



WORLD
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INSTRUCTION MANUAL

ISO-H₂S-2

Hydrogen Sulfide Sensor for use with WPI Analyzers

Serial No. _____

www.wpiinc.com

030316

Other WPI Favorites

Free Radical Analyzer

- Real-time detection using electrochemical microsensors
- Measure nitric oxide from $< 0.3 \text{ nM}$ to $100\mu\text{M}$.
- Measure hydrogen peroxide $< 10 \text{ nM}$ to 100mM
- Measure hydrogen sulfide
- Measure glucose
- Measure oxygen from 0.1% to 100%
- Integrated system includes one temperature sensor, your choice of two additional sensors, and a start-up kit
- Current measurement range from 300 fA to $10 \mu\text{A}$ (four ranges) permits wide dynamic range for detection.
- Wide bandwidth allows recording of fast events
- Isolated architecture allows Lab-Trax interface to simultaneously measure free radical and independent analog data (i.e., ECG, BP, etc.) data on any channel.



MACRO SENSORS

SPECIES	Carbon Monoxide	Nitric Oxide	Hydrogen Peroxide	Oxygen	Hydrogen Sulfide
Order Number	ISO-COP-2	ISO-NOP	ISO-HPO-2	ISO-OXY-2	ISO-H2S-2
Available Diameters	2 mm	2 mm	2 mm	2 mm	2 mm
Response Time	$< 10 \text{ sec}$	$< 5 \text{ sec}$	$< 5 \text{ sec}$	$< 10 \text{ sec}$	$< 5 \text{ sec}$
Detection Limit/Range	10nM to $10\mu\text{M}$	1 NM to $40\mu\text{M}^*$	$< 100\text{nM}$ to $100\mu\text{M}$	0.1% - 100%	$< 5\text{nM}$ - $100\mu\text{M}$
Sensitivity	$\sim 0.5 \text{ pA/nM}$	$\leq 2 \text{ pA/nM}$	$8 \text{ pA}/\mu\text{M}$	$0.3\text{-}0.6\text{nA}/\%$	2 pA/nM
Drift	$< 1\text{pA/min}$	$< 1\text{pA/min}$	0.1pA/min	$< 1\%/min$	
Temperature Dependent	Yes	Yes	Yes	Yes	Yes
Physiological Interference	nitric oxide	NaNO_2 (10^{-6} or better)	None	None	None
Replacement Sleeves (pkg of 4)	#95620	#5436	#600012	#5378	#600016
Filling Solution	#95611	#7325	#100042	#7326	#100084
Start-up Kit	#95699	#5435	#600011	#5377	#600015

**Higher detection limit available on request — call for custom pricing.*

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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—The ISO-H2S-2 sensor works with WPI free radical analyzers to monitor hydrogen sulfide.

INTRODUCTION

The basic structure of the **ISO-H2S-2** sensor is shown in Fig. 2. It consists of an internal H_2S -sensing working/reference electrode combination. This electrode fits inside a disposable protective stainless steel sleeve (WPI #**600016**) which must contain fresh electrolyte (WPI #**100084**) and is separated from the external environment by a gas-permeable polymeric membrane covering the end of the sleeve. The other end of the sleeve is flanged. The locking cap is used to attach the sleeve to the probe handle.

Notes and Warnings

NOTE: The sensor must be polarized for at least 12 hours in 0.1M PBS solution prior to use.

NOTE: The sensor membrane and membrane coating are extremely delicate. Improper handling will lead to damage of the sensor.

NOTE: For optimal use of each sensor and sensor membrane sleeve, the sensor must be stored properly. See "Maintenance" on page 7. Pay special attention to the sticker on the box. It says, "THE SENSOR SLEEVE IS FILLED WITH ELECTROLYTE. IF THE SENSOR IS NOT TO BE USED WITHIN ONE WEEK, REMOVE THE SLEEVE, CLEAN THE SENSOR WITH DEIONIZED WATER, AND STORE THE SENSOR DRY."

Parts List

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

- (1) **ISO-H2S-2** H₂S sensor
- (1) Probe Unpacking Instructions (Read this before handling the probe.)
- (1) Spare membrane sleeve
- (1) 1 cc Syringe
- (1) **MF28G67** MicroFil
- (1) **100084** 10mL vial of filling solution
- (1) Instruction Manual
- (1) Sensor Performance Evaluation

Unpacking

Upon receipt of this sensor, 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 15 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 15 of this manual.

INSTRUMENT DESCRIPTION

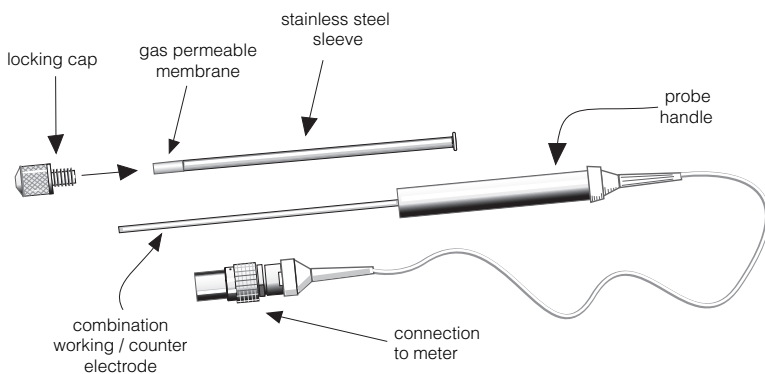


Fig. 2—The locking cap slides over the sensor sleeve. The sleeve is filled with electrolyte and slides over the ISO-H2S-2 electrodes. The locking cap securely attaches the sleeve to the sensor handle.

Gas permeable membrane–The membrane covers the end of the stainless sleeve to separate it from the external environment.

Stainless steel sleeve (WPI#**600016**, pkg. of 4)–The sleeve houses the sensitive electrode pair. The sleeve is flanged to properly connect with the locking cap. It must contain fresh filling solution (WPI#**100084**).

Working/Counter electrode–This is an internal H₂S-sensing pair of working and counter (reference) electrodes.

Locking cap–This cap attaches the sleeve to the probe handle.

When the sensor is assembled (with locking cap and sleeve in place) the internal electrode should press gently against the polymeric membrane, which will be *slightly stretched*. This ensures that the electrolyte diffusion is as thin as possible, minimizing sensor response time.

NOTE: Once a membrane is stretched it is permanently deformed and cannot be reused if the sleeve is removed from the electrode.

Additional membrane sleeves are available in packages of 4 (WPI #**600016**). The **ISO-H2S** start-up kit (WPI #**600015**) also includes replacement membrane sleeves, along with all the accessories to fill them properly with electrolyte solution.

OPERATING INSTRUCTIONS

Polarizing the Sensor

1. Place the sensor in 0.1M PBS solution
2. Plug it into the free radical analyzer.
3. Turn on the free radical analyzer.
4. Set the poise voltage on the free radical analyzer. On the **TBR4100/1025**, set the Probe Select switch to **H₂S** (150mV).
5. Plug in the **ISO-H2S-2** sensor and allow it to polarize up to 12 hours to reach a stable baseline current of <75000pA before it is used for measurement.
6. During initial polarization the current is typically high, but within an hour it will begin to decrease rapidly. In order to observe this phenomenon, start by setting the range to 100nA. After several hours the current will fall. The range should always be set to 100nA, regardless of how low the baseline falls.
7. If the stabilized baseline value exceeds 75000pA, see “Replacing the Membrane Sleeve” on page 8.

NOTE: This polarization procedure assumes the temperature is 25°C. At 37°C the baseline current is higher.

Calibrating the Sensor

Preparing Na₂S Stock Solution

1. Use a 100mL volumetric flask. Dissolve 5mg EDTA in 100mL distilled water.
2. Purge the solution vigorously with argon gas for 15 minutes.
3. Weigh 24.0mg of sodium sulfide (Na₂S .9 H₂O) (reagent plus, 99.99+% from Sigma) and dissolve it in the solution under argon atmospheres.
4. Seal the flask with a rubber stopper. The solution is 1.0mM Na₂S. Store the solution in a dark bottle and refrigerate at 2–8°C.

Calibration Procedure

The sensor should be immersed in PBS and plugged into the free radical analyzer. Set the range to 100nA and the poise to H₂S (150mV). Once the sensor is polarized, it can be calibrated. The following example describes the fundamental concepts behind a standard calibration protocol. Known concentrations of H₂S are generated in PBS by adding a known volume of the Na₂S stock solution.

1. Place a 20mL vial of PBS on the magnetic stirring plate with the small stir bar still inside. Turn on the stirrer so that the bar is stirring at a moderate rate.

NOTE: This rate of the stirrer should NOT be modified during calibration.

NOTE: The calibration should be carried out at the same temperature at which the experimental measurements are to be made. This can be accomplished by placing the vial and stand in a water bath at the appropriate temperature and allowing the temperature of the solution in the vial to equilibrate with the water bath.

2. Immerse the **ISO-H2S-2** sensor in the solution and secure it in an electrode holder such as WPI's **Pro-Guide** (WPI #**47510**, **47520**, **47530**, **47540**) or a micro-manipulator. The sensor tip should be immersed about 2–3mm into the solution. It should not contact the stir bar, which could damage the membrane.
3. Connect the H₂S sensor into a channel of a free radical analyzer. Set the poise voltage to +150mV. For the **Apollo 4000**, this is accomplished in the software. Use the custom option to manually set the voltage to 150mV. For the **Apollo 1000** select poise from the main menu and set it to "internal". Then, adjust the poise voltage using a small screwdriver to turn the "poise adjust" potentiometer on the front panel. For the **TBR4100**, simply set the "probe select" control on the front panel to H₂S. Allow the sensor to polarize for 12 hours.

The quiescent baseline current is an indicator of the health of the sensor. A correctly operating sensor has a baseline current of < 75,000pA.

4. To the vial containing 20mL PBS, sequentially inject three aliquots of Na₂S solution (20μL, 40μL and 80μL). The current output increases after each addition and then plateaus. As soon as it reaches a plateau, inject the next aliquot.

The reaction produces H_2S gas. When H_2S gas passes through the gas permeable membrane, it generates an output current that is measurable, and the results can then be graphed. This corresponds to $1\mu\text{M}$, $2\mu\text{M}$ and $4\mu\text{M}$ final concentrations, respectively.

The output from the **TBR4100/1025** looks similar to the example shown in Fig. 3. Here three sequential additions of H_2S were made to the PBS.

6. Calculate the concentration of H_2S in solution after the each aliquot injection of to 20mL of PBS. The concentration of H_2S produced can be calculated as follows:

$$M_1V_1 = M_2V_2 \text{ where } M=\text{molarity and } V=\text{volume}$$

$$\begin{aligned} \text{1st Aliquot: } & 20\mu\text{L} + 20\text{mL} = 20,020\mu\text{L} \\ & 1000\mu\text{M}(20\mu\text{L}) = M_2(20,020\mu\text{L}) \\ & 20,000\mu\text{M}/20,020 = 0.999\mu\text{M} = 999\text{nM} \end{aligned}$$

$$\begin{aligned} \text{2nd Aliquot: } & 40\mu\text{L} + 20,020\mu\text{L} = 20,060\mu\text{L} \\ & 1000\mu\text{M}(40\mu\text{L}) = M_2(20,060\mu\text{L}) \\ & 40,000\mu\text{M}/20,060 = 1.994\mu\text{M} = 1994\text{nM} \end{aligned}$$

$$\begin{aligned} \text{3rd Aliquot: } & 80\mu\text{L} + 20,060\mu\text{L} = 20,140\mu\text{L} \\ & 1000\mu\text{M}(80\mu\text{L}) = M_2(20,140\mu\text{L}) \\ & 80,000\mu\text{M}/20,140 = 3.972\mu\text{M} = 3972\text{nM} \end{aligned}$$

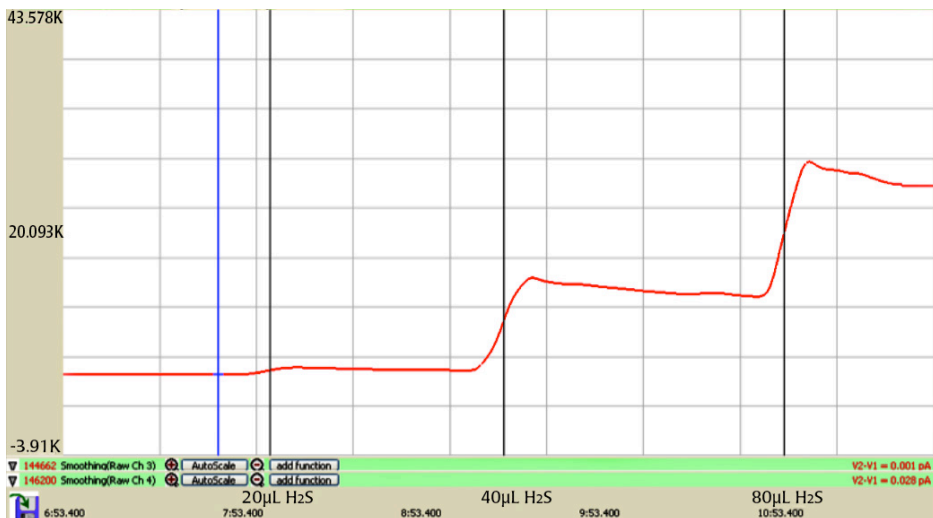


Fig. 3—The calibration curve shows that three aliquots were added.

7. Record the calculated concentration values and the resulting sensor currents in a table similar to the one below. Record the **difference** in current output (pA) generated by the addition of the known quantities of H_2S in a table similar to the one below.

Amount added	[H ₂ S] nM	Response (pA)
20μL	999	3986.76
40μL	1994	6717.67
80μL	3972	10077.81

8. Construct a standard calibration curve using the recorded data. Using a third party spreadsheet with graphing capability like Microsoft® Excel, it is possible to generate a linear regression analysis that will display the equation and the R² coefficient. To do this in Excel, enter the data and generate a "scatter plot" graph. Then, select the line and right click. Choose **Add Trendline**. The **Add Trendline** dialog box appears. On the **Type** tab, select **Linear**, and on the **Options** tab, select the **Display equation on chart** and **Display R-value on chart**.

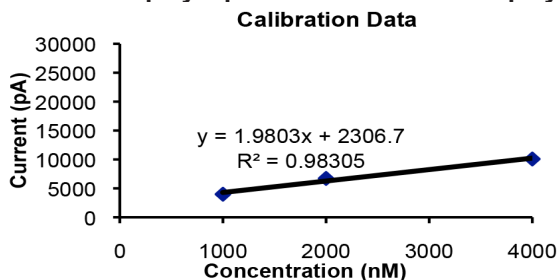


Fig. 4—Calibration Output

The slope of this curve indicates the sensitivity of the sensor. Once the sensitivity of the probe has been determined, the sensor is ready to use experimentally. (In the above example, the sensitivity was 1.98pA/nM.)

NOTE: Because the H₂S sensor is sensitive to salinity and temperature, calibration and subsequent measurements should be performed in solution with the same temperature and salinity.

***The H₂S sensor measures the dissolved H₂S gas, which is only one component of the total sulfide equilibrium system. The total sulfide concentration $[S_2] = [H_2S] + [HS^-] + [S_2^{2-}]$, so the H₂S concentration can be calculated by: $[H_2S] = [Na_2S] / \{ 1 + K_1 / [H^+] + K_1 K_2 / [H^+] \}$

For K₁ and K₂, (pK₁= 6.89, pK₂=19) see

Frank J. Millero, Tinka Plese, Marino Fernandez(1988)

Limnology and Oceanography, 33(2): 269

Giggenbach, W. (1971). *Inorg. Chem.* 10:1333.

Meyer, B.; Ward, K.; Koshlap, K.; & Peter, L. (1983). *Inorganic Chemistry* 22:2345.

Myers, R. J. (1986). *Journal of Chemical Education* 63:687.

MAINTENANCE

Durability and Handling

The sensor is relatively durable, except for the membrane sleeve. Exercise caution when handling any sensor to avoid actions that could damage the sensor tip. Pay particular attention to the sensor membrane, because the membrane is extremely delicate and improper handling will lead to damage. With proper care and by following the instructions, a membrane sleeve should last more than one month.

Refer to the Probe Unpacking Instructions that came with your sensor for handling instructions.



CAUTION: Do NOT scratch the sensor membrane sleeve. Do NOT wipe the *sensor membrane* with anything, even Kimwipes. If necessary, squirt it with distilled water or compressed air.



CAUTION: The sensor membrane is easily punctured if it comes into contact with sharp objects. For example, do NOT let the stir bar come into contact with the sensor membrane.

Cleaning the Membrane

The membrane sleeve itself requires little maintenance. The primary concern is to avoid damage to the membrane and to keep it as clean as possible. After each use, clean the membrane by immersing the tip in distilled water for 20–30 minutes to dissolve salts and remove particles which may have accumulated on the membrane.

If the sensor was used in a protein-rich solution, the membrane tip should first be soaked in a protease solution for several minutes to remove protein build-up, and then in distilled water. Enzymatic detergent (Enzol, WPI #7363) can also be used.

The membrane sleeves can also be sterilized chemically using an appropriate disinfectant (Cidex, WPI #7364).

Accumulated organic matter can be removed by briefly immersing the tip in 0.1M HCl or 0.1M NaOH solution (at times both may be necessary) for 10 seconds.

A good indication of a dirty membrane sleeve is a sluggish response or an unusually low sensitivity. If these problems are not rectified by the above cleaning procedures then replace the membrane sleeve.



CAUTION: The sensor cannot be used in organic solvents.

Replacing the Membrane Sleeve

Even with the best of care and proper maintenance, the membrane sleeve will eventually need to be replaced.

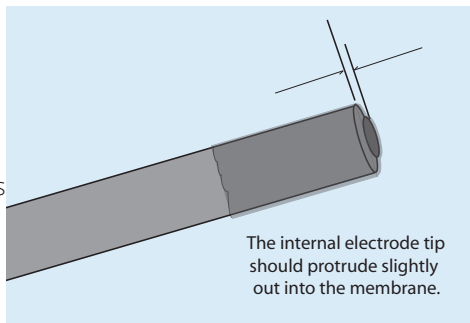
TIP: You can verify that the membrane sleeve on this sensor has not been compromised by lightly touching the sensor tip (membrane) to a Kimwipe. If the Kimwipe has a yellowish spot on it, the yellow filling solution is leaking through the membrane sleeve and the sleeve will need to be replaced.

1. Unscrew the locking cap from the handle.
2. Hold the stainless steel sleeve and remove it and the locking cap from the internal electrode assembly, being careful not to bend the internal electrode assembly when doing so.
3. Rinse the internal electrode with distilled water (particularly the tip) and let it soak for at least 15 minutes. Be careful not to let water get up into the handle.
4. Gently dry the electrode with a soft tissue (Kimwipes). Be sure to dry thoroughly the flat surface at the tip of the electrode. After drying the current should stabilize fairly quickly to a low value (for example, 0 - 20pA). If this occurs, it is a good indication that the electrode is functioning properly.
5. If the electrode is not clean, repeat steps 3 and 4.
6. Remove the locking cap from the old used sleeve, and gently slide it onto the new replacement sleeve. Additional membrane sleeves (WPI#**600016**) may be purchased separately.
7. Dip the internal electrode 1–2cm into the **ISO-H2S** electrolyte (WPI #**100084**) provided in the start-up kit. The current will rise rapidly offscale. Using the MicroFil™ nonmetallic syringe needle (WPI #**MF28G67-5**) and 1mL plastic syringe (included in the Startup kit) inject approximately 100μL of electrolyte directly into the new sleeve, starting about half way down the sleeve and drawing the MicroFil out of the sleeve as it fills. The filling process should be performed slowly enough so as not to create turbulence, which could introduce air bubbles into the electrolyte. The MicroFil (#**MF28G67**) supplied in the startup kit is less than the length of the sleeve, so that it will not puncture the delicate membrane at the tip of the sleeve during injection.

TIP: If air bubbles form in the electrolyte, gently flick or tap the side of the sleeve to remove the bubbles.

8. Slowly and smoothly insert the electrode into the sleeve, and screw the locking cap into the handle. The electrode should be observed to press gently against the membrane (**Fig. 5**).

Fig. 5—(Right) Membrane placement. The internal electrode tip should protrude slightly out into the membrane.



- 9. The current displayed on the meter at this time will be high or offscale.
- 10. Suspend the tip of the newly assembled probe in distilled water.
- 11. After 10-15 minutes the current should no longer be offscale and will gradually decrease with time. It may take several (up to 12) hours for the sensor current to reach a low stable value, at which time it will be ready for use.

TIP: The integrity of the new membrane can be determined by immersing the probe tip into a strong saline solution (1M). If the current increases dramatically or is offscale then the membrane integrity is not good and a new membrane will have to be fitted. Additional **ISO-H2S-2** membranes (packages of 4) are available (WPI #**600016**).

ACCESSORIES

Part Number	Description
5399	T-Adapter Kit (pkg. of 3)
600015	ISO-H2S-2 Startup Kit*
600016	Replacement Sleeves (pkg. of 4)
100084	ISO-H2S-2 Electrolyte Filling Solution (10mL)
7363-4	Enzol - Enzymatic detergent (1 gal.)
MF28G67-5	MicroFil electrolyte filling needle (pkg. of 5)

*The **ISO-H2S-2** start-up kit (WPI #600015) contains everything needed to begin working with this sensor, and the kit is highly recommended for first-time **ISO-H2S** users. The kit includes:

- **600016** - Five additional membrane sleeves (Sold in packages of four sleeves.)
- **100084** - Electrolyte filling solution
- **MF28G67-5** - MicroFil™ electrolyte filling needle
- **3563** - 1mL syringe
- Two sample vials with lids and stoppers
- Potentiometer adjustment tool (Tweaker) for use with the **TBR4100/1025**



Fig. 6—The startup kit is recommended for first time users.

TROUBLESHOOTING

Issue	Possible Cause	Solution
Baseline current is below specified range.	The poise voltage (sensor setting) may be incorrectly set.	Set the poise voltage to 150mV. Set the range at 100nA.
	The sensor may be nearing the end of its usable life.	Perform a standard calibration with at least three points. If the sensor responds linearly within the desired concentration range, it is still useable. The calibration should show that the sensor responds in a linear fashion.
Unstable baseline	If the baseline hasn't stabilized after 12 hours, the polarizing solution may be contaminated.	Prepare fresh polarizing solution. Use 0.1M PBS only. See "Replacing the Membrane Sleeve" on page 8.
	External electrical interferences may be the problem.	Identify and isolate electrical interferences.
Calibration data set is not linear	The dilution factors may be incorrect.	Verify the procedure used.
	Uneven aliquots may have been used.	Check the pipetter calibration.
Sensitivity below range specified	Membrane is old or worn, or electrolyte solution evaporated.	In either case, Replace the membrane sleeve. See "Replacing the Membrane Sleeve" on page 8.

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 sensor conforms to the following specifications:

Outside Diameter	2mm
Response Time	< 10 sec
Detection Llimit/Range	<5nM
Nominal Sensitivity (New sensor)	>1 pA/nM
Poise Voltage	150mV
Typical Quiescent Baseline Current, 25°C	<75000pA
Acceptable Baseline Range	1000-8000pA
Baseline Noise (pA)	<10pA
Polarization Time	12+ hours
Recommended Polarization Solution	0.1M PBS

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DECLARATION OF CONFORMITY

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.*

USA

International Trade Center, 175 Sarasota Center Blvd., Sarasota FL 34240-9258
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