



ISO-HPO-100

Microsensor for Hydrogen Peroxide



INSTRUCTION MANUAL

Serial No. _____



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Warranty

WPI (World Precision Instruments, Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of 30 days from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

- Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within 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.

Calibration Procedure

Once the background current is stable, inject 2 μL of 1mM H_2O_2 standard into the buffer. The resultant concentration will be 100nM H_2O_2 . Alternatively, you may start with a different concentration depending on the concentration you plan to measure experimentally. Allow the signal to plateau and stabilize. Inject 2 times the first amount, then allow the signal to plateau and stabilize. Continue doubling or stepping up injections until you have a sufficient number of data points to construct a calibration curve, which is typically 5 points. Be sure to calibrate the sensor before each use, the use of microsensors in the presence of biological compounds can reduce the sensor's sensitivity over time due to adsorption of macromolecules onto the surface of the membrane.

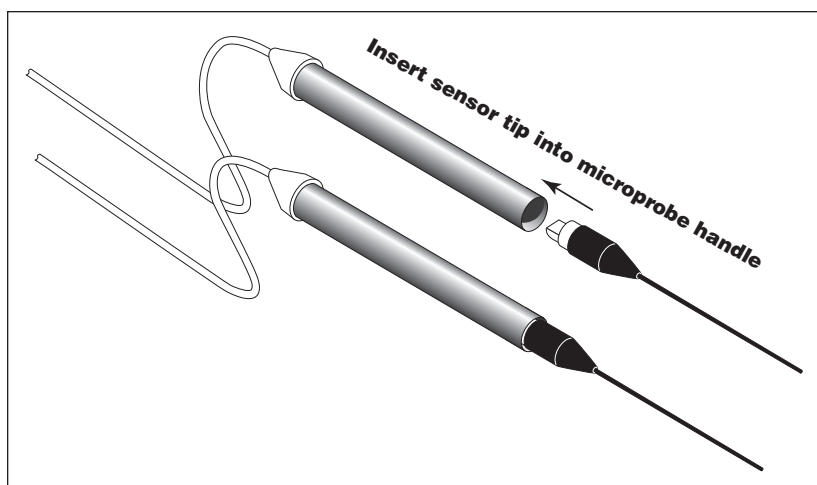
Use the recorded data to construct a calibration curve. The calibration curve can be simply constructed by plotting the signal output (*i.e.*, in pA) vs. the concentration of H_2O_2 added at that time. After the sensitivity of the H_2O_2 probe is established, the meter (Apollo 4000 or Apollo 1000) can be calibrated to display data in either concentration (*i.e.*, nM, μM) or redox current (*i.e.*, pA, nA).

Introduction

The ISO-HPO-100 is WPI's more sensitive sensor for hydrogen peroxide detection. Its design allows it to be used in tissues, vessels and similar applications. With a detection sensitivity of 1-3 pA per nM of H_2O_2 , its response is magnitudes higher than the ISO-HPO-2. In return, it does not offer the same selectivity. The ISO-HPO-2 can selectively detect H_2O_2 , concentrations over 100nM, but the ISO-HPO-100 can easily detect concentrations as low as 5 nM. Any change in the Ascorbic Acid concentration is the biggest concern for interference, where the response to changes in ascorbic acid levels can be up to 5% that of hydrogen peroxide.

Unpacking

Due to the microsensor's extremely small size and intricate construction, great caution should be used when handling it. Avoid bringing the sensor tip into contact with solid surfaces, since this may bend or break it. The sensors are packaged in foam holders so that their tips are physically isolated from contact with the container. To remove a microsensor from the package, use the thumb and index finger of one hand to separate the slit in the foam which contains the sensor and use the thumb and index finger of the other hand to grasp the sensor at its midsection and gently remove it from the container.



Attaching the Microsensor to the Microprobe Handle

Once removed from the package, the microsensor should be plugged into the microprobe handle (WPI #15810) which is connected to the Apollo 4000 or Apollo 1000, again being very careful that the sensor tip does not come into contact with anything which 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 microprobe handle. Simply realign the sensor by gently rotating it until it snaps into place.

When a non-polarized microsensor is initially connected to an Apollo 4000 or Apollo 1000, it may display a high (sometimes off-scale) background current. The polarization voltage applied by the instrument will cause a reduction of the background current to a stabilized baseline value over time. The amount of time required to reach a stable baseline current varies for each sensor. New sensors typically take longer, on the order of several hours. Once a stable baseline current is achieved (usually between 10-2000pA), the microsensor is ready for use.

Using the Hydrogen Peroxide Microsensor

First, calibrate the sensor using the calibration procedure described in this manual or using the methods described in the Apollo 4000 or Apollo 1000 manuals. After completing the calibration, you may place the sensor in the experimental set-up and commence H_2O_2 monitoring as explained in the Apollo instruction manual.

Storage and Maintenance

When not being used for a short period of time (such as overnight), the microsensor should remain attached to the microprobe handle and immersed into buffer solution, such as PBS. Before the next experiment, the sensor can be transferred from the buffer solution to the experimental solution.

If the microsensor is to be stored for a long period of time, then it may be stored dry by removing it from the microprobe handle and returning it to the case in which it was shipped, being very careful to avoid making contact with the sensor tip.

The H_2O_2 microsensor is a maintenance free consumable sensor: when it no longer functions, remove it from the microprobe handle and dispose of it, replacing it with a new one.

Calibration by Hydrogen Peroxide Standard

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 (*i.e.*, 2-4% H_2O_2). There are 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 range concentrations, therefore it is recommended to prepare a 1mM H_2O_2 standard to be used for calibration of the microsensor.

Calibration Set-up

NOTE: H_2O_2 Probes are sensitive to changes in temperature, ion concentration, and pH. Therefore, it is recommended that the calibration of your sensor is performed using the same experimental conditions, especially at the same temperature.

#1—Calibration Buffer: This can be any common buffer with a pH near the physiological range, such as PBS or Krebs' buffer. Pour 20 mL of buffer into a vial with a magnetic stir bar, and begin stirring solution. Immerse the hydrogen peroxide microsensor into the buffer solution.

#2—Standard Hydrogen Peroxide solution: Standardize a H_2O_2 stock solution. Dilute with deionized water to 1mM final concentration just before calibration of the sensor.