



# INSTRUCTION MANUAL

**ISO-NOP**

Macrosensor for NO Measurement



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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-NOP sensor*

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## INTRODUCTION

The **ISO-NOP** is a nitric oxide sensor with a replaceable stainless steel membrane sleeve filled with an electrolyte solution. Replacement membrane sleeves can be purchased in packages of four (WPI #**5436**), along with filling solution (WPI #**7325**). A startup kit for this sensor is available and is recommended for the first purchase of this sensor type (WPI #**5435**).

The **ISO-NOP** is the original nitric oxide probe, ideal for cell cultures, cell suspensions and many other applications. It incorporates WPI's proprietary combination electrode technology whereby the nitric oxide-sensing element and separate reference electrode are encased within a single shielded sensor design.

## Notes and Warnings

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**CAUTION:** DO NOT EXPOSE SENSOR TO ORGANIC SOLVENTS.

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**CAUTION:** Carefully read the "Probe Unpacking" instructions (found in the sealed sensor case) before handling the sensor.

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**CAUTION:** If the sensor membrane is punctured, calibration solution ( $H_2SO_4$ ) may leak into the sensor sleeve, which can lead to a high background current. Discoloration of the sensor is a good indication that this has occurred.

Immediately after calibration or use, rinse the sensor with deionized water and dry with a soft tissue or KimWipe. Permanent damage to the sensor will occur if the sensor is left in the sleeve under this exposure.

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**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 "Storing the Sensor" on page 11. 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-NOP** NO sensor
- (1) Probe Unpacking Instructions (Read this before handling the probe.)
- (1) Spare membrane sleeve

- (1) 1 cc Syringe
- (1) **MF28G67** MicroFil
- (1) **7325** 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 19 of this manual. Please contact WPI Customer Service if any parts are missing at 941-301-1003 or customerservice@wpiinc.com.

The **ISO-NOP** 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. The test date and serial number of each sensor is printed on the bottom of its box. 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 19 of this manual.

# INSTRUMENT DESCRIPTION

## Structure of the Sensor

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

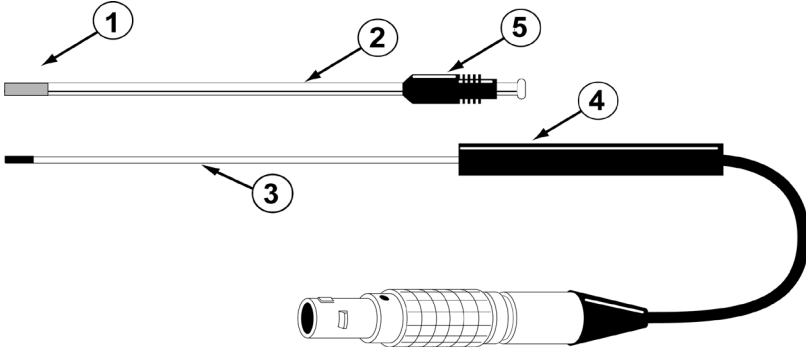


Fig. 2—ISO-NOP Sensor assembly

- 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#5436) that houses the sensitive electrode pair. The sleeve is flanged to properly connect with the locking cap. It must contain fresh electrolyte (WPI#7325).
- ③ Internal NO-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 #5436). The **ISO-NOP** start-up kit (WPI #5435) 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 NO sensors are quite sensitive: temperature and electrical interference.

### Temperature

The background current (and to a lesser degree) the selectivity of the NO sensor is affected by temperature. This is due to the effects of temperature on the partial pressure of dissolved NO 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 NO 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.)

## Polarizing the Sensor

During initial polarization the current is typically very high (about 100 nA), but within a few minutes it will begin to decrease rapidly. In order to observe this phenomenon, start by setting the range to 100 nA. After several hours the current will fall below 10 nA. At that time, adjust the range to 10 nA. The 10 nA range setting is required to provide adequate resolution for viewing the sensor's response to nitric oxide. The use of the recording can assist in trouble shooting the sensor if there are issues.

1. Support the sensor in 0.1 M PBS solution (instead of  $\text{KI-H}_2\text{SO}_4$ ).
2. Turn on the free radical analyzer.
3. On the TBR4100/1025 set the Probe Select switch to NO (0.865 V) and the display switch to Output Current. Set the range to 100 nA. Start the recording at 1 sample/sec.
4. Plug the sensor into the free radical analyzer and the displayed current should be observed to go up immediately to a high current (60-90 nA), then the current will begin to fall.


5. The ISO-NOP sensor should be allowed up to 12 hours to reach a stable baseline current of 1000-800pA before it is used for measurement.
6. After 12 hours if the baseline current value greatly exceeds the value on the included QC sheet, refer to the testing tip on page 13 before following the guide to Replace the Membrane Sleeve on page 12.

**NOTE:** KI-H<sub>2</sub>SO<sub>4</sub> can be used to polarize the sensor in, but if the membrane is broken or damaged, then the electrodes will be damaged. (See Fig. 5 on page 11.) After polarization in PBS, test the membrane's integrity before placing it in KI-H<sub>2</sub>SO<sub>4</sub>. Refer to the tip on page 13.

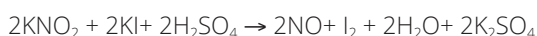
**NOTE:** This polarization procedure assumes the temperature is 25°C. At 37°C the baseline current is higher. If the current exceeds 10 nA, it may be required to adjust the current range up to 100 nA.

**TIP:** A Pre-Polarizer (WPI #NSA-3) is available to equilibrate your sensors without attaching them to your free radical analyzer. It is a small, battery-powered device that applies a potential to the NO sensors equivalent to the potential applied by the free radical analyzer. NO sensors which have been polarized with the Pre-Polarizer can be plugged into the free radical analyzer for immediate use.

## Calibrating the Sensor by Chemical Generation of NO

 **CAUTION: This calibration method may only be used with the ISO-NOP sensors. DO NOT use with other sensors. It will cause permanent damage.**

This procedure involves making two solutions (0.1 M H<sub>2</sub>SO<sub>4</sub> + 0.1 M KI, and 50 μM KNO<sub>2</sub> or NaNO<sub>2</sub>) and then running a calibration procedure. It is based on the following reaction:



A known amount of KNO<sub>2</sub> is added to produce a known amount of NO. The quantity (and so the concentration) of NO generated can be calculated directly from the stoichiometry if the concentrations of the reactants are known. Since KI and H<sub>2</sub>SO<sub>4</sub> are present in great excess, the limiting reagent is KNO<sub>2</sub>. Experiments have demonstrated that the nitric oxide generated from this reaction will persist long enough to calibrate the NO sensor easily and accurately. Since the reaction goes to completion, the equation above states that the ratio between KNO<sub>2</sub> and NO is 1:1. Therefore the amount of NO generated in the solution will be equal to the amount of KNO<sub>2</sub> added. The final concentration of NO will be equal to the diluted concentration of KNO<sub>2</sub> in the solution.

### Preparing Solution #1: 0.1 M H<sub>2</sub>SO<sub>4</sub> + 0.1 M KI

**NOTE:** Prepare fresh solution daily.

1. Pour 20 mL of 0.1 M H<sub>2</sub>SO<sub>4</sub> into a 20 mL glass vial.
2. Drop a small stirring bar into the solution and position the vial on top of a magnetic stirring plate. Turn on the stirrer so that the bar is stirring at a moderate rate.
3. Add 0.33 g KI and mix.

## Preparing Solution #2: 50 $\mu\text{M}$ $\text{KNO}_2$ (or $\text{NaNO}_2$ )

The recommended method for preparing this solution is to purchase an ion chromatography liquid nitrite standard ( $\text{NaNO}_2$  or  $\text{KNO}_2$ ) and dilute it as appropriate. Standard nitrite is available from WPI (#7357).

Alternatively, crystalline reagent  $\text{KNO}_2$  may be used, however,  $\text{KNO}_2$  is extremely hygroscopic and degrades once exposed to atmospheric moisture. Therefore, WPI recommends purchasing the reagent packaged under argon if the crystalline reagent is used. (It is available from Eastman Kodak Chem #105 7462.) Store it in a desiccator. While this extends the life of the reagent, it needs to be replaced more frequently than the liquid standard. Store the standard nitrite solution prepared from this compound in a gas-tight bottle in the refrigerator.

## Calibration Procedure

Once the sensor is polarized, it can be calibrated. The polarized sensor should already be immersed in Solution #1 and plugged into the free radical analyzer. Set the range to 10 nA and the poise voltage to 865 mV. The following example briefly describes the fundamental concepts behind a standard calibration protocol. Known concentrations of NO are generated in Solution #1 by adding a known volume of a the NO standard (Solution #2).

**NOTE:** For additional sensor calibration procedures and calibration theory, refer to the free radical analyzer manual. Most WPI manuals can be downloaded directly from [www.wpiinc.com](http://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](mailto:technicalsupport@wpiinc.com).

1. Place the 20 mL vial of Solution #1 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 once it is set.

**NOTE:** The calibration should be carried out at the same temperature at which the experimental measurements of NO 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-NOP 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. Or, use one of the septa (WPI #15575) included with the start-up kit. The sensor tip should be immersed about 2-3 mm into the solution. It should not contact the stir bar, which could damage the membrane.



**CAUTION:** If using a septum, DO NOT PUSH THE SENSOR TIP THROUGH THE HOLE. Slide the electrode laterally through the sliced side of the septum.

Carefully lower the sensor into the vial sealing the opening with the septum. Carefully insert the sensor. Make sure that the tip of the sensor does NOT make contact with the cap and/or bottom of the jar. This can damage the membrane.

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**NOTE:** Generally, it is not necessary to pre-purge the calibration solution, since the NO decays slowly in this solution. However, if you feel it is necessary to de-gas Solution #1 prior to calibration, insert one of the long stainless steel needles included with the calibration kit through the septum so that the tip is in the solution. Attach the needle through appropriate tubing to a source of pure argon gas (nitrogen may also be used). Insert one of the short needles included with the kit through the septum so that the needle tip is clearly exposed inside the vial (not in the solution). The small needle allows gas to escape, thereby avoiding a buildup of pressure. Purge the solution at low pressure (5 PSI or less) for 15 minutes. Once purging is complete and the gas source is turned off, remove the purging and pressure relief needles.

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. The quiescent baseline current is an indicator of the health of the sensor.
4. To the vial containing 20 mL of Solution #1, sequentially inject three aliquots of Solution #2 (50  $\mu$ L, 100  $\mu$ L and 200  $\mu$ L) into the glass vial. Typically, each aliquot is twice the volume of the previous one. A standard protocol might include three successive additions of  $\text{KNO}_2$ . (For example, 50  $\mu$ L, 100  $\mu$ L, 200  $\mu$ L.) 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 NO gas. When NO gas passes through the gas permeable membrane, it generates an output current that is measurable, and the results can then be graphed. The output from the TBR4100/1025 looks similar to the example shown in **Fig. 3**. Here three sequential additions of  $\text{KNO}_2$  were made to Solution #1.

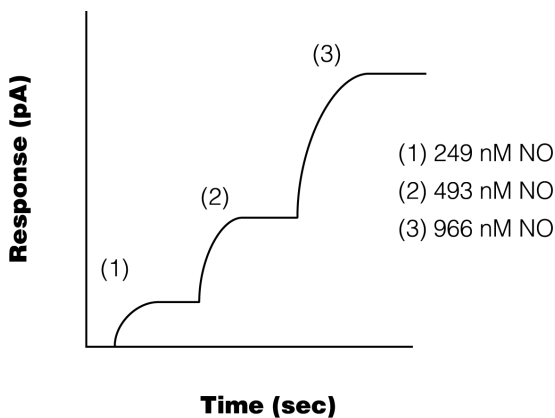


Fig. 3—Calibration Curve

5. Calculate the concentration of NO in solution after the each aliquot injection of Solution #2 to 20 mL of Solution #1. The concentration of NO produced can be

calculated as follows:

$M_1V_1 = M_2V_2$  where M=molarity and V=volume

1<sup>st</sup> Aliquot:  $50 \mu\text{L} + 20 \text{ mL} = 20,050 \mu\text{L}$   
 $50 \mu\text{M} (50 \mu\text{L}) = M_2(20,050 \mu\text{L})$   
 $2500 \mu\text{M}/20,050 = 0.125 \mu\text{M} = 125 \text{ nM}$

2<sup>nd</sup> Aliquot:  $100 \mu\text{L} + 20,050 \mu\text{L} = 20,150 \mu\text{L}$   
 $50 \mu\text{M} (100 \mu\text{L}) = M_2(20,150 \mu\text{L})$   
 $5,000 \mu\text{M}/20,150 = 0.248 \mu\text{M} = 248 \text{ nM}$

3<sup>rd</sup> Aliquot:  $200 \mu\text{L} + 20,150 \mu\text{L} = 20,350 \mu\text{L}$   
 $50 \mu\text{M} (200 \mu\text{L}) = M_2(20,350 \mu\text{L})$   
 $10,000 \mu\text{M}/20,350 = 0.491 \mu\text{M} = 491 \text{ nM}$

6. Record the calculated values in a table similar to the one below. Record the difference in current output (pA) generated by the addition of the known quantities of  $\text{KNO}_2$  in a table similar to the one below.

Amount added	Total Volume	[NO] nM	Response (pA)
50 $\mu\text{L}$	20,050 $\mu\text{L}$	125	332
100 $\mu\text{L}$	20,150 $\mu\text{L}$	248	746
200 $\mu\text{L}$	20,350 $\mu\text{L}$	491	1486

7. 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  $R^2$  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**.

ISO-NOP Calibration Curve Based on Tabulated Data

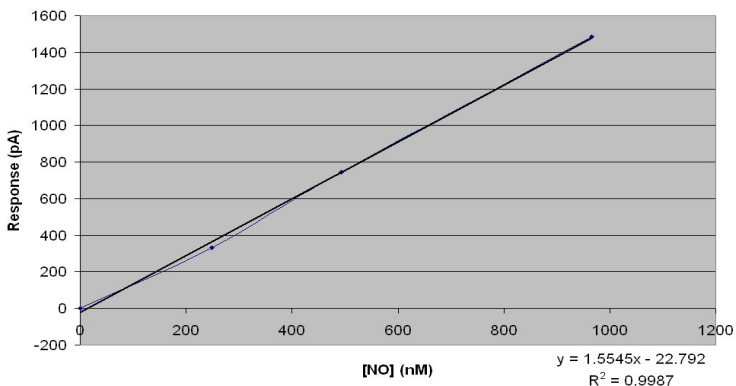


Fig. 4—Calibration Output

The slope of this curve indicates the sensitivity of the sensor. Once the sensitivity of the probe has been ascertained, the sensor is ready to use experimentally. (In the above example, the sensitivity was 1.55 pA/nM.)

## SENSOR CARE AND MAINTENANCE

### Durability and Handling

The sensor is relatively durable, except for the membrane sleeve. Exercise caution when handling any NO sensor to avoid actions that could damage the sensor tip. Pay particular attention to the sensor membrane, because the membrane coatings are 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.

### Cleaning the Sensor

Immediately after calibration, rinse the sensor with deionized water and dry it with a soft tissue or KimWipe. If the sensor membrane is punctured, calibration solution

(H<sub>2</sub>SO<sub>4</sub>) may leak into the sensor sleeve, which can lead to a high background current. Discoloration of the sensor is a good indication that this has occurred. Permanent damage to the sensor will occur if the sensor is left in the sleeve under this exposure.



Fig. 5—The good sensor is shown on the top, and the sensor on the bottom has been exposed to the calibration solution for far too long.

## 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. **Do NOT use the probe in organic solvents.**

## 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 sensors, and do NOT expose them to organic solvents

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## Replacing the Membrane Sleeve

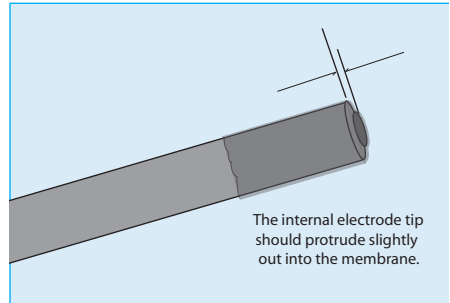
Even with the best of care and proper maintenance, the membrane sleeve will eventually need to be replaced.

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#**5436**) may be purchased separately.
7. Dip the internal electrode 1-2cm into the **ISO-NOP** electrolyte (WPI #**7325**) provided in the **ISO-NOP** start-up kit. The current will rise rapidly offscale. Using the MicroFil™ nonmetallic syringe needle (WPI #**MF28G67-5**) and 1 mL 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. 6**).
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-NOP** membranes (packages of 4) are available from WPI (#5436).



*Fig. 6—Membrane placement. The internal electrode tip should protrude slightly out into the membrane.*

## ACCESSORIES

**Table 1: Accessories**

Part Number	Description
5399	T-Adapter Kit (pkg. of 3)
5435	ISO-NOP Startup Kit*
5436	Replacement Sleeves (pkg. of 4)
7325	ISO-NO Electrolyte Filling Solution (10mL)
7357	Nitrite Standard Solution, 1g/L (100mL)
7363-4	Enzol - Enzymatic detergent (1 gal.)
7521	ISONO Electrolyte, CO <sub>2</sub> insenstive Filling Solution (10mL)
MF28G67-5	MicroFil electrolyte filling needle (pkg. of 5)
NSA-3	Pre-Polarizer – 3 channel, ISO-NO Activator

\*The **ISO-NOP** start-up kit (WPI #5435) contains everything needed to begin working with this sensor, and the kit is highly recommended for first-time **ISO-NOP** users (Fig. 7). The kit includes:

- **5436** - Five additional membrane sleeves (Sold in packages of four sleeves.)
- **7325** - 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**
- Two 25g needles and one male luer fitting (1/8" ID barb) used when purging a solution with NO gas as described in the TBR4100/1025 manual in the "Preparing an NO Standard using NO Gas" section.



Fig. 7–ISO-NOP Startup kit

## 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 865 mV. (For the TBR, choosing the NO sensor setting selects 865 mV automatically.) Set the range at 10 nA.
	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. See “Calibrating the Sensor by Chemical Generation of NO” on page 6. 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 0.1 M PBS only. 0.1 M H <sub>2</sub> SO <sub>4</sub> + 0.1 M KI.
	External electrical interferences may be the problem.	Identify and isolate electrical interferences.

Calibration dataset is not linear	The dilution factors may be incorrect.	See “Calibrating the Sensor by Chemical Generation of NO” on page 6. 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 12.
High background current	Calibration solution may have leaked through a hole in the membrane and be contaminating the sensor.	If the membrane is punctured and calibration solution leaks into the sensor, it can cause discoloration and permanent damage to the sensor. Clean the sensor with deionized water. If the background current remains high, replace the sensor. See “Cleaning the Sensor” on page 10.

## Dry Sensor Test

To determine if the sensor itself is defective or has a short, you may perform a dry sensor test on an ISO-NOP sensor without a sleeve.

- Carefully remove the membrane sleeve. See “Replacing the Membrane Sleeve” on page 12.

**NOTE:** If the sensor has not been used for several months, the electrolyte solution may have dried out. If this is the case, the membrane sleeve is more difficult to remove, because it tends to stick to the sensor. Remove it gently to avoid damaging the delicate sensor tip.

- Remove any crystals and lightly wash the sensor with distilled water.
- Allow sensor to air dry.
- When the sensor is completely dry, plug it into the free radical analyzer.
- Turn on the free radical analyzer. You should see a near zero reading of less than 200 pA. If the reading is higher, the sensor is defective and needs to be replaced.
- If the sensor passed the test in step 5, insert the sensor into a vial with ISO-NOP filling solution. The free radical analyzer should immediately display a high nanoamp reading. If the analyzer is set in the 10nA range, the reading should be off scale. If the reading is not off scale, the sensor is defective and needs to be replaced.

- 
7. If the sensor reading was near zero when dry and extremely high in solution, the sensor itself is probably working properly. Install a new membrane sleeve. See “Replacing the Membrane Sleeve” on page 12.

**TIP:** Do not reuse an old membrane, because the membrane itself is stretched once it is used. After it is removed, it maintains the surface deformity and will not provide accurate test results if reused.

**NOTE:** If you have a problem/issue with your **ISO-NOP** sensor that falls outside the definitions of this troubleshooting section, first perform the Calibration Procedure exactly as describe on page 7 of this manual and contact the WPI Technical Support team at 941-301-1003 or [technicalsupport@wpiinc.com](mailto:technicalsupport@wpiinc.com).

## SPECIFICATIONS

The **ISO-NOP** sensor conforms to the following specifications:

Outside Diameter	2 mm
Response Time	< 5 sec
Lowest Detection Limit/Range	1 nM
Upper Detection Limit/Range	80 $\mu$ M (beyond 80 $\mu$ M, sensor accuracy is not guaranteed)
Nominal Sensitivity (New sensor)	$\leq 2$ pA/nM
Interferent (Selectivity Coefficient)	NaNO <sub>2</sub> ( $10^{-6}$ or better)
Drift	<1 pA/min.
Poise Voltage	865 mV
Typical Quiescent Baseline Current, 25°C	3,000 pA
Acceptable Baseline Range	1000-8000 pA
Polarization Time	12+ hours
Recommended Polarization Solution	0.1 M PBS
Temperature Dependent	Yes
Physiological Interference	None

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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 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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