



**OPERATING INSTRUCTIONS
DRUMMOND "NANOJECT II"
AUTOMATIC NANOLITER INJECTOR
CATALOG NUMBER 3-000-204/205A/206A**



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OPERATING INSTRUCTIONS
DRUMMOND "NANOJECT II"
AUTOMATIC INJECTOR

FOR 220 UNIT ONLY
(3-000-205A & 3-000-206A)



The triangle label found on the control box cautions the user to refer to the accompanying documents.
This equipment is rated for 230VAC, 50hz, 26W.
This equipment is rated for indoor use only.
This equipment may be used in temperatures from 5 C to 40 C.
This equipment has been tested to operate in humidity of 92.5%.
This equipment is rated for installation category 2, Pollution Degree 2.

If this equipment is used in a manner not specified by the manufacturer(Drummond Scientific Co.), the protection provided by the equipment may be impaired.

Introduction:

Drummond Scientific has a long and outstanding history in the manufacturing of microinjection apparatus. Several years ago, we became aware that our 10uL positive displacement microdispenser was being modified to inject *Xenopus* oocytes. We modified this unit by redesigning the microdispenser to eliminate any tip movement caused by plunger rotation.

Further modifications seemed uneconomical which prompted a complete redesign. Drummond developed a positive displacement motorized unit which was capable of repetitively dispensing precisely 46 nL., a volume optimized for oocyte injection. This was to be the first Nanoject.

The next model offered the scientist a variable volume, ranging from 4.6 nL. to 73.6 nL. Hence, the Nanoject Variable.

The introduction of the Nanoject II offers volumes ranging down to 2.3 nL. and up to 69 nL. and a higher torque motor, and a two speed injection rate. This new motor is quieter and smoother in operation further reducing tip movement. The development of a new optional collet configuration will hold the micropipet more securely and thereby reduce air infiltration and oil leakage, all factors which enhance the precise delivery of sample.

By reducing air infiltration into the pipet and by enabling the researcher to inject at a slower speed, smaller pipet tips will perform better than previous units. Earlier models, as well as the Nanoject II, are excellent for *Xenopus* injections however, 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.

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

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AUTOMATIC NANOLITER INJECTOR
Catalog Number 3-000-204/205A/206A

This equipment is ready for use as received. It will require pulled capillary tips. It is essential that only the capillaries supplied or exact replacement capillaries are used for pulling micropipets.

I. Contents:

- a. Injector head with cord.
- b. Control box.
- c. Power supply with cord.
- d. Capillary glass; 2 vials of 100 pieces per vial:
 - 1. 3.5" capillaries, 100 pcs. Cat. No. 3-000-203-G/X
 - 2. 7.0" capillaries, 100 pcs. Cat. No. 3-000-203-G/XL
- e. Replacement O-ring kit, standard. Cat. No. 3-000-002-KN
- f. 1 30g x 2" needle for backfilling pipets. Cat. No. 3-000-027
- g. 1 Universal Adapter Cat. No. 3-000-024-A

II. Operation:

The injector head should be mounted in a suitable micromanipulator or stereotaxic unit. Marzhauser model MM33 is quite suitable and frequently used. The aluminum barrel provides an ideal clamping surface. Its diameter measures 11mm.

The precise operation of the Nanoject II depends greatly on the use of tips prepared from the glass provided. We cannot guarantee that glass micropipets pulled from capillaries with other dimensions will work. Never attempt to use micropipets pulled from filament glass. Damage to the wire plunger will result and injection volumes will not be accurate.

A. Micropipet Pulling and Backfilling:

The Nanoject II requires the use of pulled micropipets from the glass provided. Ideally, the tip size should be 10-30 microns in size. The capillary glass provided is produced from N-51-A material and 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 lightweight mineral oil (or other non-compressible fluid) before attachment to the injector. Silicone or mineral oil is frequently used. Backfilling is facilitated by using the 30g x 2" needle and a syringe. Disposable spinal needles are also frequently used.

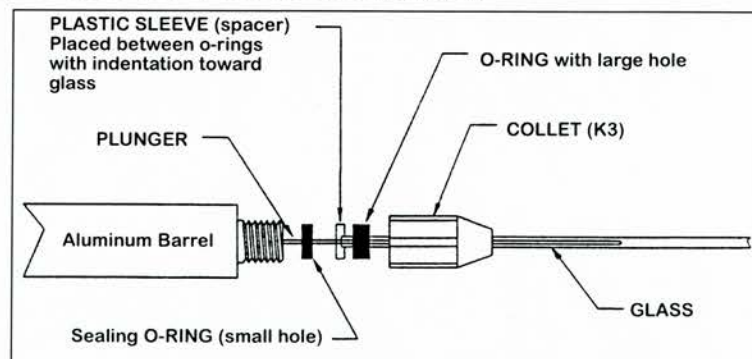
NOTE: THE NANOJECT II WILL NOT OPERATE PROPERLY WITHOUT BACKFILLING THE MICROPIPET.

B. Securing the Micropipet to the Injector:

The injector is supplied with the standard collet/O-ring configuration, and an extra O-ring kit with allen wrench.

1. Standard Collet/O-ring, Orientation:

Once the micropipet 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, or slightly extended, is also acceptable). This is referred to as the "home" position. Push the micropipet 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 micropipet a pull to confirm it is securely mounted. See fig. 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 pipet.



(fig. 1)
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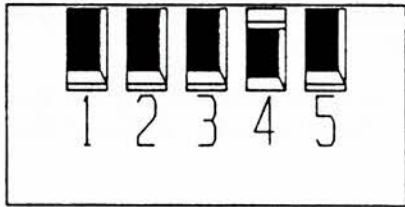
C. Filling the Micropipet:

Once the micropipet 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 pipet and any excess oil will be expelled. Hold the EMPTY button until an audible beep is heard. The plunger is now fully extended (approx. 27mm from the end of the collet).

Now place the tip of the pipet into your sample and press and hold the FILL button. The plunger will retract drawing with it the sample. The micropipet when fully filled, will contain approximately 5uL of sample. At any time you can stop filling by letting go of 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 samples to equilibrate in the tip before continued filling. Do not allow air bubbles to form in the micropipet. These bubbles can cause inaccurate injection volumes.

D. Injection:

Injection volumes are determined by the position of the dip switches. Only dip switches #1-#4 control the volume (see fig. 3). Dip switch #5 controls the injection rate and the fill rate.



(fig. 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 injection of the micropipet into the oocyte or tissue is produced by the micromanipulator.

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

The control buttons work as follows:

(1) FILL:

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

#5 UP = 46 nL/second fill rate

#5 DOWN = 23 nL/second fill rate

(2) EMPTY:

Extends wire plunger while position is depressed. “Beep” will sound at fully extended position (approx. 27mm from end of collet). Empty speed is approximately 92 nL/second. *Fast empty* (203 nL/sec.) 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.

(3) FAST FILL & FAST EMPTY:

Dip switch #5 does not have any 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. Depending on the position of dip switch #5 the fast rate will vary.

#5 UP = 230 nL/second

#5 DOWN = 92 nL/second

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

E. Setting the 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.

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

5 INJECTION RATES:

U FAST = 46nL/sec.

D SLOW = 23 nL/sec.

Note: The dip switch settings are completely different from the previous model, Nanoject XV.

fig. 3

F. 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 the Nanoject II. The control box can be cleaned with a clean dry cloth.

G. Servicing the Unit:

There are only two common service functions that the end user will perform. (1) replacement of o-rings and (2) replacement of the wire plunger.

(1) 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. An extra o-ring kits is supplied. Refer to fig. 1 for proper installation. This step is critical for proper operation. Note the white spacer in fig. 1 must be properly oriented.

(2) Replacement of wire plunger:

To change the wire plunger (cat. no. 3-000-000-203-X) 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 the 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.

Any additional service functions, should be performed by Drummond Scientific. Unauthorized tampering can void the warranty. Contact Drummond at:

1-800-523-7480 or 1-610-353-0200

H. Plunger in “HOME” position:

When you plug in the Nanoject II, 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. If the plunger does not return to this position when the fill is pushed and held, the home position must be reset. To reset the plunger to this position, push the *FILL* to retract and simultaneously hold the *INJECT* button. When in the *home* position, release both buttons and the unit is reset. NOTE: Resetting the home position is only necessary when the unit does not return to home position when fully retracted.

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

I. Optional Accessories:

An optional foot switch is available that plugs into the control box that enables you to *inject* using the foot switch. Catalog No. 3-000-026

J. 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”.

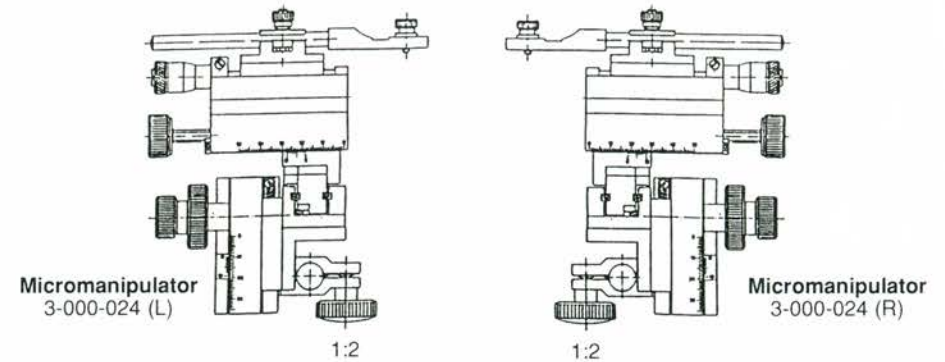
5. Virtually any lightweight oil appears suitable for backfilling.

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.

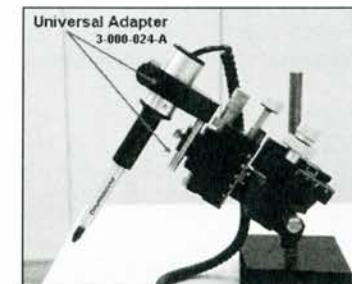
7. An excellent reference to all procedures involving the *Xenopus* oocyte is:

Kay, B. and H. Peng. “*Xenopus laevis*: Practical Uses in Cell and Molecular Biology”. Methods in Cell Biology. Vol. 36. Academic Press

MICROMANIPULATOR



Micromanipulator Support Base
3-000-025-SB



Universal Adapter
3-000-024-A

This micromanipulator, Marzhauser MM33, seems ideally suited to the Drummond NANOJECT. It will not drift under a load of 3.5 oz. (NANOJECT) and the integral clamp will easily hold the injector barrel (11mm OD) without modification. X, Y, and Z controls remain easily accessible with the NANOJECT in place. We offer this micromanipulator with or without a base, in the latter case a 1/2" clamp is supplied for attachment to a support rod.