



ISO-H2S-100

Microsensor for H₂S measurement

INSTRUCTION MANUAL

Serial No. _____

020812

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—The ISO-H2S-100 sensor is a platinum wire electrode that functions similar to WPI's ISO-H2S-2 sensor.

INTRODUCTION

The **ISO-H2S-100** is a hydrogen sulfide sensor with a 100 μ m diameter tip. It is a dry microsensors, however, it functions like a traditional **ISO-H2S-2** sensor. The sensor can be ordered in a variety of lengths from 1–5mm. It incorporates WPI's proprietary combination electrode technology in which the hydrogen sulfide-sensing element and separate reference electrode are encased within a single shielded sensor design.

The **ISO-H2S-2** is WPI's original 2mm H₂S sensor, that incorporates a removable, electrolyte-filled membrane sleeve. The new **ISO-H2S-100** offers several advantages:

- It is much easier to use, because requires no sleeves or filling solutions.
- Because the **ISO-H2S-100** is constructed with platinum wire, it is more durable.
- It offers a bigger linear range than the **ISO-H2S-2**, and the range is based on the length of the sensor tip.

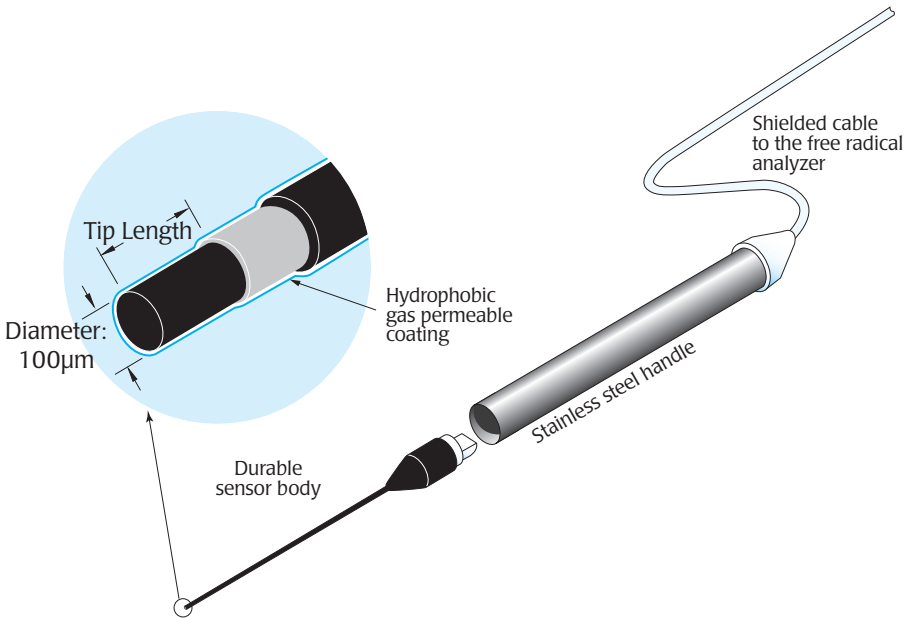


Fig. 2—The ISO-H2S-100 is used like the other microsensors.

Notes and Warnings

The H₂S microsensor is robust, but not indestructible. Exercise caution when handling the sensor to avoid actions that could damage the tip. Do not bring the tip into contact with hard surfaces like stir bars. See "Unpacking" on page 3.



CAUTION: DO NOT EXPOSE SENSOR TO ORGANIC SOLVENTS.

Parts List

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

- (2) ISO-H2S-100 microsensors
- (2) Sensor Performance Evaluations (each sensor is tested individually at WPI)
- (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 11 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 11 of this manual.

OPERATING INSTRUCTIONS

Attaching the Sensor to the Microsensor Handle

Once removed from the package, plug the microsensor into a microsensor cable (WPI #91580) connected to the free radical analyzer (Fig. 3).* **Be very careful that the sensor tip does not come into contact with anything that could damage it.** The sensor should plug in easily. If you encounter resistance, it is probably due to misalignment of the sensor plug with the socket connector inside the microsensor cable. Simply realign the sensor by gently rotating it until it snaps into place.

***NOTE:** Current WPI free radical analyzers include the **TBR4100** and **TBR1025**. Previous analyzers manufactured by WPI (like **Apollo1000**, **Apollo4000**) may also be used.

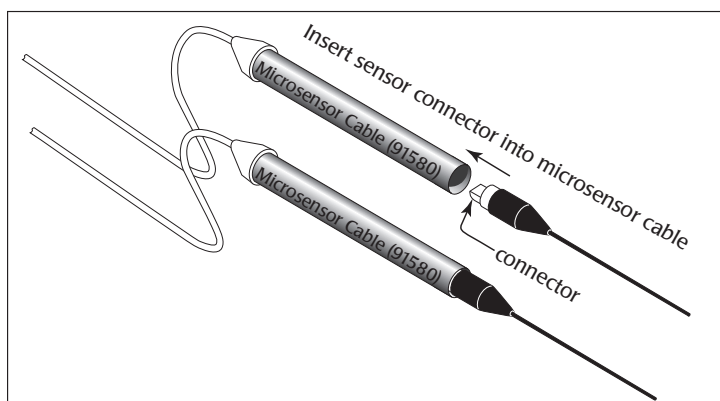


Fig. 3—Microsensors may be changed or replaced quickly and easily

Polarizing the Sensor

When a non-polarized microsensor is initially connected to a free radical analyzer, it will display a high (sometimes off-scale) background current. It takes time for the background current to drop down to a stabilized baseline value. Set the poise voltage to 0.15V. (For the **TBR4100/1025**, set the **Probe Select** dial to **H₂S**.) The amount of time required to reach a stable baseline current varies for each sensor. New sensors typically take longer, on the order of four hours.

The Performance Evaluation included with your sensor shows the baseline current and the sensitivity of your sensor when it was quality tested at WPI. (In addition, it shows the polarization time of your sensor in the WPI labs.) The baseline value attainable in your lab may be slightly higher or lower, depending on the temperature* and composition of the test media. For initial performance verification of an **ISO-H2S-100** in your lab, WPI recommends using the Na₂S calibration method described below. Once a stable baseline current is achieved (usually between

100–2,000pA), the microsensor is ready for use.

It is possible for the background current to be negative after the polarizing. This does not affect the functionality of the sensor.

***NOTE:** The background current of the sensor will usually increase with increasing temperature of the experiment. Although the sensitivity of the sensor does not change significantly within the range 20-37°C, it is recommended that the calibration procedure be performed at the same temperature and salinity as the experiment.

Calibrating the Sensor

Once the sensor is polarized, it can be calibrated. The following example briefly describes the fundamental concepts behind a standard calibration protocol. Known concentrations of H₂S are generated in buffer solution by adding a known volume of the prepared Na₂S stock solution.

NOTE: To avoid thermal artifacts during the calibration procedure, both the calibration media and Na₂S stock solution should be maintained at the same temperature.

NOTE: For additional information, refer to the free radical analyzer manual. Most WPI manuals can be downloaded directly from www.wpiinc.com. This information can also be e-mailed when you contact the WPI Technical Support team at 941.371.1003 or technicalsupport@wpiinc.com.

Preparation of Na₂S Stock Solution

1. Dissolve 5mg EDTA in 100mL distilled water (18mΩ) in 100mL volumetric flask.
2. Purge the solution vigorously with argon gas for 15 minutes.
3. Weigh 48.0mg sodium sulfide (Na₂S .9 H₂O–reagent plus, 99.99+% – Sigma #431648) and dissolve it in the solution under argon atmospheres.
4. Seal the flask with a rubber stopper. The solution is 2.0mM Na₂S. Keep the solution refrigerated (2-8 °C) and away from light.

Calibration Procedure

The polarized sensor should already be immersed in solution and plugged into the free radical analyzer. Set the range to 10nA and the poise voltage to +150mV.

The H₂S sensor is sensitive to salinity and temperature, and H₂S solubility is sensitive to temperature. Calibration and subsequent measurements should be performed in a solution with the same temperature and salinity.

1. Place 20mL PBS buffer solution (pH 7.2, 0.05M) in a 20mL vial, drop a small stirring bar into the PBS solution and place the vial on a magnetic stirring plate.
2. Immerse the tip of the H₂S sensor in the solution and secure it in an electrode

holder such as WPI's Pro-Guide (WPI #47510, 47520, 47530, 47540) or a micromanipulator. The sensor tip should be immersed about 10–15mm into the solution. It should not contact the stir bar, which could damage the tip.

3. Wait until the current on the display stabilizes again before continuing and record the value. This may take several minutes if the sensor has undergone a large temperature change.
4. To the vial containing 20mL of PBS solution, sequentially inject four aliquots of the Na₂S solution (5μL, 10μL, 20μL and 40μL) into the glass vial. Typically, each aliquot is twice the volume of the previous one. The current output jumps rapidly after each addition and then plateaus. As soon as it reaches a plateau, inject the next aliquot.

The reaction produces H₂S gas. When H₂S gas passes through the gas permeable coating, it generates an output current that is measurable, and the results can then be graphed using a third party spreadsheet with graphing capability like Microsoft® Excel.

NOTE: The volume of injected calibration aliquots may be adjusted to accommodate the anticipated concentration range for the experiment.

5. Construct a calibration curve by plotting the signal output (pA) against the concentration (nM) of H₂S. 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**.

***The H₂S sensor measures the dissolved H₂S gas, which is only one component of the total sulfide equilibrium system. The total sulfide concentration [S²⁻] = [H₂S] + [HS⁻] + [S²⁻], so the H₂S concentration can be calculated by: [H₂S] = [Na₂S]/{ 1 + K₁/[H⁺] + K₁K₂/[H⁺]²}

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

Storing the Sensor

STANDBY: When not being used for a short period of time (such as overnight), the microsensors should remain attached to the microsensor cable and kept in solution. Before the next experiment, immerse the sensor in the experimental solution (like, Krebs' Buffer). The background current increases until it reaches a stable value. Do not be alarmed if the background current fluctuates. This is associated with the hydration of the sensor and will not negatively affect the sensor's performance.

LONG-TERM: If the microsensor will not be used for more than three or four days, then it may be stored dry. Remove it from the microsensor cable and clean it. (See below.) Return it to the case in which it was shipped, being very careful to avoid making contact with the sensor tip.

NOTE: ALWAYS rinse with distilled water before storing the sensor dry.

The microsensor is a maintenance-free consumable sensor. When its performance is no longer satisfactory, remove it from the microsensor cable and dispose of it, replacing it with a new one.

Cleaning the Sensor

The sensor should be cleaned after each use by suspending the tip in distilled water for 20–30 minutes to dissolve salts and remove particles which may have accumulated on the tip. If the sensor was used in a protein-rich solution, the tip should first be soaked in a protease solution for several minutes to remove protein build-up and then rinsed with distilled water. Enzymatic detergent (Enzol, WPI #7363-4) can also be used. The sensor can be sterilized chemically using an appropriate disinfectant (Cidex, WPI #7364). If necessary, gently dab the sensor with Kimwipes® to remove residue.

NOTE: ALWAYS rinse with distilled water before storing the sensor dry.

ACCESSORIES

Table 1: Accessories

Part Number	Description
7363-4	Enzol Enzymatic Detergent, 1 gallon
7364	Cidex Disinfecting Solution
91580	Microsensor Cable
47510	ProGuide Position/Holder with Base
47520	ProGuide Position/Holder
47530	ProGuide Plus Position/Holder with fine adjustment
47540	ProGuide Plus with Base

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. (For the TBR, choosing the H ₂ S sensor setting selects +150 mV automatically.) Set the range at 10nA.
	The sensor may be nearing the end of its usable life.	Perform a 5-point calibration set using the standard. If the sensor responds linearly within the desired concentration range, it is still usable. See "Calibrating the Sensor" on page 5.
Unstable baseline	If the baseline hasn't stabilized after 4 hours, the polarizing solution may be contaminated.	Prepare fresh polarizing solution.
	External electrical interferences may be the problem.	Identify and isolate electrical interferences.
Calibration data set is not linear.	Uneven aliquots may have been used.	Check the pipetter calibration.
	The stock solutions have deteriorated.	Prepare fresh standard solution. See "Calibrating the Sensor" on page 5.
Sensitivity below range specified	Foreign materials have been adsorbed on the sensor's surface.	If the foreign materials are proteins, use and enzymatic cleanser like Enzol (WPI #7363-4) to remove the contaminant.
	The sensor has reached end of its usable life.	Replace the sensor.

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

SPECIFICATIONS

This sensor conforms to the following specifications:

Outside Tip Diameter.....	100µm
Available Length.....	5mm
(length varies in 1mm increments—for example, 1mm, 2mm, 3mm... 5mm is standard. Sensor length is proportional to sensitivity.)	
Response Time	20–60sec.
Lowest Detection Limit/Range.....	<5nM
Nominal Sensitivity-New Sensor.....	1–4pA/nM
Baseline Drift	<2pA/min.
Poise Voltage.....	0.15V
Typical Quiescent Base-line Current, 25°C.....	1,000pA
Acceptable Baseline Range	100–2,000pA
Polarization Time	4+ hrs
Overall Sensor Length.....	5cm
Sensor Size	
Diameter	0.6mm
Active Sensor Length.....	5mm
Flex Body	35mm
Sensor Life.....	1–3 months
Operating Conditions	20°–40°C (68°–104°F)
Storage Conditions	4°–25°C (50°–77°F)



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

International Trade Center, 175 Sarasota Center Blvd., Sarasota FL 34240-9258
Tel: 941-371-1003 • Fax: 941-377-5428 • E-mail: sales@wpiinc.com

UK

1 Hunting Gate, Hitchin, Hertfordshire SG4 0TJ
Tel: 44 (0)1462 424700 • Fax: 44 (0)1462 424701 • E-mail: wpiuk@wpi-europe.com

Germany

Zossener Str. 55, 10961 Berlin
Tel: 030-6188845 • Fax: 030-6188670 • E-mail: wpide@wpi-europe.com

China & Hong Kong

WPI Shanghai Trading Co., Ltd.
Rm 20a, No8 Dong Fang Rd., Lu Jia Zui Financial District, Shanghai PRC
Tel: +86 688 85517 • E-mail: chinasales@china.wpiinc.com

Internet

www.wpiinc.com