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# ISO-NOP200

*Microsensor for NO Measurement*



## INSTRUCTION MANUAL

Serial No._____

031502

***World Precision Instruments***

## 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.*



## Unpacking the ISO-NOP200 Sensor

Due to the ISO-NOP200's extremely small size and intricate construction, great caution should be used when handling it. Avoid bringing the sensor tip into contact with anything, since this may break it. The sensors are packaged so that their tips are exposed and protected from contact with the container. To remove an ISO-NOP200 sensor from the package, use the thumb and index finger of one hand to separate the slit in the sponge 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 ISO-NOP200 to the Microprobe Handle

Once removed from the package, the ISO-NOP200 sensor should be plugged into the microprobe handle which is connected to the ISO-NO meter, again being very careful that the sensor tip does not come into contact with anything. The sensor should plug in easily. If you encounter resistance, it is probably due to the misalignment of the sensor with the connector inside the microprobe handle. Simply realign the sensor by gently rotating it until it snaps into place.

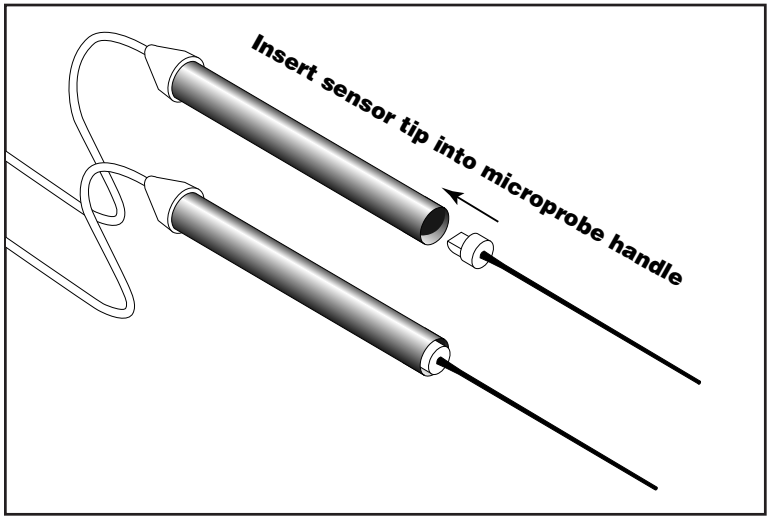



*Fig. 1 — The ISO-NOP200 offers unprecedented performance in making real-time* in situ *measurements — thanks to its proprietary selective coatings and unique construction, combining both working and reference electrode as well as electrostatic shielding into a single sensor structure.*

With the ISO-NOP200 sensor connected to the meter you may observe the current go offscale. The background current decay period varies in length, but for a new ISO-NOP200 sensor being polarized for the first time it may take several hours. Once the background current has decayed to a low stable value (usually less than 2 nA) the ISO-NOP200 sensor is ready for use.

## Using the ISO-NOP200

First, calibrate the sensor using the NO calibration kit included with the ISO-NO. (The calibration kit, part #15829, may also be purchased separately.) See page 3 for instructions on calibrating the sensor.

After completing the calibration, you may place the sensor in the experimental set-up and commence NO monitoring as explained in the ISO-NO instruction manual.

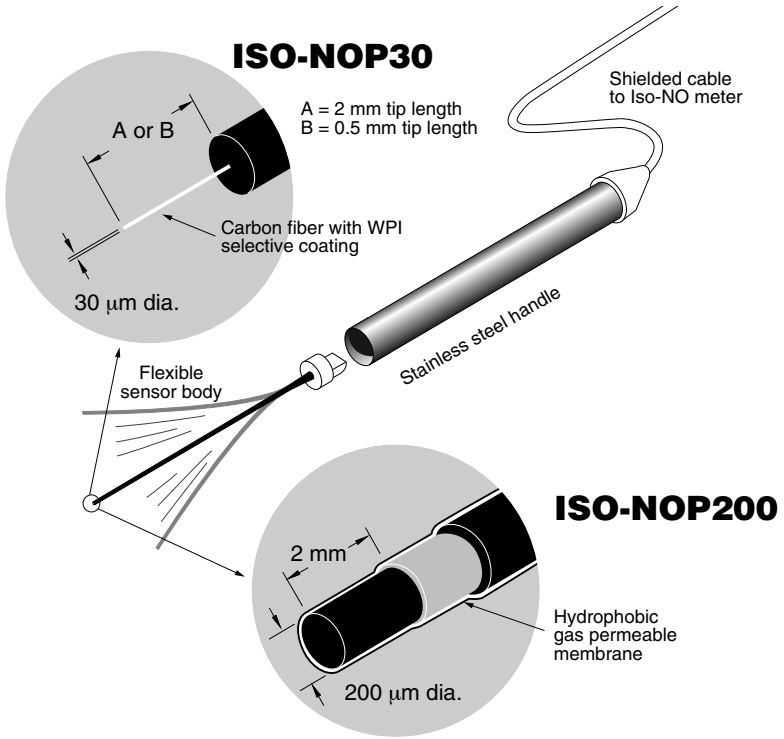
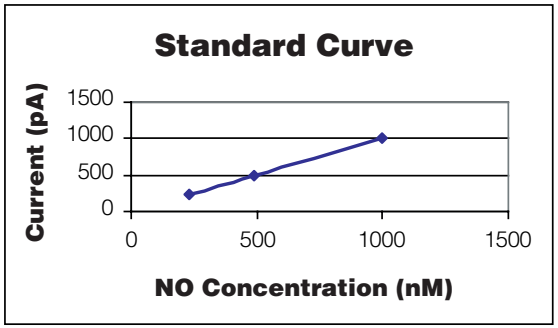



*Fig. 2 — ISO-NO's microsensors may be changed or replaced quickly and easily.*

| [SNAP] | [NO] = 0.539 X [SNAP] | Output Current |
|---|---|---|
| 232.4 nM | 125.3 nM | 230 pA |
| 462.0 nM | 249.0 nM | 488 pA |
| 916.8 nM | 494.2 nM | 1001 pA |




$Y = -52.1 + 2.1 X \quad R^2 = 0.997$

The data from calibration curve indicates that this procedure allows precise calibration of NO probes ($R^2$= 0.997). The accuracy of calibration is not as significant as precision and could deviate approximately +/- 10% from mean. The source of error is most probably arises from gravimetric measurement of standard reagent, SNAP. In addition, purity of SNAP as well as partial oxidation of O generated NO in the calibration solution could have been other plausible sources of error. Such deviation may not be so important when NO is quantified in biological systems because usually the ability to measure changes in the basal level of NO is more significant than the absolute level of NO.

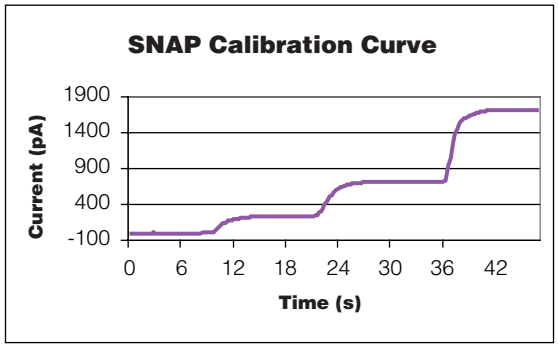
| % of detectable NO / mole of SNAP | | % of detectable NO / mole of SNAP | |
|---|---|---|---|
| Exp.1 | 59.1 | Exp.11 | 57.9 |
| Exp. 2 | 49.2 | Exp. 12 | 53.1 |
| Exp. 3 | 55.5 | Exp. 13 | 51.4 |
| Exp. 4 | 48.9 | Exp. 14 | 44.4 |
| Exp. 5 | 61.6 | Exp. 15 | 59.8 |
| Exp. 6 | 57.7 | Exp. 16 | 56.0 |
| Exp. 7 | 46.5 | Exp. 17 | 48.8 |
| Exp. 8 | 54.8 | Exp. 18 | 51.7 |
| Exp. 9 | 58.7 | Exp. 19 | 53.0 |
| Exp. 10 | 56.6 | Exp. 20 | 53.6 |
| AVG | 53.9 +/- 2.1 | SD 4.70 | S.E.M. = 1.05 |

At 95% confidence interval:

| | | | | | |
|---|---|---|---|---|---|
| Mean | = | 53.9 | S.E.M. | = | 1.05 |
| SD | = | 4.70 | N | = | 20 |

Example for creating a calibration curve and related computations:

1. SNAP weight = 6.4 mg.
2. SNAP was dissolved in 250 mL solution #1 to obtain the standard stock solution.
3. 10 μL, 20 μL, and 40 μL of SNAP stock were added sequentially into 10 mL of solution # 2.
4. The current was continuously recorded during the course of the calibration.
5. A standard calibration curve was constructed according to the recorded date.




## Storage and maintenance of the ISO-NOP200

When not being used for a short period of time (such as overnight), the ISO-NOP200 should remain attached to the microprobe handle and kept dry (not immersed in solution). Before the next experiment, immerse the sensor in the experimental solution (e.g., Kreb's Buffer); the background current will increase until reaching a stable value. Do not be alarmed if the background current becomes elevated. This is associated with the hydration of the sensor and will not negatively affect the sensor's performance.

If the ISO-NOP200 sensor 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 ISO-NOP200 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. If you exercise caution when working with the ISO-NOP200, this unique sensor may last for an extended period of time.

***WARNING: Nitric oxide must be handled only in a well-ventilated area, usually a laboratory fume hood with forced ventilation****. The U.S. Occupational Safety and Health Administration has set a time-weighted average maximum NO value as 25 ppm. That is to say that 25 ppm is cited as the maximum concentration to which workers may be continually exposed. Brief inhalation of concentrations as low as 200 ppm could produce delayed pulmonary edema which may be fatal after an asymptomatic period of up to 48 hours after the initial exposure.* ***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.*

## Calibration with aqueous standards prepared with NO Gas

*(NO sensors that can be calibrated with this method: ISO-NOP, ISO-NOP200, ISO-NOP3020, ISO-NOP3005)*

## Preparing an NO Standard

This method has the advantage of allowing the user to calibrate ISO-NO in the same environment in which the experimental measurements will be made. It has the disadvantages of added cost, inconvenience, and greater hazard to the user. All of these factors must be taken into consideration. The setup for preparing a saturated NO aqueous solution is illustrated in Figure 3.

1. **Be certain the fume hood is functioning.** See figure 3.
2. Make sure that all fittings and connections are secure. The tubing to be used should not be permeable to NO. We recommend Tygon® tubing if a polymer tubing is to be used; this is permeable to NO but has the best performance compared to other polymer tubing of which we are currently aware. Ideally glass tubing should be used. If Tygon® tubing is used, note that prolonged exposure to NO affects its properties; therefore it is recommended that the tubing be inspected frequently and that it be replaced when it appears to be brittle. The

**Lecture bottle of NO (14.2 liters, 98.5%) obtained from Aldrich, catalog #29.556-6; telephone 800-558-9160*



## Predicting the Level of Detectable NO According to the Molar Ratio of SNAP in the Presence of Catalyst

A calibrated NO sensor was used in a series of experiments to measure SNAP decomposition in the presence of copper sulfate. The data from these experiments indicated that SNAP is decomposed instantaneously under the following set of parameters:

| | |
|---|---|
| Temperature | 25 degree Celsius |
| Catalyst | 0.1M copper sulfate |
| pH | 4.0 |
| SNAP concentration | 0.0 – 2.0 μM |
| Note: | Copper sulfate is at equilibrium with ambient air. |

SNAP (RSNO) decomposes to NO and a disulfide byproduct according to the following equation:

***2RSNO  2NO + RS-SR***

Theoretically, the concentration of generated NO should be equal to the final concentration of SNAP in the copper sulfate solution in the calibration vial if the decomposition goes to completion and if the generated NO is detected quickly before it is getting oxidized to nitrite and nitrate.

It is expected that the level of detectable NO will be below the theoretical vale because the copper sulfate solution was at equilibrium with ambient air, and consequently a portion of generated NO had already been converted to nitrite and nitrate even before it was measured by the calibrated probe. In addition, it is possible that decomposition of SNAP does not go to completion even in the presence of a catalyst. Further investigation is necessary to evaluate the kinetics of SNAP decomposition in the presence of a catalyst in an anoxic environment.

The following data is only applicable for calibration of a probe in a solution which is at equilibrium with ambient air. The data suggests 53.9% of SNAP can generate detectable levels of NO. In other words, only 0.539 mole of NO is generated per mole of SNAP under the proposed set of parameters. The other 46.1 % of SNAP is either not decomposed or it is decomposed to NO and RS-SR but the NO oxidized prior to being detected by the probe.

Table below represents the spread of normalized data for the mole percentage of measured NO per mole of SNAP in 20 different experiments.

### Calibrating a probe

Place 10.0 mL of solution #2 in a 20 mL vial ( supplied in the calibration kit). Drop a small stirring bar into the solution, and place the vial on the top of a magnetic stirring plate. Immerse a NO probe into this solution, and while stirring, allow the background current to stabilize for about 3-5 minutes. As soon as the background current becomes stable start the recorder.

Next, sequentially inject three aliquots of SNAP solution, 5μl, 10 μL, and 20 μL, into the vial containing copper sulfate solution. The background current rapidly increases upon addition of first aliquot and will reach a plateau within a few seconds. Inject the second aliquot, 10 μL, as soon as the first signal reaches a plateau. Finally add the third aliquot as the second signal reaches its plateau. If aliquots are not added promptly when reaching the previous plateau, the signal will slowly decline because generated NO is quickly oxidized to nitrite and nitrate which will no longer can be detected by the probe.

*Note: You can change the volume of injected aliquots according to the concentration of SNAP stock solution. Also, decrease the volume of aliqot if the electrode is very sensitive or increase the volume of aliquot if the electrode is less sensitive.*

Because NO sensors can be calibrated in a linear fashion, the magnitude of every signal should almost double as the volume of SNAP solution added is doubled in the course of the calibration. Use the recorded data to construct a calibration curve. The calibration curve can be simply constructed by plotting the magnitude of a signal in picoamperes vs. final concentration of SNAP for that particular signal. Note that every addition of SNAP solution corresponds to a particular NO concentration. This will be discussed below. After the sensitivity of a probe is determined, the meter can be calibrated accordingly to display concentration rather than the redox current.

The standard SNAP solution can be used for the calibration of NO probes throughout the day. Store the solution in the dark and refrigerate when not in use. Prepare a fresh stock solution of SNAP in the beginning of every day to ensure minimal decomposition of SNAP in the stock solution. Concentration of SNAP decreases to 5-10% of its nominal value after approximately 4-5 hours.



pressure regulator and tee purge adaptor should be stainless steel. This is because nitric oxide is a corrosive gas.

3. Prepare 100 mL of a 10 % (by weight) KOH solution and place it in the sidearm flask as illustrated above. The flask should be sealed with a stopper through which the tubing passes by means of a Luer fitting to a syringe needle which extends almost to the bottom of the flask. Tubing is used to connect the side arm of the flask to the vial containing the water to be equilibrated with NO. The KOH solution is used to remove other nitrogen oxides from the NO gas.
4. Place 20 mL of distilled (preferably deionized) water in a small glass vial. Seal the vial with a stopper and insert through the stopper a long syringe needle which extends almost to the base of the vial. Connect this syringe needle to the tubing from the KOH flask as illustrated. Insert an additional shorter syringe needle which **should not extend into the solution.** This acts as a pressure relief during purging.
5. Place the distilled water vial in an ice-water bath. Reducing the temperature increases the solubility of NO in solution. Thus when the solution is used at room temperature you will be assured of a saturated NO solution.

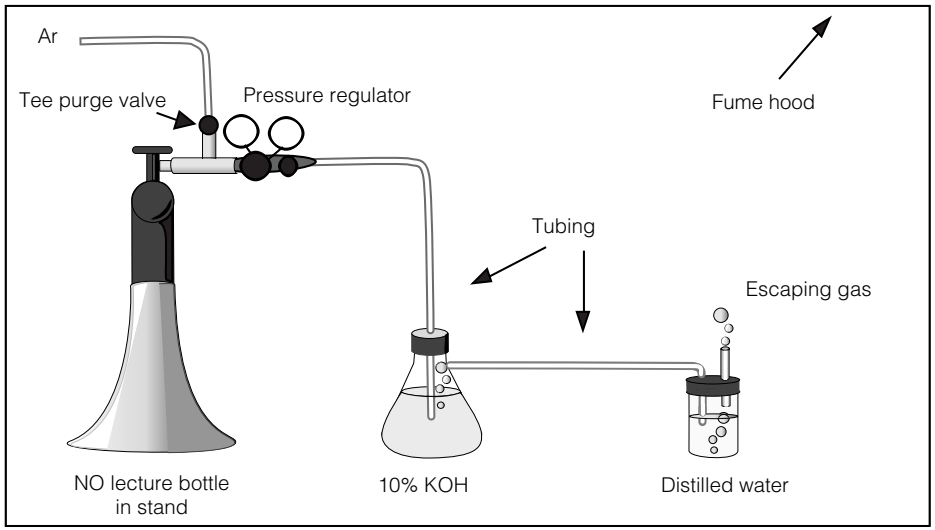



**Figure 3. Setup for preparing a saturated NO aqueous solution must be in a fume hood with forced ventilation. Nitric oxide is highly toxic and, as illustrated, escapes into the atmosphere during preparation of the standards.**

6. Purge the system with argon (or nitrogen) gas for a period of 30 minutes at a moderate flow rate such that the pressure is maintained at a safe level (1-2 psi). When purging it should be observed that gas is indeed bubbling through the KOH solution as well as the distilled water. After 30 minutes turn off the argon source, and switch the tee purge valve to the correct position for purging with NO from the lecture bottle.
7. Purge the system with NO for 5-10 minutes if using a pure source (longer if the NO source is not pure). Again make sure that gas is bubbling through both solutions. **Warning: NO is now escaping from the pressure relief needle in the stopper of the distilled water vial. It is imperative that the fume hood be running at maximum capacity with the front panel closed (in the down position).**
8. After the time in step 7 has elapsed turn off the NO source.
9. Immediately remove the two needles from the distilled water vial.
10. Set the tee purge valve for purging with argon (or nitrogen) gas, and turn on the argon source. Purge the system for 5-10 minutes at a moderate flow rate. Gas should be bubbling through the KOH and then escaping from the flask into the atmosphere. Again be sure that the fume hood is ventilating well.
11. Turn off the argon (or nitrogen) source, and allow the fume hood to continue to ventilate for 10-15 minutes so as to ensure that all traces of NO gas are removed from the atmosphere.
12. The solution of distilled water should now be saturated with NO. The concentration of NO produced by this saturation is dependent upon the temperature. At 0° C, the concentration is approximately 3.3 mM, and at 20°C the concentration is approximately 1.91 mM.
13. Dilutions of known concentration can be prepared from this saturated solution In preparing a dilution, **be careful not to unseal the vial,** for this exposes the solution to atmospheric oxygen.

Once the dilutions are prepared, it is a simple matter to calibrate the instrument.



## Calibrating by decomposition of a S-nitrosothiol compound, SNAP

*(No sensors that can be calibrated with this method: ISO-NOP, ISO-NOP200, ISO-NOP3020, and ISO-NOP3005)*

S-Nitriso-N-acetyl-D,L-penicillamine (SNAP) is a stable NO containing compound that can be used for quantitative generation of NO in solution. SNAP decomposes to NO and a disulfide byproduct when dissolved in water. However, the rate of decomposition is very slow. The kinetics of decomposition for this reagent is a function of several parameters including pH, presence of a catalyst, and temperature.

In the procedure described below, SNAP is used in combination with a catalyst, copper sulfate, to generate a known quantity of NO in solution. **Note that this protocol does not investigate the effects of all parameters involved in SNAP decomposition nor does it propose a model by which NO is decomposed.** The presented procedure provides an empirical estimation of the amount of generated NO based on the molarity of a standard stock solution of SNAP under a controlled set of parameters.

## Getting Started

### Prepare the following solutions:

Solution #1: Adjust the pH of 250 mL of water (preferably HPLC grade) to 9.0. Dissolve 50 mg EDTA in this solution.

Solution #2: Prepare 250 mL 0.1 M copper sulfate in distilled water. Adjust the pH to 4.0 by addition of Sulfuric Acid

### Preparing standard SNAP solution:

To prepare the stock solution of SNAP, weigh approximately 5.0mg +/- 2.0 mg of SNAP and add it to solution #1. Calculate the Molarity of SNAP solution (SNAP f.w. = 220.3). Decomposition of SNAP in the stock solution proceeds very slowly due to the basic pH of the solution and also because of the presence of chelating reagent, EDTA. Thus the rate of decomposition is negligible and the stock solution of SNAP remains relatively stable for at least 3-5 hours.

Note: The purity of standard reagent, SNAP, is very important for the reported data. Use high grade SNAP with minimal purity of 95% or better. SNAP can be purchased from WPI. Catalog # SNAP10 - SNAP25 - SNAP100.