ISO-COP-2
MacroSensor for CO Measurement

Serial No.______________
Free Radical Analyzer

- Real-time detection using electrochemical microsensors
- Measure nitric oxide from < 0.3 nM to 100µM.
- Measure hydrogen peroxide < 10 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 µA (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

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>Carbon Monoxide</th>
<th>Nitric Oxide</th>
<th>Hydrogen Peroxide</th>
<th>Oxygen</th>
<th>Hydrogen Sulfide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Available Diameters</td>
<td>2 mm</td>
<td>2 mm</td>
<td>2 mm</td>
<td>2 mm</td>
<td>2 mm</td>
</tr>
<tr>
<td>Response Time</td>
<td>&lt; 10 sec</td>
<td>&lt; 5 sec</td>
<td>&lt; 5 sec</td>
<td>&lt; 10 sec</td>
<td>&lt; 5 sec</td>
</tr>
<tr>
<td>Detection Limit/Range</td>
<td>10nM to 10µM</td>
<td>1 NM to 40µM*</td>
<td>&lt; 100nM to 100µM</td>
<td>0.1%-100%</td>
<td>&lt; 5nM-100µM</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>~0.5 pA/nM</td>
<td>≤ 2 pA/nM</td>
<td>8 pA/µM</td>
<td>0.3-0.6nA/%</td>
<td>2 pA/nM</td>
</tr>
<tr>
<td>Drift</td>
<td>&lt;1pA/min</td>
<td>&lt;1pA/min</td>
<td>0.1pA/min</td>
<td>&lt; 1%/min</td>
<td></td>
</tr>
<tr>
<td>Temperature Dependent</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Physiological Interference</td>
<td>nitric oxide</td>
<td>NaNO₂ (10^-6 or better)</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

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—ISO-COP-2 sensor
INTRODUCTION

The ISO-COP-2 is a carbon monoxide sensor with a replaceable stainless steel membrane sleeve filled with an electrolyte solution that measures CO in vivo or in vitro in real time!

Replacement membrane sleeves can be purchased in packages of four (WPI #95620), along with filling solution (WPI #95611). A startup kit for this sensor is available and is recommended for the first purchase of this sensor type (WPI #95699).

Sensor Design

The ISO-COP-2 is an amperometric sensor designed for use in cell culture and similar applications.

In principle, CO diffuses through the gas-permeable membrane and is then oxidized to CO$_2$ on the working electrode of the sensor. This oxidation creates a current with a magnitude directly related to the concentration of CO in solution.

It is designed for use with WPI’s TRB4100 (4-Channel Free Radical Analyzer), TBR1025 (1-Channel) or the Apollo1000.

Notes and Warnings

WARNING: CARBON MONOXIDE GAS MUST BE HANDLED ONLY IN A WELL-VENTILATED AREA, TYPICALLY A LABORATORY FUME HOOD WITH FORCED VENTILATION. THE U.S. OCCUPATIONAL SAFETY AND HEALTH ADMINISTRATION HAS SET A TIME-WEIGHTED AVERAGE (8-HOUR) (TWA) PERMISSIBLE EXPOSURE LIMIT (PEL) OF CARBON MONOXIDE AS 50PPM. THE ACGIH THRESHOLD LIMIT VALUE (TLV) FOR CARBON MONOXIDE IS 25PPM TWA. THAT IS TO SAY, 50PPM IS CITED AS THE MAXIMUM CONCENTRATION TO WHICH WORKERS MAY BE CONTINUALLY EXPOSED. OVEREXPOSURE MAY RESULT IN HEADACHES, DIZZINESS, CONVULSIONS, LOSS OF CONSCIOUSNESS AND DEATH. CARBON MONOXIDE IS ALSO EXTREMELY FLAMMABLE. IT IS THEREFORE CRITICAL THAT THE PERSONNEL HANDLING THE GAS BE THOROUGHLY FAMILIAR WITH THE MATERIAL SAFETY DATA SHEET (MSDS) AND PROPER HANDLING PROCEDURES. THE PRECAUTIONS RECOMMENDED BY THE GAS MANUFACTURER MUST BE FOLLOWED.

CAUTION: DO NOT EXPOSE SENSOR TO ORGANIC SOLVENTS.
**CAUTION:** Carefully read the “Probe Unpacking” instructions (found in the sealed sensor case) before handling the sensor.

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

**NOTE:** The sensor membrane and membrane adhesive are extremely delicate. Improper handling will lead to damage of the sensor. The ISO-COP-2 is designed for use in liquids.

**NOTE:** For optimal use of each sensor and sensor membrane sleeve, the sensor must be stored properly. See “Storing the Sensor” on page 8.

### Parts List

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

1. ISO-COP-2 CO sensor
2. Probe Unpacking Instructions (Read this before handling the probe.)
3. ISO-COP-2 Data sheet
4. Spare membrane sleeve
5. 1 cc Syringe
6. MF28G67 MicroFil
7. 95611 10mL vial of filling solution
8. Instruction Manual

### 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.

The ISO-COP-2 sensor is shipped in a sealed, rigid plastic, hinged box with foam padding to avoid damage to your sensor during shipment. The tip of the sensor is protected by inserting it into a small, plastic vial containing deionized water (DIW) to keep the membrane from drying out.

To open the package, carefully cut the seals on either side of the sensor box. Inside the box, on top of the sensor, are the important documents to read.
before handling the sensor, including the instructions for removal of the sensor tip from the protection vial. Keep the sensor tip in this vial until the sensor is ready for use.

KEEP THE SENSOR STORAGE BOX and all the documentation in a safe place. Use of the sensor should begin within 30 days of receipt.

**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. For further details, please read the section entitled “Claims and Returns” on page 15 of this manual.
INSTRUMENT DESCRIPTION

Structure of the Sensor

The basic structure of the ISO-COP-2 sensor is shown below (Fig. 2).

Fig. 2—ISO-COP-2 Sensor assembly

1. Gas permeable, polymeric membrane covering the end of the stainless sleeve to separate it from the external environment

2. Disposable, protective stainless steel sleeve (WPI#95620) that houses the sensitive electrode pair. The sleeve is flanged to properly connect with the locking cap. It must contain fresh electrolyte (WPI#95611).

3. Internal CO-sensing pair of working and counter (reference) electrodes

4. Probe handle

5. Locking cap attaches the sleeve to the probe handle

When the sensor is fully 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 # 95620). The ISO-COP-2 start-up kit (WPI #95699) also includes replacement membrane sleeves, along with all the accessories to fill them properly with electrolyte solution.
OPERATING INSTRUCTIONS

Environmental Influences

There are two environmental parameters to which CO sensors are quite sensitive: temperature and electrical interference.

Temperature

The background current (and to a lesser degree) the selectivity of the CO sensor is affected by temperature. This is due to the effects of temperature on the partial pressure of dissolved CO gas in liquid samples, on the permeability of the membrane and on the conductivities of various sensor components. It is recommended that the calibration procedure be performed at the same temperature as the experiment and that temperature be held constant during CO measurement.

Electrical Interference

External, electrical noise sources (like fluorescent lights, MRI machines, electric motors, computers, pumps and other electrical instruments) may couple into the sensor signal path electromagnetically and impose undesirable signals in the output record. The magnitude of this external noise depends on the environment of the laboratory. If the interference introduced by the electrical signals in the environment is large, the first step towards eliminating it is to ground and shield the system properly.

TIP: Refer to your free radical analyzer manual for proper grounding and shielding techniques. (In the TBR4100 or Apollo1000 manuals, see “Grounding and Noise Concerns” in the Operating Instructions section.)

Setup

The CO electrolyte filling solution (WPI #95611) must be made fresh when you are ready to use the sensor, so the ISO-COP-2 sensor is shipped with a dry membrane sleeve. Before using it, you must fill the sleeve with the CO electrolyte filling solution. Use the membrane sleeve that was shipped on your sensor and follow the instructions for replacing a membrane sleeve (page 9) to setup your sensor for its polarization, calibration and first use.

NOTE: Since the membrane sleeve that was on the sensor during shipping was dry, the membrane was not damaged by placing it on the sensor. That membrane sleeve is still good to use.
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 to 950mV.
5. The ISO-COP-2 sensor should be allowed up to 12 hours to reach a stable baseline current of 1000-8000pA before it is used for measurement.
6. During initial polarization the current is typically very high (well above 10nA), 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 below 10nA. At that time, adjust the range to 10nA. The 10nA range setting is required to provide adequate resolution for viewing the sensor’s response to carbon monoxide.
7. If the stabilized baseline value exceeds 8000pA, see “Replacing the Membrane Sleeve” on page 9.

NOTE: This polarization procedure assumes the temperature is 25°C. At 37°C the baseline current is higher. If the current exceeds 10nA, it may be required to adjust the current range up to 100nA.
SENSOR CARE AND MAINTENANCE

Durability and Handling

The sensor is relatively durable, except for the membrane sleeve. Exercise caution when handling any CO 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.

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

With proper care and by following the instructions below a membrane sleeve should last more than one month.

**STANDBY:** If the electrode is being used on a daily basis, it should be left connected to the free radical analyzer in the ON position with the tip suspended in distilled water.

**LONG-TERM:** For long-term storage of more than one week, remove the membrane sleeve, clean the sensor tip with deionized water and dry carefully. Protect the tip and store the sensor with the membrane removed in a dry, cool environment.

Cleaning the Membrane

The membrane sleeve itself requires very little maintenance. The primary concern is to avoid damage to the membrane and to keep it as clean as possible. After each use the membrane should be cleaned by suspending the tip in distilled water for 20-30 minutes to dissolve salts and remove particles which may have accumulated on it. If the probe 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 in distilled water. Enzymatic detergent (for example, Enzol, WPI#7363) can also be used.
Accumulated organic matter can be removed by briefly immersing the tip in a 0.1M HCl or 0.1M NaOH (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 cleaning, then the membrane sleeve should be replaced.

**Sterilizing the Membrane**

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

**CAUTION**: Do not use alcohol on the sensor.

**Replacing the Membrane Sleeve**

Even with the best of care and proper maintenance, the membrane sleeve will eventually 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 kits (WPI #95620) may be purchased separately. Filling a single ISO-COP-2 sensor sleeve requires about 1mL.
7. Dip the internal electrode 1-2cm into the ISO-COP-2 electrolyte (WPI #95611 provided in the ISO-COP-2 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 freshly mixed CO electrolyte filling solution 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. 3).

![Fig. 3—(Right) Membrane placement. The internal electrode tip should protrude slightly out into the membrane.](image)

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-COP-2 membranes (packages of 4) are available from WPI (#95620).
## ACCESSORIES

### Table 1: Accessories

<table>
<thead>
<tr>
<th>Part Number</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>5399</td>
<td>T-Adapter Kit (pkg. of 3)</td>
</tr>
<tr>
<td>95699</td>
<td>ISO-COP-2 Startup Kit*</td>
</tr>
<tr>
<td>95620</td>
<td>Replacement Sleeves (pkg. of 4)</td>
</tr>
<tr>
<td>95611</td>
<td>ISO-CO Electrolyte Filling Solution (10mL)</td>
</tr>
<tr>
<td>7363-4</td>
<td>Enzol - Enzymatic detergent (1 gal.)</td>
</tr>
<tr>
<td>MF28G67-5</td>
<td>MicroFil electrolyte filling needle (pkg. of 5)</td>
</tr>
</tbody>
</table>

*The ISO-COP-2 start-up kit (WPI # 95699) contains everything needed to begin working with this sensor, and the kit is highly recommended for first-time ISO-COP-2 users (Fig. 4). The kit includes:

- Five additional membrane sleeves (Future refills are sold in packages of four sleeves - 95620.)
- 95611—Electrolyte filling solution
- MicroFil™ electrolyte filling needle (Future refills are sold in packages of five—MF28G67-5)
- 3563—1mL syringe
- Two sample vials with lids and stoppers
- Potentiometer adjustment tool for use with the TBR4100/1025

Fig. 4—ISO-COP-2 Startup kit
## TROUBLESHOOTING

<table>
<thead>
<tr>
<th>Issue</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline current is below specified range.</td>
<td>The poise voltage (sensor setting) may be incorrectly set.</td>
<td>Set the poise voltage to 950mV. Set the range at 10nA.</td>
</tr>
<tr>
<td></td>
<td>The sensor may be nearing the end of its usable life.</td>
<td>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.</td>
</tr>
<tr>
<td>Unstable baseline</td>
<td>If the baseline hasn’t stabilized after 12 hours, the polarizing solution may be contaminated.</td>
<td>Prepare fresh polarizing solution. Use 0.1M PBS only.</td>
</tr>
<tr>
<td></td>
<td>External electrical interferences may be the problem.</td>
<td>Identify and isolate electrical interferences.</td>
</tr>
<tr>
<td>Calibration data set is not linear</td>
<td>The dilution factors may be incorrect.</td>
<td>Verify the procedure used.</td>
</tr>
<tr>
<td></td>
<td>Uneven aliquots may have been used.</td>
<td>Check the pipetter calibration.</td>
</tr>
<tr>
<td>Sensitivity below range specified</td>
<td>Membrane is old or worn, or electrolyte solution evaporated.</td>
<td>In either case, Replace the membrane sleeve. See “Replacing the Membrane Sleeve” on page 9.</td>
</tr>
</tbody>
</table>

**NOTE:** If you have a problem/issue with your ISO-COP-2 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

The sensor conforms to the following specifications:

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outside Diameter</td>
<td>2mm</td>
</tr>
<tr>
<td>Response Time</td>
<td>&lt; 10 sec</td>
</tr>
<tr>
<td>Detection Limit/Range</td>
<td>~10 nM</td>
</tr>
<tr>
<td>Linear range</td>
<td>10 nM–10 µM</td>
</tr>
<tr>
<td>Nominal Sensitivity (New sensor)</td>
<td>~0.5 pA/nM</td>
</tr>
<tr>
<td>Poise Voltage</td>
<td>950 mV</td>
</tr>
<tr>
<td>Typical Quiescent Baseline Current, 25°C</td>
<td>3,000 pA</td>
</tr>
<tr>
<td>Acceptable Baseline Range</td>
<td>1000–8000 pA</td>
</tr>
<tr>
<td>Polarization Time</td>
<td>12+ hours</td>
</tr>
<tr>
<td>Recommended Polarization Solution</td>
<td>0.1M PBS</td>
</tr>
<tr>
<td>Temperature Dependent</td>
<td>Yes</td>
</tr>
<tr>
<td>Physiological Interference</td>
<td>NO (nitric oxide)</td>
</tr>
</tbody>
</table>
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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 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 pitted 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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