



Nanoliter 2000

Microprocessor controlled nanoliter injection

INSTRUCTION MANUAL

Serial No. _____

021012

www.wpiinc.com

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ABOUT THIS MANUAL

The following symbols are used in this guide:

 This symbol indicates a CAUTION. Cautions warn against actions that can cause damage to equipment. Please read these carefully.

 This symbol indicates a WARNING. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and TIPS contain helpful information.

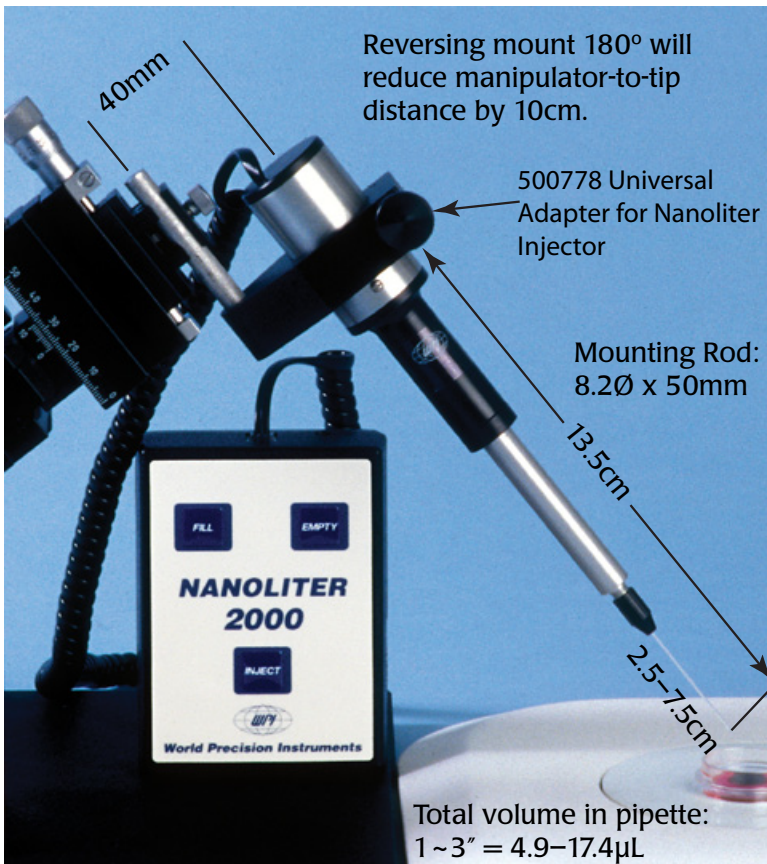


Fig. 1–Nanoliter 2000 setup. Micromanipulator is not included .

INTRODUCTION

The **Nanoliter 2000** microinjection system provides you with the capability to inject liquids in discrete aliquots ranging from 2.3nL to 69nL. The **Nanoliter Injector** utilizes a stainless steel plunger to create a mechanical displacement of fluid within a micropipette, thus pushing the contents out through the pipette tip.

General Notes

- 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.
- The preferred tip opening size for *Xenopus* injection is about 20 μ m. Some researchers report using 30 μ m tips with no increase in mortality.
- Injections of 25nL or less generally require smaller tips, normally 10 μ m.
- A broken tip is preferable for oocyte injection. Sharp polished tips tend to deflect.
- An excellent reference for 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.

Parts List

After unpacking, verify that there is no visible damage to the instrument. Verify that all items are included:

- (1) Injector head
- (1) Control unit
- (1) Power supply
- (1) **500778** Universal Adapter for Nanoliter Injector
- (2) Capillary glass vials
 - (1) 3.5" capillaries, 100 pieces
 - (2) 7.0" capillaries, 100 pieces
- (1) Replacement O-ring kit, standard
- (1) Allen wrench for replacing wire plunger
- (1) **MF34G** MicroFil™ non-metallic filling needle (used for backfilling)

(1) **1TIP10XV119** pre-pulled pipette (10 μ m)

(1) Instruction Manual

Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 19 of this manual. Please contact WPI Customer Service if any parts are missing at 941.371.1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100mm (four inches) of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 19 of this manual.

INSTRUMENT DESCRIPTION

Instrument Description

The **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 correct diameter (1.14mm OD and 0.5mm ID) in two lengths: 3.5" (WPI #4878) and 7" (WPI #4879). In addition, pulled micropipettes (WPI #TIP10XV119) are available.

The injector head can be mounted in a micromanipulator. WPI's **M3301** model is quite suitable and frequently used. The **500778** Universal Adapter for the Nanoliter Injector (shaft diameter of 8.2mm) is used for mounting the Nanoliter Injector to a micromanipulator.

The **Nanoliter 2000** is comprised of a controller (**Fig. 3**) and an injector handle (**Fig. 4**). **Fig. 3** shows the right side of the controller where the DIP switches are located.



Fig. 2—Nanoliter 2000 controller showing the Fill, Empty and Inject buttons on the front of it



Fig. 3—The right side of the controller box has five DIP switches for controlling aliquot injection volumes and injection speeds

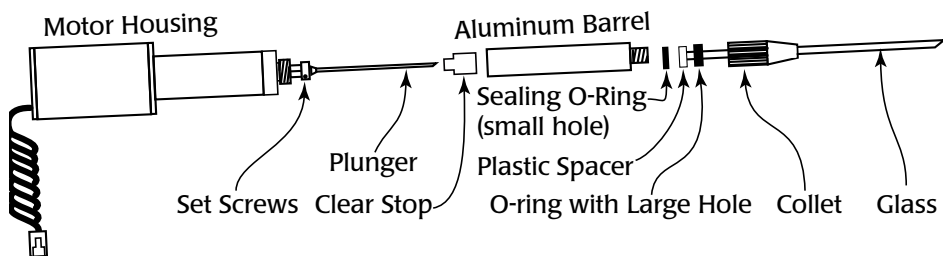


Fig. 4–Nanoliter 2000 (exploded view) shows the parts of the Nanoliter 2000 injector handle

Controller Buttons

Fill–The Fill button retracts the wire plunger by one aliquot volume when the button is depressed. A beep sounds at the extreme fill position. DIP switch #5 (**Fig. 3, 7**) controls the rate of filling.

- #5 up = 46nL/s fill rate
- #5 down = 23nL/s fill rate

Empty–The Empty button extends the wire plunger by one aliquot volume when the button is depressed. A beep sounds at the fully extended position (approximately 27mm from end of collet). The empty speed is approximately 92nL/s.

Fast Fill and Fast Empty–DIP switch #5 has no effect on the fast empty mode. To empty rapidly, hold down the Empty button and touch the Fill button one time. The empty speed increases to 230nL/s. The plunger maintains the faster speed until the Empty button is released.

The fill mode also has a fast speed. Hold down the Fill button and touch the Empty button one time. The fast rate varies, depending on the position of DIP switch #5 .

- #5 up = 230nL/second
- #5 down = 92nL/second

NOTE: When performing fast empty or fast fill, a beep sounds when the plunger reaches the end of travel.


Inject–The aliquot volume is selected using the DIP switches on the right side of the control unit. Then, each time the Inject button is depressed, an audible beep is heard and the selected volume is dispensed. See “Setting the Aliquot Volume and Injection Speed” on page 10.

Plunger in “HOME” Position

When you plug in the **Nanoliter 2000**, it assumes 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 it is not in use. To reset the plunger to this position, push the Fill button to retract or the Empty button to extend the plunger and simultaneously hold the Inject button. When in the home position, release both buttons to reset the unit.

OPERATING INSTRUCTIONS

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.

 **CAUTION:** 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

Ideally, the tip size should be pulled to 10-30 μ m. The capillary glass provided has a softening point of 780°C. Many researchers pull the tips smaller than required and then (using forceps) break them off at the desired size. The sharp broken edge works well to pierce the cell membrane.

Once the tips are pulled, they are typically “backfilled” with oil 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 the standard collet/O-ring configuration.

Standard Collet/O-ring Configuration

1. Once the micropipette is backfilled, loosen the collet (**Fig. 5**). 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.
2. Push the micropipette onto the wire plunger. As you push the tip on, feel it go through the large O-ring and seat in the white spacer. See **Fig. 5** for the proper configuration of the O-rings and the white spacer. It is absolutely essential that these components are properly configured.

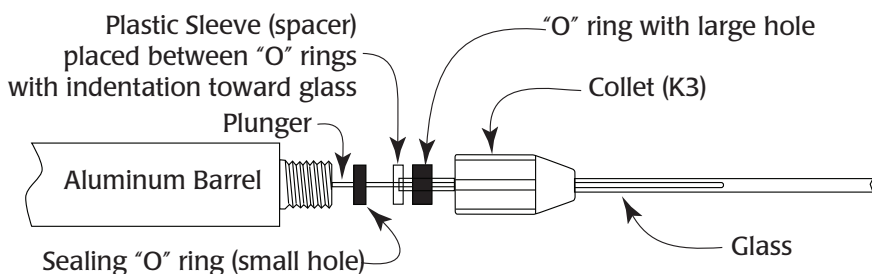


Fig. 5—Standard configuration for installing the micropipette

3. Once positioned, tighten the collet securely. Give the micropipette a pull to confirm it is securely mounted.

NOTE: The white spacer has one flat side and one side with a recess machined around the hole. This recess receives the back end of the pipette and protects the plunger sealing O-ring from damage.

Micropipette Filling Techniques

There are various methods for loading liquid samples into micropipettes for microinjection. A pulled micropipette has a back side and a front side.

- Back side—the non-pulled end, which has the relatively large opening that allows for rapid bulk filling
- Front side—the pulled end of the micropipette, the sharp side

The filling method of choice is usually dictated by experimental requirements and is chosen at your discretion.

Back Filling

The term “back filling” refers to the process of filling the pipette from the large, non-pulled end. Back filling offers a great advantage when performing a bulk fill, because the large opening allows you to use a syringe and MicroFil flexible needles. (See “Optional Accessories” on page 12.) The bulk filling process occurs rapidly with little mess.

1. Attach a MicroFil needle to a syringe, immerse the tip of the needle into the filling liquid and use the syringe to pull the fluid into the MicroFil needle.
2. Place the MicroFil needle into the micropipette from the back side so that the needle’s tip is pushed all the way into the front part of the pipette near the pulled end.

3. Eject the liquid into the micropipette and slowly withdraw the MicroFil needle as the liquid fills the micropipette.

Front Filling

“Front filling” is the term to describe filling a micropipette through the small, pulled front end of the pipette. Front filling is achieved by creating a vacuum at the back end of the pipette that pulls the filling liquid into the micropipette. Once the microliter is installed on the **Nanoliter 2000**, it is possible to front fill. Front filling is advantageous for small volumes or expensive samples. Because of the small opening in the front, it is not preferred for bulk filling of large volumes.

Typical Technique

The technique described below is a typical process for filling a micropipette with a small volume for microinjection.

1. Backfill the micropipette with mineral oil.
2. Use the **Nanoliter 2000** controller to eject the plunger from the home position. This is necessary in order to allow the plunger to move backwards to create a vacuum for front filling.
3. **Fig. 5** and **Fig. 6** show the assembly of the micropipette to the **Nanoliter 2000**.
4. Once the micropipette is installed, submerge the micropipette tip into the sample.
5. Press the Fill button on the **Nanoliter 2000** controller to pull the sample into the tip of the micropipette.

Injecting

Injection aliquot volumes are determined by the position of the DIP switches, located on the right side of the **Nanoliter 2000**. DIP switches #1–4 control the aliquot volume (**Fig. 6**). DIP switch #5 selects the fast injection rate and fill rates. See “Setting the Aliquot Volume and Injection Speed” on page 10.

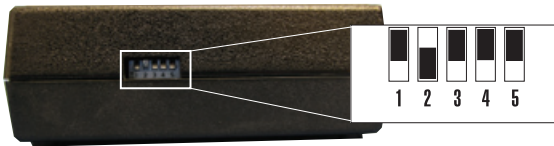


Fig. 6—The DIP switches, located on the right side of the control unit, determine the injection volumes. See Table 1.

Once the desired aliquot volume is selected, then each time the Inject button is depressed, an audible beep is heard and the aliquot is dispensed.

Pressing Inject before the first aliquot is dispensed will not produce a second injection. Wait for the beep (which indicates the injection is complete) before beginning a second injection.

Setting the Aliquot Volume and Injection Speed

Aliquot volumes and injection speeds are controlled by the positions of the DIP switches located on the side of the control box. Table 1 lists the DIP switch settings to use for DIP switches 1–4 to select the various aliquot volumes. (“U” = up, “D” = down). DIP switch 5 determines the fast injection and fill rates.

- Up (Fast) = 46nL/s
- Down (slow) = 23nL/s

TIP: To set the DIP switches, use a ball point pen or a jeweler’s flat head screw driver.

NOTE: DIP switch settings for the **Nanoliter 2000** are completely different from the previous model, the Nanoliter Injector A203.

Table 1: DIP Switch Settings

Volume (nL)	DIP Switch Settings			
	1	2	3	4
2.3	U	U	U	U
4.6	D	U	U	U
9.2	U	D	U	U
13.8	D	D	U	U
18.4	U	U	D	U
23.0	D	U	D	U
27.6	U	D	D	U
32.2	D	D	D	U
36.8	U	U	U	D
41.4	D	U	U	D
46.0	U	D	U	D
50.6	D	D	U	D
55.2	U	U	D	D
59.8	D	U	D	D
64.4	U	D	D	D
69.0	D	D	D	D

MAINTENANCE

Replacing O-Rings

After a period of time or heavy use, you may observe some leakage around the seals. To correct this, replace the O-rings. Replacement O-rings are included with your **Nanoliter 2000**. Additional O-rings (WPI #5340) may also be ordered. See “Securing the Micropipette to the Injector” on page 7. **Fig. 5** shows the proper installation configurations. Correct orientation of the O-rings is critical for proper operation.

Replacing the Wire Plunger

Refer to **Fig. 4** on page 5 to see the exploded diagram of the Nanoliter 2000 injection handle.

1. Verify that the plunger is in the home position.
2. Unscrew the collet one turn and remove the O-rings.
3. Hold the motor housing with one hand while loosening the aluminum barrel. To remove it, unscrew it counter-clockwise with your other hand. This exposes a brass fitting with two Allen screws on opposite sides of the brass fitting and the clear stop.



CAUTION: Do NOT disassemble the Motor Housing. Doing so may damage the plunger drive assembly.

4. Using the hex wrench provided with the replacement plunger, loosen the two set screws.
5. Remove the plunger.
6. Insert the new plunger and tighten the set screws snugly. Do NOT overtighten. Set screws must be flush with the edge or the aluminum barrel will not seat properly.
NOTE: The pointed end of the plunger is normally oriented out, but it can be inserted either way.
7. Slide the clear stop over the plunger so that the narrow side is toward the brass fitting. This stop prevents the plunger from advancing too far into the aluminum barrel.
8. Slide the aluminum barrel over the tip of the plunger. Hold the handle so the plunger is point straight up. Gently shake the unit until the barrel hole lines up with the plunger and slides down over it.



CAUTION: DO NOT FORCE THE ALUMINUM BARREL OVER THE PLUNGER. The plunger is easily bent.

-
9. Tighten the aluminum barrel in place.
 10. Carefully position the washers and collet on the tip of the plunger. Make sure the washers are assembled in the proper order. Do not puncture new holes in the washers or fluid will leak through them.
 11. Tighten the collet.

Cleaning Recommendations

The injector can be cleaned by removing the collet, O-rings and spacers and wiping them with alcohol.



CAUTION: Do NOT soak the **Nanoliter 2000** parts in liquid. Do NOT autoclave the **Nanoliter 2000**.

The control box may be cleaned by wiping it with a clean, damp cloth.

OPTIONAL ACCESSORIES

Table 2: Accessories

Part Number	Description
4878	Replacement 3.5-in. glass capillaries (300)
4879	Replacement 7-in. glass capillaries (300)
5340	Spare Parts Kit (includes MicroFil™ MF34G, displacement plunger, five O-ring sets)
13142	Footswitch for Nanoliter 2000
15867	Footswitch for Micro4 Controller
300033	Adapter cable, Nanoliter 2000 to Micro4
500299	Replacement Plungers, 5-pack
500778	Replacement Nanoliter Injector Universal Adapter
MF34G-5	MicroFil, 34g (pkg. of 5)
MICRO4	Micro4 Controller, 4-Channel
TIP10XV119	Pre-pulled Micropipettes for Nanoliter Injector (10)

TROUBLESHOOTING

Issue	Possible Cause	Solution
Glass pipette pushes off the pump tip	The front O-ring seal is worn out	Replace the O-ring seals. See “Replacing O-Rings” on page 11.
	The wire plunger is bent enough to add friction to the glass, which pushes it off	If it is bent, replace the wire plunger. See “Replacing the Wire Plunger” on page 11.
	O-ring seal is soaked in oil and tightening the collet is not enough to secure the glass	Wash the front O-ring in alcohol before placing it on the glass pipette shank.
Plunger doesn't retract completely	Home position is set incorrectly	See “Plunger in “HOME” Position” on page 6 for instruction on resetting the home position.

NOTE: If you have a problem/issue with that falls outside the definitions of this troubleshooting section, contact the WPI Technical Support team at 941.371.1003 or technicalsupport@wpiinc.com.

SPECIFICATIONS

The **Nanoliter 2000** conforms to the following specifications:

Injection Volume	Variable in discreet aliquot volumes (See Table 1, page 10)
Remote Control	Yes
Glass OD	1.14mm
Glass ID	0.5mm
Step	12.7 μ m/step
Injection Speed	Slow: 23nL/s
	Fast: 45nL/s
Fill Speed	Slow: 23nL/s
	Fast: 46nL/s
Empty Speed	92nL/s
Smallest Volume	2.3nL
Injections Per Filling, Max.	100 injections
Shipping Weight	3 lb. (1.1kg)

APPENDIX: NANOLITER 2000/MICRO4 VOLUME SETTINGS

When using the **Micro4** to control injections with the **Nanoliter 2000**, take care when entering the injection volume. The **Nanoliter 2000** injector's volume per step is based on the movement of the plunger wire inside a pulled glass pipette. This plunger moves 0.0005" (12.7µm) for each step of the motor. The volume of 2.3nL per step is based on the inside diameter of a 0.5mm pipette and the 12.7µm movement of the plunger wire. As shown in the table below, use Type K when programming the **Micro4** for use with the **Nanoliter 2000**.

NOTE: FOR FULL OPERATING INSTRUCTIONS FOR THE MICRO4 AS IT RELATES TO THE NANOLITER 2000 OPERATION, REFER TO THE UMP3 INSTRUCTION MANUAL.

Table 3: Micro4 Volume Setting Table

Type	Syringe Volume	Scale Length	ID (mm)	nL / step REV K	Max. Rate nL/s	Max. Rate Microstep Mode nL/s
A	0.5µL	54.1mm	0.1085	0.0294	20	1
B	1.0µL	54.1mm	0.1534	0.0587	40	2
C	5µL	54.1mm	0.343	0.2934	202	14
D	10µL	54.1mm	0.485	0.5868	451	29
E	25µL	60mm	0.73	1.329	1022	66
F	50µL	60mm	1.03	2.646	2035	132
G	100µL	60mm	1.46	5.315	4088	265
H	250µL	60mm	2.3	13.191	9999	659
I	500µL	60mm	3.26	26.501	9999	1325
J	1000µL		4.61	52.995	9999	2649
K	Nanoliter 2000		0.48 plunger in 0.50 glass	2.3 nL /step (0.0005" step)	884	115
L	10µL	60mm	0.4607	0.5293	407	29
M N O P	User Defined			See page 14	custom rate*	
**	ILS005		0.4856	0.5880 compensates for length as TYPE M		

*The rate maximum is calculated internally and is determined by the nL/step value times 10000 and divided by 13. The microstepping maximum rate is nL/step*10000/200.

A203XVY (original **Nanoliter**) uses type M, N, O or P with a value=1.15nL/step.

Setting the Correct Volume on the Micro4

Since the aliquot volume per step is 2.3nL, the volume to be entered on the **Micro4** touch panel must be a multiple of 2.3. The normal injection error is < 2%.

TECHNICAL NOTE: For example, if you wish to inject 100nL, the setting on the **Micro4** panel will be calculated as $100/2.3$ or 43.47 steps. The motor can only step in whole numbers, so the volume must be adjusted, up or down, to the nearest whole step value. Increasing to 44 steps gives a volume of 101.2nL ($44 * 2.3=101.2$). Decreasing to 43 steps gives a volume of 98.9nL ($43 * 2.3=98.9$). One of these two volumes should be used to insure a proper injection. By default, if you leave the value on the **Micro4** at 100nL, 98.9nL is injected.

Difficulty can arise when the volume value is half or more of the next 2.3nL step. For example, setting the **Micro4** for an injection of 10nL results in a theoretical injection volume of 9.2nL, produced by 4 whole steps of the injector. Five whole steps results in 11.5nL. Entering a value of 11.0nL in the controller, however, generates a spurious value in the **Micro4** display (10.35nL), but the actual injection is still only 9.2nL. To avoid this error, enter only multiples of 2.3nL when calculating required volumes.



Fig. 8—Micro4- an optional microprocessor-based controller, can provide an intelligent and easy-to-use interface for up to four Nanoliter 2000 injections.



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DECLARATION OF CONFORMITY



WORLD PRECISION INSTRUMENTS, INC.

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DECLARATION OF CONFORMITY

We: World Precision Instruments, Inc.
175 Sarasota Center Boulevard
Sarasota FL 34240-9258
USA

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.

Issued on: 18th February 2000


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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 30 days* 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 ten (10) days after receipt of shipment. Claims for lost shipments must be made within thirty (30) days of receipt of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim is settled. 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

Do not return any goods to us without obtaining prior approval and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. Goods accepted for restocking will be exchanged or credited to your WPI account. Goods returned which were ordered by customers in error are subject to a 25% restocking charge. Equipment which was built as a special order cannot be returned.

Repairs

Contact our Customer Service Department for assistance in the repair of apparatus. Do not return goods until instructions have been received. Returned items must be securely packed to prevent further damage in transit. The Customer is responsible for paying shipping expenses, including adequate insurance on all items returned for repairs. Identification of the item(s) by model number, name, as well as complete description of the difficulties experienced should be written on the repair purchase order and on a tag attached to the item.

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



World Precision Instruments, Inc.

USA

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