



INSTRUCTION MANUAL

ISO-HPO-2

Hydrogen Peroxide Sensor for Use
with WPI Analyzers

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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-HPO-2 sensor works with WPI free radical analyzers to monitor hydrogen peroxide.

INTRODUCTION

The **ISO-HPO-2** is a 2.0 mm stainless steel sensor, with replaceable membrane sleeves (#**600012**) and an internal refillable electrolyte (#**100042**). The sensor is designed for use in cell cultures and similar applications.

The basic structure of the **ISO-HPO-2** sensor is shown in Fig. 2. It consists of an internal H_2O_2 -sensing working/reference electrode combination. This electrode fits inside a disposable protective stainless steel sleeve (WPI #**600012**) which must contain fresh electrolyte (WPI #**100042**) 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 2 hours in 0.1M PBS solution prior to use.

NOTE: The sensor membrane is 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 “Sensor Care and Maintenance” on page 6. If your sensor was shipped wet, it includes a fluorescent sticker on the box that 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.” See “Cleaning the Membrane” on page 7.

Parts List

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

- (1) **ISO-HPO-2** H₂O₂ sensor
- (1) Probe Unpacking Instructions (Read this before handling the probe.)
- (1) Spare membrane sleeve
- (1) 1 cc Syringe
- (1) **MF28G67** MicroFil
- (1) **100042** 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 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.

INSTRUMENT DESCRIPTION

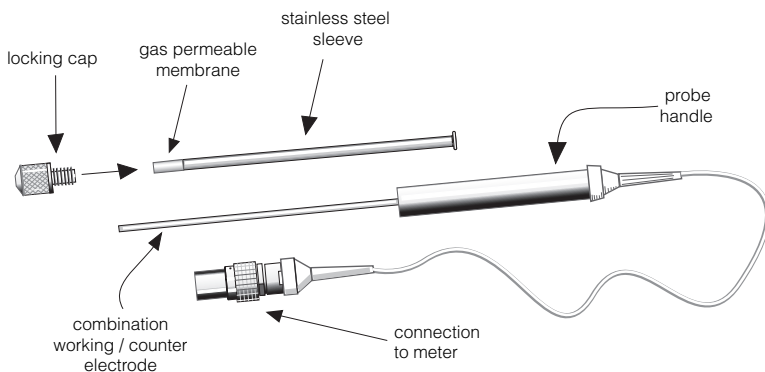


Fig. 2—The locking cap slides over the sensor sleeve. The sleeve is filled with electrolyte and slides over the ISO-HPO-2 electrode. The locking cap securely attaches the sleeve to the sensor handle.

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

Stainless steel sleeve (WPI#**600012**, 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#**100042**).

Working/Counter electrode–This is an internal H₂O₂-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 #**600012**). The **ISO-HPO-2** start-up kit (WPI #**600011**) also includes replacement membrane sleeves, along with all the accessories to fill them properly with filling solution (electrolyte).

OPERATING INSTRUCTIONS

Polarizing the Sensor

1. Measure an appropriate volume (for example, 10mL) of 0.1M PBS buffer solution into a glass vial. Place the vial on a magnetic stirring plate and put a small stir bar into the vial. Set the stir rate at a medium speed.
2. Remove the sensor from the filling solution in which the tip has been immersed during storage. See the *Probe Unpacking Instructions* included with your sensor.

NOTE: Some sensors are shipped or stored dry. For these sensors, you will need to install the membrane sleeve (included). See “Replacing the Membrane Sleeve” on page 7.

3. Immerse the **ISO-HPO-2** sensor tip in PBS buffer solution. The sensor tip should be immersed about 0.3-0.5mm into the solution, and should not be touched by the stir bar.
4. Connect the **ISO-HPO-2** sensor to the input on the free radical analyzer.
5. Turn on the free radical analyzer (**TBR4100/1025**).
6. Select the poise voltage for hydrogen peroxide on the free radical analyzer. On the **TBR4100/1025**, set the *Probe Select* switch to *HPO* (+450mV).

NOTE: It is important that the poise be set correctly to avoid erroneous results.

7. Set the current range to 10nA

8. Allow the sensor to polarize for at least 2 hours to reach a stable baseline current of about 800–900pA before it is used for measurement.
9. During initial polarization the current is typically high, but within an hour it will begin to decrease rapidly. After several hours the current will fall. The range should always be set to 10nA, regardless of how low the baseline falls.
10. In two hours or less, the sensor should achieve a stable baseline current value. A typical baseline value is about 800–900pA. At this time, the sensor is ready for calibration. If the baseline value does not drop within 2 hours, see “Replacing the Membrane Sleeve” on page 7.

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

Calibrating the Sensor

Hydrogen peroxide (H_2O_2) is a very important product in the biological system. The determination of H_2O_2 requires an accurate method of calibration. Amperometric (electrochemical) determination using the **TBR4100/1025** is a very reliable method to measure H_2O_2 . The instrument measures the amount of H_2O_2 oxidized on the surface of the sensor using a poise voltage of +450mV. The oxidation of H_2O_2 at the sensor surface produces a small current (pA), which is detected by the **TBR4100/1025**. The amount of current produced is linearly proportional to amount of H_2O_2 in the experiment.

Items Required

- One glass vial
- 1.0mM H_2O_2 standard solution (To make standard solution, see “Making Standard 1mM HPO Solution” on page 4.)
- 0.1M PBS buffer solution (PBS buffer tablets) (To make standard solution, see “Making of 0.1M PBS Buffer Solution” on page 5.)

NOTE: The multi-port measurement chamber (WPI #**NOCHM-4**) can be used as an alternative calibration kit, specifically for use at different temperature condition. Calibration temperatures from 4 - 40°C can be controlled using an external circulating bath (contact WPI for information).

Making Standard 1mM HPO Solution

Hydrogen peroxide solution is commercially available and is commonly sold with a stabilizer compound in solution. Usually these solutions are within a known concentration range (for example, 2-4% H_2O_2). There are many published procedures for standardizing H_2O_2 solutions, such as the titration method using potassium permanganate. The H_2O_2 sensor can detect low nM concentrations, so, WPI recommends the following procedure.

1. Weigh 67.5mg Acetanilide (Sigma-Aldrich 397237) and dissolve it into 250mL of deionized water in a volumetric flask.
2. Remove 1mL of this solution and put it in a clean container.

3. Add 231 μL of 2-4% H_2O_2 (Sigma-Aldrich 323381) to the flask.
4. Then, use the removed 1 mL of solution from step 2 to dilute the solution in the flask to exactly 250 mL again.
5. Store this standard solution in an amber bottle, if available, or alternatively, wrap aluminum foil around the bottle to limit light intrusion. This solution should be refrigerated.

Making of 0.1M PBS Buffer Solution

Dissolve 1 Sigma tablet (Sigma-Aldrich P4417-100TAB) into 200 mL deionized water (DIW). This solution yields 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride, pH 7.4, at 25 °C.

Calibration Procedure

1. Polarize the sensor. See “Polarizing the Sensor” on page 3.
2. Record the current value after a 10–15 minute settling period. If the current is offscale or unstable after a half hour in solution, it is likely that the membrane has been damaged and the sleeve needs to be changed. (See “Replacing the Membrane Sleeve” on page 7.)

NOTE: The calibration should be carried out at the temperature at which the samples of H_2O_2 are to be measured. 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 bottle to equilibrate with the water bath.

Creating a Calibration Curve

To create a calibration curve, measure the current (pA) generated by the addition of increasing amounts of H_2O_2 to the calibration solution.

1. Wait for current output to stabilize.
2. Consecutively add known volumes of the H_2O_2 standard solution to the PBS buffer solution. For example, add 0, 5, 10, 20, 40 and 80 μL H_2O_2 standard solution (1.0 mM) into the 10 mL PBS buffer solution. The additions can be added once every 20-30 seconds. The resulting output should look similar to the example in Fig. 3.

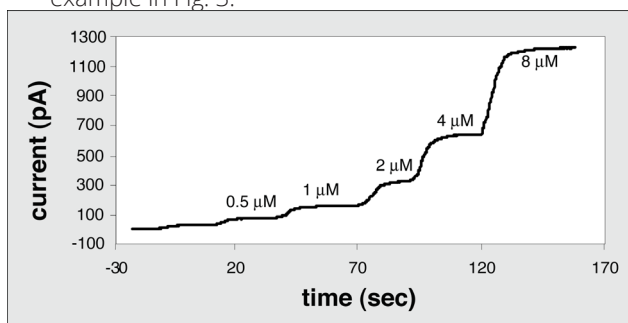


Fig. 3—Calibration Curve

From this output, a calibration curve Fig. 4 can be created by plotting the changes in current (pA) against the changes in concentration (mM). The slope of this curve indicates sensitivity of the probe.

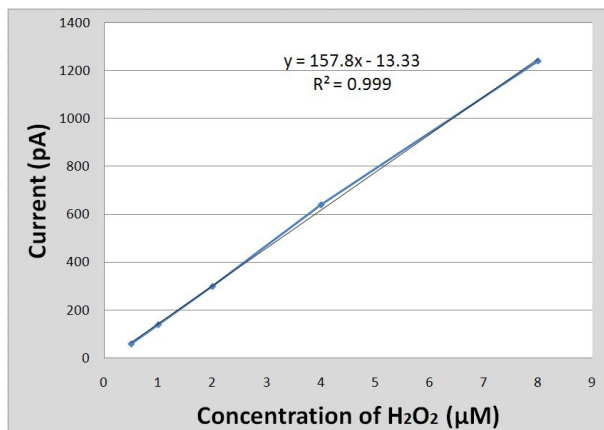


Fig. 4—Calibration Data

Interference Temperature

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 any calibration should be performed at the same temperature as the experiment.

The sensor works best between pH 3–10. Changing the pH of the solution does not affect the sensitivity. However, if the pH is below 3.0, the noise of the sensor will increase. At pH 10.0 and higher, the response of the sensor will diminish significantly.

SENSOR CARE AND 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.

Storing the Sensor

When the **ISO-HPO-2** sensor is not being used (for short-term storage) it should be connected to the **TBR4100/1025** with power ON and with the tip suspended in 0.1M PBS solution. (Do not use water.) This will keep the sensor polarized and ready for immediate use. For long term storage, remove the membrane sleeve, rinse with distilled water and store dry in a cool, dark location. Protect the inner electrode by using an old membrane sleeve with the membrane removed or place it inside a suitable box to prevent damage.

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 sterilant. 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 spot on it, the 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#**600012**) may be purchased separately.
7. Dip the internal electrode 1–2cm into the **ISO-HPO** electrolyte (WPI #**100042**) 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).

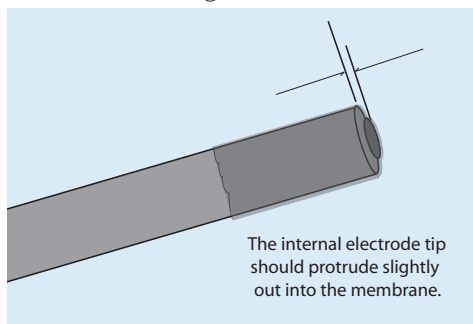


Fig. 5—Membrane placement. The internal electrode tip should protrude slightly out into the membrane.

9. When connecting to the analyzer, the current displayed on the meter at this time will be high or offscale.
10. Suspend the tip of the newly assembled probe in 0.1M PBS to polarize it or in distilled water for storage.

11. After 10–15 minutes the current should no longer be offscale and will gradually decrease with time. It may take a couple (up to 2) 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-HPO-2** membranes (packages of 4) are available (WPI #600012).

ACCESSORIES

Part Number	Description
5399	T-Adapter Kit (pkg. of 3)
600011	ISO-HPO-2 Startup Kit*
600012	Replacement Sleeves (pkg. of 4)
100042	ISO-HPO-2 Electrolyte Filling Solution (10mL)
7363-4	Enzol - Enzymatic detergent (1 gal.)
MF28G67-5	MicroFil electrolyte filling needle (pkg. of 5)

*The **ISO-HPO-2** start-up kit (WPI #600011) contains everything needed to begin working with this sensor, and the kit is highly recommended for first-time **ISO-HPO** users. The kit includes:

- (1) **600012** - Four additional membrane sleeves
- (1) **100042** - 10mL Electrolyte filling solution
- (1) **MF28G67** - MicroFil™ electrolyte filling needle, 28 AWG (67mm long)
- (1) **3563** - 1mL syringe
- (2) sample vials with lids and stoppers
- (1) Male luer fitting, 1/8" ID barb
- (1) 25g Needle



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 450mV. Set the range at 10nA.
	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 2 hours, the polarizing solution may be contaminated.	Prepare fresh polarizing solution. Use 0.1M PBS only. See "Replacing the Membrane Sleeve" on page 7.
	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 7.

NOTE: If you have a problem/issue 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	2 mm
Response Time	< 5 sec
Detection Limit/Range	<100 nM to 100 μ M (Higher detection limit (up to 100 mM) is available upon request with custom pricing.)
Nominal Sensitivity (New sensor)	>8 pA/ μ M
Poise Voltage	450 mV
Typical Quiescent Baseline Current, 25°C	800–900 pA
Acceptable Baseline Range	20-1000 pA
Polarization Time	2+ hours
Recommended Polarization Solution	0.1 M PBS

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.*



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