SpectroPipetter SPT & SPT-2

Miniature sample cell for microliter sampling

INSTRUCTION MANUAL

Serial No._____

042302

World Precision Instruments, Inc.

Contents

General Warnings and Cautions

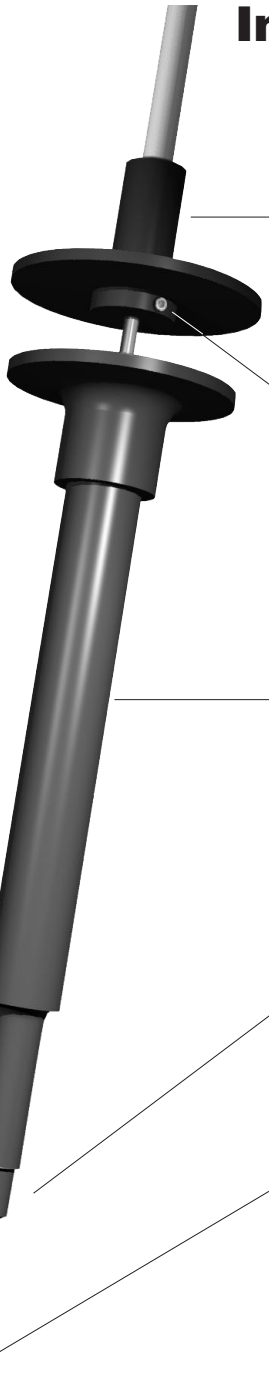
CAUTION: Do not try to dissamble the **SpectroPipetter** (**SPT**, **SPT-2)**. Disassembly by untrained personnel will damage the unit and invalidate the warranty.

CAUTION: Do not apply any mechanical force to the tip of the SpectroPipetter. Store it in the stand provided or in a secure place with the protective tip in place.

CAUTION: Clean the SpectroPipetter only with diluted **Cleaning Solution Concentrate** (**#15807**). Do not use concentrated acids or bases; these solutions may change the optical characteristics of the waveguide.

NOTE: Dilute hydrochloric acid (5-10% w/w) can be used to enhance the efficiency in the detection range below 300 nm if measurement has fallen by more than 30% of initial readings. Immediately **flush** with cleaning solution and then with distilled water.

Instrument Description



Plunger handle — As in a standard pipetter, pushing and releasing the plunger handle drives a piston that draws in and expels fluid from the sample cell.

Set screw — Two set screws on the plunger handle control the zero position of the plunger, and holds the plunger handle to the main body. It is used mainly for calibration.

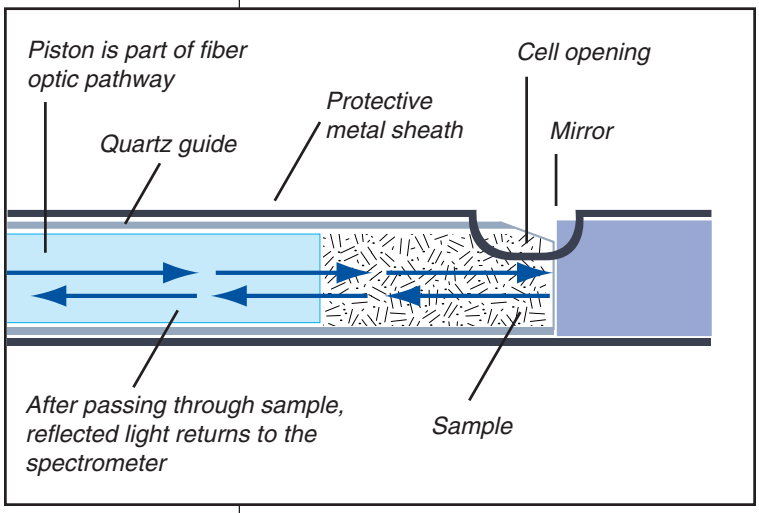
Main body — Serves as a base for the sample cell and plunger and acts as the primary handle for the instrument.

Tip retainer — Holds the sample cell in place.

Sample cell — Sample fluid is drawn into and expelled from this microchamber by means of a tiny side opening. A transparent sleeve (not shown here) protects the sample cell from accidental breakage when not in use. See tip diagram, page 2.

WPI's **SpectroPipetter** is a spectrophotometry sampling device for spectral analysis on fluid samples of less than two microliters. In its outward design, the SpectroPipetter resembles a standard laboratory pipetter. Fluid is taken up and expelled by means of a manual plunger, just as in a standard pipetter. The plunger is made of a bundle of fiber optics protected by stainless steel tubing. In addition to moving the sample in and out, it delivers and collects the light used for absorbance measurements. The SpectroPipetter is connected to a spectrophotometer by a fiber-optic cable, which passes through the pipetter's core and carries light to the delicate tip module. The tip module (see diagram), which resembles a standard pipette tip, is actually a tiny photo-optic component which serves as the sample cell in place of a cuvette. The fiber optic cable from the pipette splits into two cables each with a SMA connector termination. The SPT cable labeled with "Launch" is a 400 micron core fiber [a bundle of three 200 micron fibers in the SPT-2] that should be connected to the light source(s). The cable labeled with "Return" is a 100 micron core fiber [a 200 micron fiber in the SPT-2] that should be connected to the spectrometer. During the measurement, light is launched into the "Launch" fiber and then transmitted to the pipetter where it passes through fluid held in the sample cell, strikes a mirror, and reflects back to the "Return" fiber. Returning light is routed to a fiber optic spectrometer.

SpectroPipetter tip



In addition to the standard accessories, the optional **Ultrasonic Cleaning Kit** (WPI part **#UCK**) is highly recommended for keeping the mirror clean and for removing any residual sample fluid or dye from the sample cell. The kit contains an ultrasonic cleaning bath for the tip module; a special stand for placing the SpectroPipetter in the ultrasonic bath; and a bottle of concentrated cleaning solution. The compact ultrasonic bath is specially designed for cleaning the SpectroPipetter. A built-in timer provides 20 seconds' cleaning with a high intensity ultrasonic wave each time it is activated.

Wetting the sample cell for the first time

The SpectroPipetter is shipped dry and the sample cell requires a special wetting procedure before its first use. To wet the sample cell, gently depress the plunger handle with the thumb until the piston just touches the end of the sample cell. Do not use force. When you feel resistance, the piston is in contact with the mirror. Immerse the pipette tip in distilled water, and slowly release the plunger. Water enters the sample cell through a side opening near the tip. Using a laboratory tissue, blot the tip end while slowly depressing the plunger to expel the water droplet. Refill and examine as described in "Air bubbles in the sample cell" (page 8). If air bubbles persist, re-insert the tip into water while holding the plunger handle depressed and lightly tap the main body to free bubbles clinging to the inner surface, then slowly release the plunger.

Removing the air completely may be difficult at first, and these tactics may need to be repeated several times. After this first wetting procedure, however, you will find less trapped air when filling the tip.

Once the inner surface of the sample cell has been wetted and all air has been expelled, keep the sample cell wet at all times by storing the pipetter in its stand with its tip immersed in distilled water. If left exposed to air, the sample cell will dry out very quickly, leaving a film on the mirror. This film, which will cause light scattering, impairs the instrument's sensitivity. A dry film such as this is difficult to re-dissolve and may require extended cleaning in an ultrasonic cleaning bath. It is important, therefore, to replace the pipetter in its stand (with the tip submerged in a vial of distilled water or sample solution) as soon as one spectrum has been taken, and to keep the sample cell continuously filled between one spectrum and the next.

NOTE: If you plan to use the instrument over a period of several days, it is good practice to keep the tip wet throughout this time to avoid repeatedly dealing with air bubbles.

Proper handling of the pipetter

The sample cell and piston, which make micro-volume measurements possible, form the heart of SpectroPipetter. Although simple in appearance, they are delicate optical components and should be handled carefully. Replacing these precision components is very costly and will not be covered by WPI warranty if damage occurs through abuse or improper usage. *The following precautions should be observed:*

- The SpectroPipetter is shipped with a clear plastic protective sleeve. Remove the sleeve and keep the pipetter in its stand with tip immersed in distilled water between samples and when not in use. Save the protective sleeve — you will need it when transporting or storing the SpectroPipetter.
- After initial wetting, keep the sample cell wet.
- Do not disassemble the pipetter or tamper with its adjustments. Contact WPI if you are experiencing problems with the device.
- Avoid dropping or bumping the pipetter. Although it can withstand a light touch, bumping the sample cell or dropping the instrument onto a hard surface will cause permanent damage.
- Never force the piston. When filling or emptying the sample cell, depress the plunger only until you feel resistance, indicating that the piston has touched the mirror at the tip end. Excess force can crack the piston, scratch the mirror, or break the tip.
- Use safe, appropriate cleaning solutions. Cleaning solutions safe to use with SpectroPipetter include distilled water, detergent, alcohol, acetonitrile, and properly diluted WPI **Cleaning Solution Concentrate** (in the optional **Ultrasonic Cleaning Kit**, part **#15807**). See below for further precautions.
- Rinse the sample cell in distilled water between one sample solution and the next. This helps to reduce contamination.
- Use the ultrasonic cleaning bath supplied with your system to remove any residue that does not rinse out easily with cleaning solution. To maintain sensitivity and consistency and to prevent residue accumulation in the sample cell, clean the pipetter tip in the ultrasonic cleaning bath before switching between different solutions. See also the manual supplied with your ultrasonic cleaner.

- Use the ultrasonic cleaning bath supplied with your system to remove any residue whenever cross-contamination must be strictly avoided.
- Never fill or immerse the sample cell in strong acid or strong alkaline solutions. These will damage the metallic coating of the piston.
- Never fill the sample cell with solutions containing solid particles. Solids may be very difficult to remove and may become trapped in the sample cell. Solids entering the sample cell may abrade the optical surface and permanently impair sensitivity. Solutions should be carefully filtered before use in SpectroPipetter.
- *Never fill the sample cell with solvents containing fluoride (such as artificial blood)!* Contact WPI if you have any doubt about how the solvent you plan to use will affect the sample cell.
- Never fill or immerse the sample cell in hydro fluoride acid. This will corrode the optical fiber.

Keeping the tip clean

A small amount of residual fluid remains in the sample cell each time a sample is expelled. This is generally less than 5% by volume and can be detected by checking the absorbance baseline with the sample cell filled with reference solution (*i.e.*, distilled water). A small shift from zero generally indicates residual fluid in the sample cell.

Routine rinsing with distilled water: Since most fluids are easily rinsed away, form the habit of routinely rinsing the sample cell between one spectrum and the next when there is any change in sample fluid. The simplest rinsing method involves immersing the pipetter tip in clean, distilled water and drawing in and expelling water from the sample cell. It is recommended that you repeat this 3-5 times, which takes only a few seconds.

Each rinse with cleaning solution reduces the residue by at least a factor of ten. After five rinses, the residue will be less than 10^{-5} by volume of the sample concentration.

To correct a baseline shift caused by residual fluid, set the spectrometer to scan in repeat mode. Immerse the sample cell in distilled water. Draw in and expel water from the sample cell repeatedly, while monitoring the absorbance baseline on screen. You will notice that the baseline returns to its original level. Once the

baseline shift is corrected, the sample cell is clean and ready to be used for testing another sample.

Using alcohol or acetonitrile as cleaning solutions: Certain residues, of course, cannot be removed easily with distilled water. These primarily include molecules that are neutral in charge and/or are only slightly polarized, as, for example, Brilliant Green dye. The best way to remove residues of this type is to substitute alcohol or acetonitrile for distilled water. To do this, expel the sample fluid, then draw in and expel alcohol or acetonitrile. Repeat from three to five times, then use the on-screen display to recheck the baseline in reference solution.

Cleaning organic solvents: If the SpectroPipetter is used with non-water soluble organic solvents such as acetone, THF, benzene, etc., a thin layer of solvent (or chemicals that dissolved in the solvent) will remain on the surface of the cell when it is used later for aqueous solution sample. That thin layer can severely reduce the transmission of the light especially in the ultraviolet range. It may also affect the baseline stability of the spectrum. To remove this layer, use the ultrasonic cleaner to clean the pipette in an organic solvent that can dissolve both the thin layer and the water. Acetonitrile, DMF and DMSO are good solvents for this purpose. Another cleaning method is to gradually increase the polarity of the washing solvent in several steps.

When to use the ultrasonic bath: Sample residue that persists after rinsing can be removed by using the ultrasonic bath. This device sends ultrasonic waves through the pool of cleaning solution, causing implosion of microscopic bubbles in the cleaning solution. The collapsing bubbles create a vacuum that draws contaminants away from the surfaces being cleaned. Residues are removed from the mirror and inner surface of the sample cell. The ultrasonic bath should be used whenever a residue cannot be removed by simple rinsing, or whenever cross-contamination must be eliminated completely.

Any of the solvents mentioned previously (distilled water, detergent, alcohol, acetonitrile, or diluted WPI ultrasonic cleaning solution) can be used with an ultrasonic cleaner. We have found that properly diluted WPI Cleaning Solution Concentrate is effective for most applications.

Setting up an ultrasonic bath: Put the diluted cleaning solution into a 20 mL glass scintillation vial, and set the vial in the ultrasonic tank which is partially filled with distilled water. (Follow manufacturer's directions for diluting commercial solutions.)

In this way, you can quickly change cleaning solution by switching one vial with another if the first should become contaminated or prove to be unsuitable.

Using the ultrasonic bath: To use the ultrasonic cleaner, turn it on (*i.e.*, pulsing) and set spectrometer to scan in repeat mode. Immerse the sample cell into the cleaning solution, wait a moment, and then depress and release the plunger several times. The spectrum will appear quite erratic. This is because the ultrasonic cleaner creates air bubbles which cause instability in the SpectroPipetter. After a few seconds, turn off the ultrasonic bath and draw in cleaning solution. Examine the spectrum. If it has reached its previous baseline level, the tip is clean and ready to be used to measure another sample. If it has not reached its previous baseline level, repeat the procedure. The tip can usually be cleaned in less than 10 seconds. However, at times, removing a residue may take several minutes.

Note: Depressing the reset button activates the ultrasonic cleaner for about 20 seconds before it automatically shuts off.

Maintaining stability

The SpectroPipetter's unique optical system entails challenges to stability that are very different from those encountered in conventional spectrophotometry. Controlling the baseline shift is of first importance to SpectroPipetter users. Instability arising from conditions within the pipetter can be pronounced if the instrument is poorly understood or improperly used.

Understanding the various factors contributing to baseline shift can help you to avoid them for the most part and can help you to diagnose and correct any problems that do occur. Very often, multiple factors are involved. To aid you in troubleshooting stability problems, we have included the following discussion of possible causes. This list of contributing factors is not all-inclusive, however. In all likelihood, as applications for SpectroPipetter continue to be developed, more factors contributing to baseline shift will be documented.

Air bubbles in the sample cell: The main characteristic of air bubble-induced drift is that it is unstable — the baseline shifts by different amounts each time the plunger is pumped. Larger air bubbles can cause a baseline shift of more than 1 AU. Large air bubbles can be removed by tapping the body of the pipette while lightly depressing the plunger against the mirror, when the sample cell is

submerged in liquid. This generally shakes the bubble free of the tip.

Smaller bubbles affect stability less than larger ones but are considerably more difficult to detect and remove. On screen, the air bubble will reveal its presence by scattering the bright light during the scan and evenly shifting the baseline up. To remove small air bubbles, expel all of the sample. Dip the tip into reference solution and pump several times. If air is still present, remove the tip from the solution, depress the plunger, and dry the tip by blotting the opening with a laboratory tissue. Before releasing the plunger, insert the tip in the reference solution. Then carefully refill the sample cell with reference solution very slowly. If irregular baseline drift still occurs when the pipette is pumped, air bubbles are probably still present, and this procedure may need to be repeated several times. If none of the above methods are working, clean the tip in the ultrasonic bath with the ultrasonic cleaning solution and refill the tip with reference solution. The surfactant in the ultrasonic cleaning solution will help to remove the air bubbles.

The best way to avoid air bubble-induced drift is to prevent air from getting into the sample cell. First, the sample cell should be wetted in reference solution and any trapped air bubbles should be removed. Second, the sample should be introduced into the sample cell by slowly releasing the plunger. Third, if the solution contains small air bubbles, degas it before filling the sample cell.

Avoid baseline shift due to cable movement and bending: Although the SpectroPipetter uses special optic fibers that are the least sensitive to movement and bending, the light transmission of the cable may still be affected by a position change of the fiber optic cable. To reduce the problem, **1** lay the cable as straight as possible, avoiding sharp bends, and **2** take the reference measurement at the same position as the sample measurement.

Differences in refractive index or light scattering properties: If the sample has a different refractive index than the reference solution (*e.g.*, a different salt concentration), the baseline will shift slightly. This shift is usually flat and small. Unlike air bubble-induced instability, the baseline shift is sample dependent and reproducible. The best way to avoid refractive index baseline shift is to use a reference solution that has the same index of refraction as the sample solution.

If the sample contains molecules or substances comparable in size to the wavelength of light (for example, large DNA molecules, membrane fragments, or even air bubbles), light scattering effects can occur, causing a baseline shift. This

scattering is wavelength dependent, shorter wavelengths shift more than the longer wavelengths.

The sample cell is contaminated: Baseline shifts can be caused by microorganisms, solid particles, or dried sample residues in the sample cell. Generally, the mirror is much more easily contaminated than the cell wall. This contamination usually causes a stable shift or steady drift towards lower sensitivity, which reveals itself as a steady increase in the absorption baseline. This type of shift can be removed by a 20-second ultrasonic cleaning.

The more contaminated the sample cell, the worse the drift. This is because contamination tends to trap more contamination. It is important therefore to keep the sample cell clean. Using the ultrasonic bath before taking reference spectra and after measuring samples (especially those which contain substances that easily stick to the sample cell) is the most effective way to keep the sample cell clean. A change in baseline (away from zero) following ultrasonic cleaning is a good criteria for judging whether the sample cell is clean. When the sample cell is clean, the baseline will remain unchanged after ultrasonic cleaning.

The plunger is damaged: Under normal use, the plunger will last for a very long time. However, depressing the plunger with excess force may damage the piston tip. In this case, the instrument may still be functional, but it will likely have reduced sensitivity that cannot be recovered by any correction method mentioned above. Please call WPI if you suspect the piston is damaged.

Scattering for UV measurements

When taking absorbance measurements of extremely low concentration, low volume samples, scattering may become an issue. This is often the case for UV analysis of small DNA or protein samples and can give the appearance of increased absorbance due to the loss of light. One likely cause of scattering is small pockets of air in the solution caused by shaking. Scattering may be suspected if an absorbance spectrum tends to increase without peaking as the wavelength decreases (higher in the blue region than in the red). Normally, the effective change in absorbance due to scattering is much smaller than the absorbance of the sample. However, at very low concentrations (0.5 micrograms/mL of DNA for example), the effect may be equal in magnitude to the absorbance of the sample (on the order of 0.01 AU). To reduce scattering due to tiny air bubbles in your solution, do not shake low concentration samples. Use a clean, dry

pipette to mix the solution by gently drawing up and releasing it five to ten times. If air bubble scattering is still present, running the solution in a centrifuge for a couple of minutes will usually help to pull some of the air out and reduce the problem.

Sterilization

The SpectroPipetter may be sterilized by Et-OH, alcohol or a bactericide (for example, Cidex or Sporicidin). It should not be sterilized by heat or Gamma radiation.

Storage

Store the SpectroPipetter in the special stand provided. It is advisable to keep this instrument in a location with as little congestion as possible to minimize the chance for any damage to occur.

Keep the protective tip in place with SpectroPipetter is not in use.

It is good practice to keep the tip wet. Immerse in a vial of distilled water.
NOTE: Do *not* store the tip assembly in the ultrasonic cleaning solution.

Troubleshooting

The SpectroPipetter is a highly sensitive device. It is extremely important to keep it clean. This is especially important in the ultraviolet range, where unexpected results may often be produced by contamination of the experimental solution. The high sensitivity of the SpectroPipetter may create some problems that can be easily overcome with care and forethought – the user may need to develop new skills in handling both the equipment and samples being examined.

Typical Contamination Effects

Phenomena: Transmission in the UV and visible ranges becomes low or very unstable.

> **Possible cause:** A contamination layer (a biofilm) sticking to the surface of the mirror inside the tip; or a particle trapped inside the tip assembly.
>
> **Solution:** Place the tip assembly into a vial of 5-10% w/w hydrochloric acid solution and run the ultrasonic cleaner for a minimum of 30 seconds up to two minutes. Immediately flush the tip with distilled water. Follow with another cycle if light levels are not up to initial scan levels.

Phenomena: Transmission of UV, less than 300 nm, is low. Visible range is OK and stable.

> **Possible cause:** Optical fibers are coated with a thin film of metal corrosion.
>
> **Solution:** Using a fiber optic swab, clean the launch and return SMA connectors along with external SMA connections to the spectrometer.

Specifications

	SPT	SPT-2
Wavelength Range (with **TIDAS II**)	230 - 1000 nm	230 - 1000 nm
Sample Volume	2 microliter	2 microliter
Light Path Length Of Cell	1.0 cm	1.0 cm
Temperature Range	4 to 99 °C	4 to 99 °C
Optical Fiber Length	1.3 m	1.3 m
Core Diameter of Launch Fiber(s)	400 µm	200 µm bundle (3)
Core Diameter of Return Fiber	100 µm	200 µm

Accessories and Kits

15807 Cleaning Solution Concentrate (125 mL)

UCK Ultrasonic Cleaning Kit

References

S.X. Liu, J.P. Fabisiak, V.A. Tyurin, G.G. Borisenko, B.R. Pitt, J.S. Lazo, V.E. Kagan, “Reconstitution of Apo-Superoxide Dismutase by Nitric Oxide-Induced Copper Transfer from Metallothioneins”, *Chemical Research in Toxicology*, 2000.

S. Aleman, et al., “Long-term effects of antiretroviral combination therapy on HIV type 1 DNA levels” (1999) *Aids Research And Human Retroviruses* 15: 14.

T.M. Redmond, S. Yu, E. Lee, D. Bok, D. Hamasaki, N. Chen, P. Goletz, J.X. Ma, R.K. Crouch, K. Pfeifer, “Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle”, *Nature Genetics*, vol. 20, (1998), 344-351.

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.

• Always refer to the RMA# when contacting WPI to obtain a status of your returned item.

• For any other issues regarding a claim or return, please contact the RMA department

Warning: This equipment is not designed or intended for use on humans.

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

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