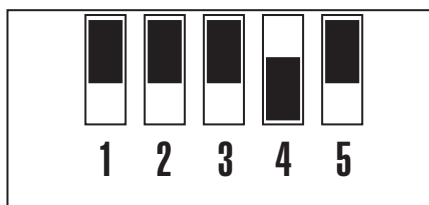


Figure 3

Once the volume has been selected, each time the INJECT button is depressed an audible beep will be heard and the selected volume will be dispensed. The actual penetration of the micropipette into the oocyte or tissue is produced by the micromanipulator.

Multiple injections can be made in one location by simply pressing INJECT again. Pressing INJECT again before the first injection is complete will not produce a second injection. Listen for the beep.

Setting Injection Volume and Injection Speed

Injection volumes and injection speeds are controlled by the positions of the DIP switches located on the side of the control box (“U” = up, “D” = down).

	DIP SWITCH:			
VOLUME:	1	2	3	4
2.3 nL	U	U	U	U
4.6 nL	D	U	U	U
9.2 nL	U	D	U	U
13.8 nL	D	D	U	U
18.4 nL	U	U	D	U
23.0 nL	D	U	D	U
27.6 nL	U	D	D	U
32.2 nL	D	D	D	U
36.8 nL	U	U	U	D
41.4 nL	D	U	U	D
46.0 nL	U	D	U	D
50.6 nL	D	D	U	D
55.2 nL	U	U	D	D
59.8 nL	D	U	D	D
64.4 nL	U	D	D	D
69.0 nL	D	D	D	D

**DIP SWITCH 5:
INJECTION RATES**
U FAST = 46 nL/sec
D SLOW = 23 nL/sec

Note: DIP switch settings are completely different from the previous model, Nanoliter Injector A203.

Operation

Nanoliter 2000 is ready for use as received, requiring only pulled capillary tips. It is essential that only the capillaries supplied (or exact replacements) are used for pulling micropipettes. WPI offers straight capillaries of appropriate diameter (1.14 mm OD and 0.5 mm ID) in two lengths: 3.5 inches (#4878) and 7 inches (#4879). In addition, pulled micropipettes are also available (#TIP10XV119).

The injector head should be mounted in a suitable micromanipulator or stereotaxic unit; model **M3301** is quite suitable and frequently used. The smaller aluminum barrel provides an ideal clamping surface. Its diameter measures 11 mm.

The precise operation of the Nanoliter 2000 depends greatly on the use of tips prepared from the glass provided. Micropipettes pulled from capillaries with other dimensions may not work. Never attempt to use micropipettes pulled from glass containing a filament: damage to the wire plunger will result and injection volumes will not be accurate.

Micropipette Pulling and Backfilling

The Nanoliter 2000 requires the use of pulled micropipettes from the glass provided. Ideally, the tip size should be 10-30 microns in size. The capillary glass provided has a softening point of 780° C. Many researchers pull the tips and then break them off with forceps. This enables piercing the cell membrane much easier with no skipping.

Once the tips are pulled, they must be “backfilled” with oil (or other non-compressible fluid) before attachment to the injector. Silicone or mineral oil is frequently used. Backfilling is facilitated by using the flexible MicroFil™ non-metallic needle and a syringe.

NOTE: NANOLITER 2000 WILL NOT OPERATE PROPERLY WITHOUT BACKFILLING THE MICROPIPETTE.

Securing the Micropipette to the Injector

The injector is supplied with two collet configurations. The configuration on the unit as delivered, and an optional configuration for use with the flared capillaries.

Standard collet / O-ring

Once the micropipette is backfilled, loosen the collet. The pointed wire plunger should be positioned so you can just see the tip flush with the end of the collet (slightly recessed is also acceptable). This is referred to as the “home” position. Push the micropipette onto the wire plunger and as you push the tip on, feel it go through the large O-ring and seat in the white spacer. Once positioned, tighten the collet securely. Give the micropipette a pull to confirm it is securely mounted. See **Figure 1** for proper configuration of the O-rings and the white spacer. It is absolutely essential that these components are properly configured. **Note: The white spacer has one flat side and one side with a recess machined around the hole. This recess is to receive the back end of the pipette.**

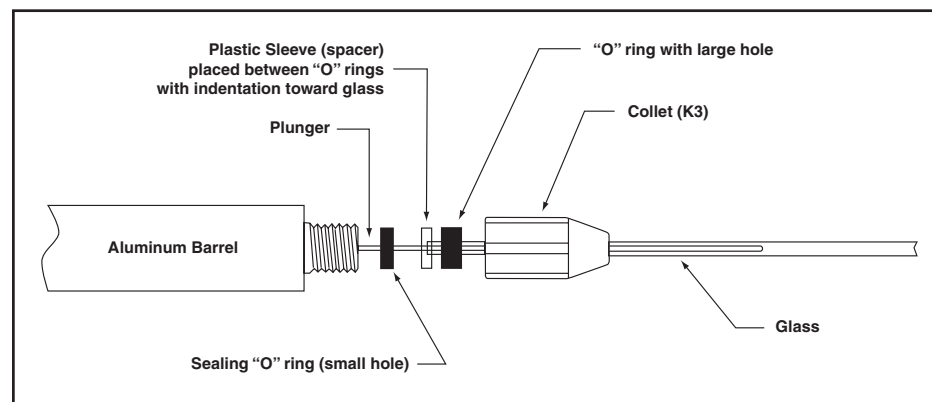


Figure 1

Filling the Micropipette

Once the micropipette is secured to the collet, press and hold the EMPTY button. This will drive the wire plunger out, forcing oil to the tip of the pipette and expelling any excess oil. Hold the EMPTY button until an audible beep is heard. The plunger is now fully extended (approximately 27 mm from the end of the collet).

Now place the tip of the pipette into your sample and press and hold the FILL button.

The plunger will retract, drawing with it the sample. When fully filled, the micropipette will contain approximately 5 μ l of sample. At any time you can stop filling by releasing the FILL button. Continued filling can be accomplished by pressing the button again. Viscous samples may require you to fill in small steps, allowing the sample to equilibrate in the tip before continued filling. Do not allow air bubbles to form in the micropipette. These bubbles can cause inaccurate injection volumes.

The control buttons work as follows:

FILL:

Retracts wire plunger while position is depressed. “Beep” will sound at extreme fill position. DIP switch #5 controls the rate of filling.

#5 up = 46 nL/second fill rate

#5 down = 23 nL/second fill rate

EMPTY:

Extends wire plunger while position is depressed. “Beep” will sound at fully extended position (approximately 27 mm from end of collet). Empty speed is approximately 92 nL/second. Fast empty (230 nL/sec) can be achieved by holding the **EMPTY** button and pressing the **FILL** button once. The plunger will maintain the faster speed until the **EMPTY** button is released. The unit will always start in the slower speed.

FAST FILL & FAST EMPTY:

DIP switch #5 has no effect on the empty mode. To empty fast, hold down the **EMPTY** button and touch the **FILL** button one time. The empty speed will increase to 230 nL/second.

The fill mode also has a fast speed. Hold down the **FILL** button and touch the **EMPTY** button one time. The fast rate will vary, depending on the position of DIP switch #5.

#5 up = 230 nL/second

#5 down = 92 nL/second

Note: The unit will always start in the slower speed.