inNO-T, Nitric Oxide Measuring System with Temperature Recording & Compensation.
&
amiNO Series of Nitric Oxide Sensors

Instruction Manual

Serial No…………………..

Model inNO-T

Innovative Instruments, Inc.
8533 Queen Brooks Court, FL 33637. USA
Phone: 813-988-2477. Fax: 813-914-8686

inNO, Nitric Oxide Measuring System and Sensors
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Introduction

inNO and the accompanied amiNO series of nitric oxide (NO) sensors constitute a complete system for the in situ detection and measurement of NO. inNO is a battery powered A/D converter that has two functions, namely, controlling the necessary instrumental parameters to measure NO (as NO meter) and a data acquisition system to record, display, and treat the data (as recorder). The system is supplied with all the software and the necessary cables. The amiNO series of NO sensors are the most sensitive and durable NO electrochemical sensor ever commercialized. When used properly, the sensors are able to provide accurate, fast, and selective NO measurements.

The amiNO series of NO sensors are amperometric sensors and covered with a series of membranes to assure the selectivity of the measurements. The basic principle of their operation is based on the diffusion of NO through these membranes from the sample solution to the sensor surface. An electrical potential is applied to the sensor's sensing element and forces NO to lose electrons to the sensing element. This results in an electrical current that is recorded. The magnitude of the electrical current is proportional to the amount of NO diffused through the membranes which is dependent on the concentration of NO in the sample. Consequently, the electrical current (analytical signal) is proportional to the concentration of NO in the sample.

It is crucial to read this manual in order to use the system successfully.

General Instructions

inNO, is a complete computerized system powered by six AA batteries. It has two major functions. First: controlling and delivering the necessary instrumental parameters to the accompanied nitric oxide (NO) sensors to measure the concentration of NO and second: operating as a data acquisition system (recorder). Consequently, no recorder or other instruments are needed except a computer operating on **Windows 95/98/2000 or NT**.You have got a complete system.

Setting Up the System:

1. Install the software into a computer. Follow the installation instructions provided on the accompanying CD.

2. Pull out the two supporting pieces underneath the front corners of the inNO system to get much better view of the front panel.

3. Connect inNO to the computer using the provided RS-232 cable. The connection port is located on the back of inNO. Connect the other side of the cable to the COM port located on the back of the computer. If your computer has more than COM port, note the port number (COM1, COM2, etc.)

4. Connect the provided meter grounding cable. Insert the pin connector into the ground port located on the back of inNO. If a lab top or a notebook computer is used, connect the alligator clip of the grounding wire to the ground port of the electrical power outlet or to the chassis of other grounded equipment. If a PC is used, connect the alligator clip of the grounding cable to the chassis of the computer, because the PC is grounded, or to the ground port of the electrical power outlet.

5. Turn power knob of inNO **ON**. NOTE: **We recommend keeping the meter ON all the time.**

6. Select the frequency of the Notch filter. 50 Hz in Europe and other countries. 60 Hz in the United States.

7. Select the current range on inNO. The lower scale can measure up to 250,000 pA (250 nA), enough current range for most of the experiments. The higher level can measure current up to 2,500,000 pA.

8. Open the inNO file as follows: From the **START** button go to **PROGRAMS**, choose inNO then double click the **inNO icon**. If the inNO software is not located in the **PROGRAMS** list and you do not know in which folder it has been installed go to **START** then **FIND** and look for inNO.

9. When the inNO window is open, and the meter is connected click the **ON** button located on the upper left corner of the screen.

10. Form the menu choose **SETUP** then **COM port**. Make sure that the meter is connected via RS-232 to the right specified port, normally COM1. If the computer has more than one COM port, the port number will be written on the back of the computer. For example, if the RS-232 is connected to COM3 port, COM3 port should be chosen from the **SETUP** menu.

11. Choose the recorder settings and parameters such as the speed of recording, current scale, line width, etc. The parameters can be changed during or after the experiment

a. The Time Scale (Chart Speed): Click once on **Time/DIV(X-axis).** A menu will appear. Choose an appropriate scale by clicking on the desired number. The smaller the number, the faster the chart speed. Normally, 50 or 100 s per division. If a very low concentration (under 10 nM) is expected, a scale of 100 or slower is recommended.

b. The Current/Concentration Scale: Click once on **pA-nM/DIV (Y-axis).** A menu will appear. Click on the desirable scale. We recommend a scale of 2000 for amiNO-IV, amiNO-700, and amiNO-FLAT. A scale of 500 for other sensors. The scale can be changed any time depending on the signal/concentration observed.

c. The Temperature Scale : Click on Temp/DIV. A menu will appear. Click on the desired scale.

d. From the **Setup** menu, select the **Sampling Rate**, normally 2/sec. If you are studying fast chemical reactions, a higher sampling rate can be chosen. Higher sampling rate will take more memory of your computer.

e. From the **Setup** menu, select the **Recording Memory**. A 100 K is enough for more than 13 hours of continuous recording at a sampling rate of 2/sec.

f. From the **View** menu, select the desired reading display, pA (current) or nM (concentration). Both of them will be displayed but, the one you select will be displayed in larger font and the ***y-axis scale will be graduated according to the selected parameter***. pA display is more common. **If you choose to display nM, make sure to change the y-axis scale.**

g. The **View** menu also allows you to set the **chart color, line width, grid on/off**. The View menu also enables the user to turn the **temperature recording display** off. In this case the system will be a single channel recorder. The View menu can be used to display a space for writing notes below the recording. To **print Notes**, go to File then **Print Notes**.

12. Zero the reading of the meter by clicking on the **ZERO** button from the menu. You can **UNZERO** (go back to the reading before zeroing) by clicking on **ZERO OFF**. Now, the system is ready.

Testing the System

After installing the software and connecting the meter to the computer, turn the meter ON (on the front panel) and the software ON (upper left corner of the software window). Connect the accompanying potential measuring cable (PMC, stereo plug in one side, two alligator clips on the other) to the front panel of the meter instead of the sensor. Connect the alligator clips to the terminals of the 10 megaohm provided resistor. If the current reading on the computer screen ranges between 80,000 and 90,000 pA then they system is functioning very well and ready for the sensors and experiments. **This test can be done any time the user suspects a problem.**

amiNO Series of Nitric Oxide Sensors

General Information

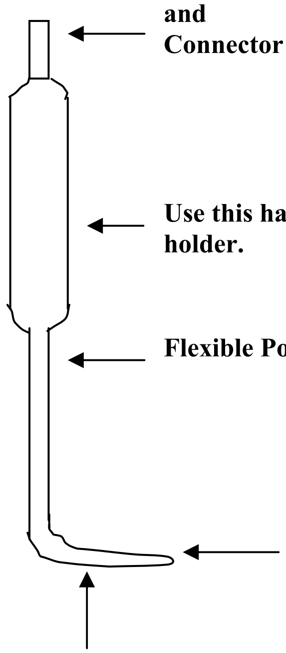
The amiNO series of nitric oxide (NO) is very useful if used properly. Make sure to read the instructions and to familiarize yourself with the system.

The current available sensors are integrated sensors, meaning that there **is no need for additional reference or counter electrode.** The sensing element, located at the tip of the sensor, is completely covered with a series of membranes including a gas permeable membrane to assure the selectivity of the measurements. The integrity of the sensor must be preserved. If any breakage or disintegration occurs, the sensor is not useable. Testing the integrity of the membrane is explained below.

Handling the Sensors:

In spite the fact that most of the amiNO sensors utilize a mechanically tough membranes, care must be taken not to damage them. All the members of the amiNO series of NO sensors, except aminNO-FLAT and amiNO-2000 have flexible bodies for easy experimental manipulations. They can be bent and made into L-shape. A certain portion of the tip must stay straight in order to avoid damaging the sensing elements as shown in the figure below.

Bending and Manipulating the Sensors



Solutions Required for Calibration

1. 1 M Sulfuric acid. 1 M concentration can be prepared from a concentrated sulfuric acid solution (96%) as follows: Add about 800 ml distilled water to a 1 liter volumetric flask. It will be very helpful to surround the flask with ice/water mixture because the dilution of the acid produces large amount of heat. Add 55.5 ml concentrated sulfuric acid **slowly and carefully** to the distilled water in the volumetric flask with **continuous mixing**. Wait until the solution cools down. Fill the flask to the mark with distilled water.
2. Solid potassium iodide. Sodium iodide can be used.
3. Nitrite Standard Solution in the range of 10.0 mM (0.0100 M).

Preparation of Calibration Solutions.

Add 18.0 ml distilled water to a 20 ml vial. Add 2.0 ml of 1 M sulfuric acid. Add approximately 20 mg potassium or sodium iodide. A small stirring bar should be used for mixing and obtaining a uniform solution. Place the vial on a magnetic stirrer.

Note:When this solution becomes light yellow, due to the formation of iodine in the solution, prepare a new solution.

Nitrite Standard: add 100.00 microliter of 0.0100 M potassium nitrite to 9.90 ml distilled water. This results in nitrite concentration of 100.0 micromolar.

Using and Calibrating the Sensors and the System:

Before calibrating and using the sensor, the sensor should have been polarized for few hours, preferably overnight, by connecting to SensoReady or to inNO system and immersed in water. The longer the polarization time, the more stable and reproducible the response of the sensor.

For NO measurements, <u>DO NOT</u> adjust the applied potential, Vw Adjust. The potential has been selected to measure NO.

<u>Suggested</u> Parameters for Calibration of the Sensors

Sensor	Current Range pA/division	Calibration Concentrations, nM	Typical Background current at 25 C, pA
amiNO-100	500	100, 200, 400	Under 10,000
amiNO-700	2000	50, 100,200	Under 40,000
amiNO-IV	2000	50,100,200	Under 40,000
amiNO-2000	500	250,500,1000	Under 5,000
amiNO-FLAT	5000	50,100,200	Under 100,000
amiNO-30	200	100,200, 400	Under 2,000
amiNO-7	200	100, 200, 400	Under 1,000

1. Connect the sensor to the meter by inserting the sensor's connector into the port labeled sensor on the far left corner of the front panel of inNO.

2. Fix the sensor on the electrode holder.

3. When the background current is stable with reasonable value (see the table in the previous page), start recording by clicking on the **REC** button. **NOTE: You can start recording at any current level.** Make sure that the recording is displayed on the screen by using the up/down vertical bar located on the right of the screen.

4. Immerse the sensor in the calibration solution by lowering the electrode holder. Immerse the tip of the sensor and part of the black flexible body in solution. If the solution is not deep enough, when measuring real samples, immerse at least the whole clear plastic tip (amiNO-700, amiNO-IV, amiNO-FLAT) ,the black tip covered with a white coating (amiNO-100), or the golden tip covered with white coating (amiNO-30 and amiNO-7). For amiNO-2000, 2-3 mm of the tip is Ok. . Avoid hitting the sensor's tip by the bottom of the vial.

5. Upon immersion in solution, the background start to increase then goes down. Observing the increase and decrease of the background will benefit your understanding of the sensors, a reason for recording!!. The time needed for such increase and decrease depends on how long the sensor was kept disconnected and/or dry. Note if the sensor was stored disconnected and dry (was not kept immersed in distilled water) for few days, we recommend connecting the sensor for longer time, e. g. overnight. There is no danger of connecting the sensor for extended period of time, a week for example. In general we recommend connecting the sensor to the system or SensoReady and immersing in the calibration or aqueous solution overnight.

6. Wait for the background to decrease to a stable value. As mentioned earlier, the longer the sensor kept immersed in solution and connected to the system, the better its performance.

7. **Zero the background by clicking the ZERO button while recording.**

8. The in situ generation of NO is achieved by the addition of standard nitrite solution to an acidified solution in the presence of a reducing agent such as iodide ion according to the following equation:

$$\mathbf{2\,NO_2^- + 2I^- + 4H^+ \rightarrow 2NO + I_2 + 2H_2O}$$

As shown in the chemical equation the mole ratio of nitrite to nitric oxide is 1:1 consequently, the amount of nitric oxide generated **equals** to the amount of nitrite added.

<u>NOTE: We recommend using calibration concentrations in the range of the test solution. For example, if the user expects an NO level around 100 nM, we recommend using calibration concentrations of 50, 100, 150, and 200 nM.</u>

9. Add 10.0 microliter (0.010 mL) of nitrite standard solution **having a concentration of 100 micromolar** to the 20.0 ml stirred calibration solution this results in the generation of 50.0 nanomolar (nM) NO in the solution. This can be calculated as follows:

   Initial concentration X initial volume = final concentration X final volume
   100 micromolar X 0.01 mL = Final concentration X 20.01 ml

   $$\text{Final concentration} = \frac{100 \times 0.01}{20.01} = 0.05 \text{ micromolar} = 50 \text{ nanomolar}$$

10. Before the second addition, it will be very helpful to understand the response of the sensor and the loss of NO from the solution. As a beginner, it is recommended to wait until the current decays back to its value before another addition of NO. Introducing another standard addition before the complete decay has minimal effect on the response or sensitivity of the sensor. **There is no problem of adding another addition before decaying of the response (Stair-shape response is observed).**

11. Add 20.0 microliter of the 100 micromolar nitrite standard. A final NO concentration of 100 nM will be obtained. Add more additions if you wish.

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IMPORTANT: How to use the EXAM and CALCULATE functions.
Click on **CALCULATE** and choose **delta** (make sure it has the check mark).
Click **EXAM**. If you want to measure the current and time values at any point simply, place the cursor on the point of interest and read the value in the **button of the screen** (under the X-axis or the space used to write notes). **If you want to measure the peak height or the difference between two points, click on the first point of selection then drag, while holding the mouse button down, to the second point of selection. <u>READ THE VALUE IN THE BUTTOM OF THE SCREEN WHILE HOLDING THE MOUSE BUTTON.</u>**

12. Click the **EXAM** button and measure the peak height of the two additions. **Example**:The 50.0 nM resulted in a current of 6,000 pA. The 100 nM resulted in a current of 12,500 pA. Average sensitivity: at 50 nM is 6000/50.0=120 pA/nM. At 100 nM is 12500/100= 125pA/nM. Average sensitivity is 122.5 pA/nM.
    <u>NOTE: The user can obtain more calibration point as needed. Plotting current vs concentration can be used as alternative to step 13 below.</u>

13. Click **CONV** button. A dialog box will appear in the upper right corner (under calculate help in the main menu) and enter in the dialog box 122.5 pA=1.0 nM. Click **CONV** again. **The sensor and the meter are now calibrated**. Notice that the displayed value of the current on the screen is 122.5 times larger than the concentration value.

The 122.5 value is used for illustration only. The sensitivity of the sensor you are using will be different.

OTHER CALIBRATION METHODS

It is up to the discretion of the user to choose other calibration procedures. Two of them are explained below:

1. Calibration Using Nitric Oxide Gas Dissolved in Aqueous Solution:

Calibration using authentic NO gas dissolved in aqueous solution is much more difficult and less accurate than its in situ generation using nitrite standard. To use this method, pure nitric oxide is bubbled through an **oxygen-free** aqueous solution in a sealed container for 15-20 minutes to produce an NO concentration of 2.0 mM (0.0020 M). **THE PROCEDURE MAUST BE DONE IN THE FUME HOOD**.The problem with this method is the lack of stability of the standard solution and the need to deoxygenate and seal the solution where the sensor is immersed.

2. Calibration Using NO-Producing Agent

Many compounds are now known to decompose in solution to produce certain amount of NO. Example is S-nitroso-acetylpencillamine (**SNAP**). The Method is convenient for users who have good experience with SNAP. The method requires a prior knowledge of the percentage of decomposition.The major limitations of using SNAP include its irreproducible percentage of decomposition and its dependence on the presence of metal ions such as copper ions, in addition of being very costly.

NOTE: The calibration should be done at the same temperature of the sample to be tested.

Calibration and Activation of the Temperature Compensated NO System, inNO-T.

Quick Tips: 2. Every NO sensor must be calibrated for temperature dependence. Each sensor has different temperature dependence. We recommend the calibration every day (or the day the sensor to be used). Connect the sensor to inNO or SensoReady and immerse it in aqueous solution for few hours for best performance.

1. Connect the NO and temperature sensors to inNO. We recommend that the sensors should have been connected for a minimum of 30 minutes before starting the measurements.
2. Attach the NO and the temperature sensors to an electrode holder.
3. Immerse the NO sensor and the temperature sensor in a vial containing about 15 ml of water. The temperature of the solution should be 1-3 degree cooler than the temperature at which your experiment will be performed. For example, if the experiment to be performed at 37 C, start with a solution at 35 C. Place the sensors tips as close as possible to each other. Stir the solution with a magnetic bar.
4. Start recording. Wait until a stable baseline is obtained.
5. Zero the reading by clicking the ZERO function of the software. Keep recording for few minutes.
6. Add 1-2 ml of hot water.
7. The NO sensor background current will increase dramatically as well as the temperature reading, of course.
8. Continue recording.
9. After 2-3 minutes of the hot water addition, click **EXAM.** Make sure that when clicking and holding on **CALCULATE**, the **DELTA** function has the check mark.
10. Place the cursor on a point just before the addition of hot water and drag to a point after the addition of the hot water, normally to the maximum. **Keep holding** the mouse and read the difference in temperature and current in the bottom of the screen. Write down the difference. **Example: If after adding hot water the background current increased by 3168 pA and the temperature changed by 1.9 C then the temperature dependence of the sensor is: 3168/1.95 = 1625 pA/C.**
11. Click on **Temp_Comp** of the software (you do not have to stop recording), and choose **Conversion Factor Setup**. A dialog box will appear. Type 1625, the factor in the above example. Of course, the factor that you find can be different.. Do not type any units or any thing else, just type1625. Click OK.
12. Click again on **Temp_Comp** and choose **Start Point Temp Setup**. A dialog box will appear. Type the temperature you wish to start compensating. In the above example, type 35.0. Usually this temperature is 2-3 degrees lower than the temperature the experiment will be performed. You can change this temperature anytime. Click OK
13. Click on **Temp-Comp** and choose **Temperature compensation mode on**. You will notice the change in your recording immediately. The system will compensate for temperature above 35.0. You will notice that after the temperature compensation

mode is turned on, the background current recording will show a “dip” just above the temperature of the solution before adding hot water, see the last page of the instruction manual. The reason for the “dip” is that the temperature sensor has much faster response time than the NO sensor. To cancel the compensation, click on **Temp-Comp** and choose **Temperature compensation mode off**.

14. After finishing the experiment and by the end of your working day or night! **We recommend turning Temperature compensation mode off.**

NOTE: If you leave Temperature compensation mode on and you shut down the system, you might find difficulty in zeroing the system the next time you start. If you forgot, and the next time you find difficulty in zeroing the system, turn Temp-Compensation mode off and you will be able to zero the system.

APPLICATIONS

1. Selection of the Right Sensor:

The following table guides the user to the selection of the right sensor to your application:

Application	First Choice	Second Choice
Measuring NO released from cultured cells/large flat tissue	amiNO-FLAT	amiNO-700 amiNO-IV
in vivo measurements and other insertion applications	amiNO-IV	amiNO-700
Following minutes changes of NO concentration	amiNO-700 amiNO-IV	amiNO-100
Measuring NO in blood	amiNO-700	amiNO-IV
Measuring NO in very small volumes or restricted spaces	amiNO-100	amiNO-30 amiNO-7
Measuring micro-molar levels of NO	amiNO-2000	amiNO-100 amiNO-30
Following the decomposition of an NO producing compound	amiNO-100 amiNO-700	amiNO-2000 amiNO-IV
Measuring very low levels of NO (below 50 nM)	amiNO-700 amiNO-IV	amiNO-FLAT amiNO-100
Measuring NO from a tissue slice	amiNO-700	amiNO-IV, amiNO-100
Gas phase NO measurements	amiNO-GAS	All others. Short term measurements only
Indirect measurements of Nitrate and nitrite	amiNO-700, depending on the expected level	amiNO-100 amiNO-2000
Training	amiNO-2000	amiNO-700

2. Measurements of NO in Real Samples:

To be successful in using these sensors, the user must be familiarized with the sensors, system, response profile, and the factors affecting the response. Despite the fact that these sensors display little temperature effect, due to their high sensitivity, the temperature of should be controlled throughout the experiment. ***The sensor must be calibrated and its sensitivity must be known before its use in any application. The calibration should be done daily.***

a. Measurements of NO Released from Biological Tissues:

Due to the short life of NO and the complex nature of biological tissues, NO sensor should be placed as close as possible to the source of NO production to minimize the its diffusion path and obtain a high signal. Two schemes can be used:

1. Place the sensor (amiNO-FLAT) is the first choice or amiNO-IV or amiNO-700 after bending them into L-shape) on the top of the tissue. Make sure that there is enough solution to cover the tissue and the electrode. The minimum amount of solution should be used to avoid the dilution of NO. Start recording. When a stable background is reached, stimulate the production of NO by adding an antagonist, drug, or arginine. The more arginine, the higher the amount of NO produced. The user can repeat the same steps but adding an inhibitor to confirm the results.

2. Place the tissue in a small test tube (5 mm ID or less) with minimum amount of solution, enough to cover the tissue and the sensor tip. It will be very helpful if you stir the solution using a small magnetic bar. Wait for a stable background and add the stimulant.

b. Measurements of NO Released from an NO-Producing Agent

Immerse the sensor the sensor in a small vial or a test tube. Wait for a stable base line. Start recording. Add the compound or the catalyst and monitor the production of NO. It is up to the user to use a stirred solution. ***Similar procedure can be used to follow chemical reactions that involve NO.***

c. Measurements of NO Released from Cell Culture

amiNO-FLAT is the best choice for this application. Place the sensor on the top of the cell. Make sure that the distance between the sensing elements and the cells is 100 micrometer or less. The stimulant should be added and distributed over the cells between and around the sensing elements.

If the user fails to observe the release of NO, the user can analyze for nitrite and nitrate in the tissue/cell containing solution, the procedure is discussed below, to figure out the problem. If no signal is observed, indicating the absence nitrite and nitrate, then NO was not produced or it is not free in solution.

d. Measurements of NO in Live organs and animals

amiNO-IV is the best choice due to the availability of a sharp metallic tip at its end which facilitates the insertion process into the tissue. It is highly recommended to have all the black portion of the tip inserted into the sample for maximum signal. If used in a long blood vessel such as an artery, insertion of the sensor parallel to the flow and the vessel is optimal. Small tissues where it is not possible to insert amiNO-IV, the shorter amiNO-700 can be used if a 1 mm hole can be drilled in the tissue. Following the introduction of the sensor into the tissue a stimulant or other treatments can be administrated while recording. The tissue can be grounded if electrical noise poses any problem.

e. Measurement of Nitrite and Nitrate

The amiNO sensors can measure down to 1 nM nitrite, the lowest detection limit for any nitrite measuring method, except gas phase chemiluminescence. The choice of the sensor depends on the expected level of nitrite. amiNO-700 can be used for lower levels. amiNO-2000 can be used for higher nitrite levels. Measuring nitrite is simple and straightforward. Use the same solution used for calibration, sulfuric acid/potassium iodide, except that a smaller volume (e. g. 1.0 ml) should be used to minimize dilution of the sample. Immerse the sensor in the acid/iodide solution. Wait for a stable background. Add a known volume of the sample to the acid /iodide solution. Nitrite in the sample is converted to NO and measured by the sensor.Nitrate in the sample can be measured following its conversion to nitrite by the available methods such as enzymatic reduction or using our nitrate reductor. Following the reduction, the produced signal upon the addition of the reduced sample represents nitrite plus nitrate in the sample.

CLEANING:

The tip of the sensor should be kept clean for optimal performance. A sluggish and slow response indicates the need to clean the sensor tip.

Almost every available cleaning method is applicable to these sensors due to their inert and rigid structure.

1. The sensor tip can be immersed in a protease solution to get rid of adsorbed proteins.
2. The sensor tip can be immersed in strong acids (3-5 M).
3. The sensor tip can be dipped in boiling water, acetone, ether, or ethanol.
4. The sensor tip can be wiped with an ethanol or acetone-soaked cotton swap or other soft material.

All other sensors, except amiNO-GAS can be cleaned using the **first three** methods listed above.

STORAGE (all sensors except amiNO-2000 and amiNO-GAS)

Between experiments, the sensors should be connected to the system or SensoReady and immersed in solution, preferably the same solution to be used in the experiments.

Short term Storage (up to 7 days):

If the user plans to use the sensors within three days, the sensor should be kept connected to the system or SensoReady. The sensors can be immersed in solution while connected few hours before the start of the experiment. There is no danger of keeping the sensors connected for longer period of time.

Long Term Storage

The sensors can be stored in their original shipping box. There is no need to keep them immersed in any solution. amiNO-2000 should kept immersed in solution to avoid the evaporation of the electrolyte. For long time storage, it can be stored dry.

Temperature Effect (If inNO-T, Temperature Compensation is not Used)

The temperature increases the background and the sensitivity. The temperature should be controlled during the experiment. In general, If the sensor you are using has a sensitivity of 100 pA/nM or more, a change of 1 C will result in an error of less than 1%.

Normally, the sudden change in temperature results is a sharp change in the background which does not decay (as long as the temperature does not change again). To know what is the response of the sensor to temperature (pA/C), immerse the sensor in solution and start recording. After a stable background, measure the temperature of the solution. Add hot water, about 0.5 ml of boiling water to 20 ml solution, while measuring the temperature. Example: If the back ground shifted by 600 pA when temperature was changed by 1.5 C, then the sensor has a temperature sensitivity of 600/1.5 = 400 pA/C. If the sensor has an NO sensitivity of 130 pA/nM then a temperature change by 1 C will result in an error of 400/130 = 3.1 nM.

Troubleshooting

Problem	Possible Cause/Solution
Current is zero when the sensor is connected to the meter (before zeroing the meter).	Possible reasons: sensor is not connected properly, the meter is off, the software is off, the sensors wiring is broken. Use the 10 megaohm resistor for testing
Current is very high (more than 200,000 pA) and is not decaying	Membrane is broken. To check for his, remove the sensor from the solution. If the background drops dramatically (from 50,000 pA to 5,000 pA as example, then the membrane is broken Other reason might be that sensor's wiring is not as it should be, in this case the current should read 362,000 or more in solution or in air.
The Sensitivity is lower than expected, the response is slow, and a little noisy.	The sensor was not polarized and/or immersed in solution for long period of time. Solution: Keep the sensor polarized (connected) and immersed in solution for longer time. The sensor tip is dirty, clean as instructed in the cleaning section.
The signal and the background is very noisy	The system is not grounded properly. Check the ground connections of the meter and sensor. Ground the sample solution. In the worst case, use Faraday cage.
computer is jammed.	Exit then re-open inNO.
The reading can not be zeroed with the zero function even if the sensor is disconnected.	Temperature compensation mode ON. Turn it OFF. Remember to turn the temperature compensation mode OFF before shutting down the computer.

SensoReady

SensoReady is a device designed to keep up to three sensors ready for immediate use. It applies the same electrical potential that is applied to the sensor when connected to inNO. When the potential is applied to the sensor, it takes long time for the background to decay and reach a stable value. By connecting to SensoReady, the sensors are given the time to decay. When disconnected from SensoReady and connected to inNO, it takes less than a minute to reach a stable background.
The sensors can be connected to SensoReady and immersed in solution.

The device uses one 9 V battery serves for long time. We recommend changing the battery anually, depending on the usage..

INSTRUCTIONS FOR:
amiNO-2000 Nitric Oxide Sensors

Structure:

The amiNO-2000 nitric oxide sensor is composed of two parts; the sensing elements and the membrane sleeve as shown below:

The membrane sleeve separates the sensing elements from the sample solution to assure selectivity and stability on nitric oxide measurements. The membrane should be free of holes or ruptures. When assembled, the sensing elements slightly extend outside the membrane sleeve and stretch the membrane for optimum performance.

Replacing the Membrane Sleeve

The membrane sleeve should be replaced when the membrane is broken (see step 9 below).
Each sensor is supplied with few membrane sleeves. Each membrane sleeve can serve for very long time as long as it is not broken. To replace the membrane sleeve, follow this procedure:

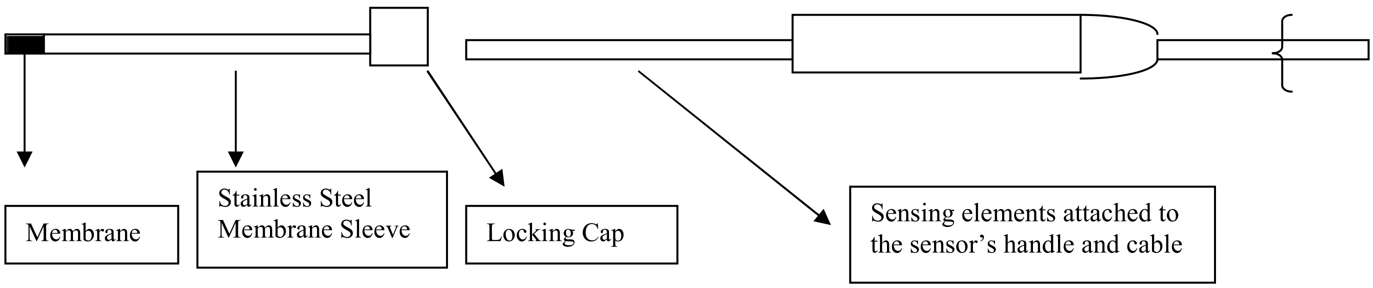
1. Unscrew the locking cap from the handle.
2. Remove the locking cap from the sleeve by sliding it toward the membrane side of the sleeve
3. Pull out the sensing elements from the stainless sleeve.
4. Wash the tip of the sensing element with distilled water.
5. Gently, dry the tip of the sensing element with a soft tissue.
6. Connect the sensor to the nitric oxide meter. The current reading should be around zero. While connected, immerse 1-2 cm of the sensing element tip into the provided electrolyte. The current reading should be very high or off-scale.
7. Pull out the sensing elements from the electrolyte bottle and insert into a new membrane sleeve and tighten the locking cap. The sensing elements will slightly extend and stretch the membrane.
8. When the background current is no longer off-scale and falling, test for the integrity of the membrane by immersing in 1 M NaCl solution or other salt or acid solutions. If the reading goes off-scale instantly, the membrane is broken and needs changing. If the background current goes up by few nanoamperes and slowly, the membrane is OK.

9. Wait for the background current to reach a low and stable value. The time needed to reach a stable value might be 30 minutes or several hours depending on how long the sensor has been kept disconnected.

Cleaning the Membrane:

If the membrane got dirty due to the adsorption of many materials it can be cleaned by rinsing with distilled water, immersing in 1 M acid solution, or soaking in protease solution to remove proteins.

Activation of the Sensor: If the sensor's sensitivity becomes low, remove the membrane sleeve, connect the sensor to the meter or SensoReady and immerse in 0.5 M sulfuric acid for 30-60 minutes, wash with distilled water, and put a new membrane sleeve and electrolyte.

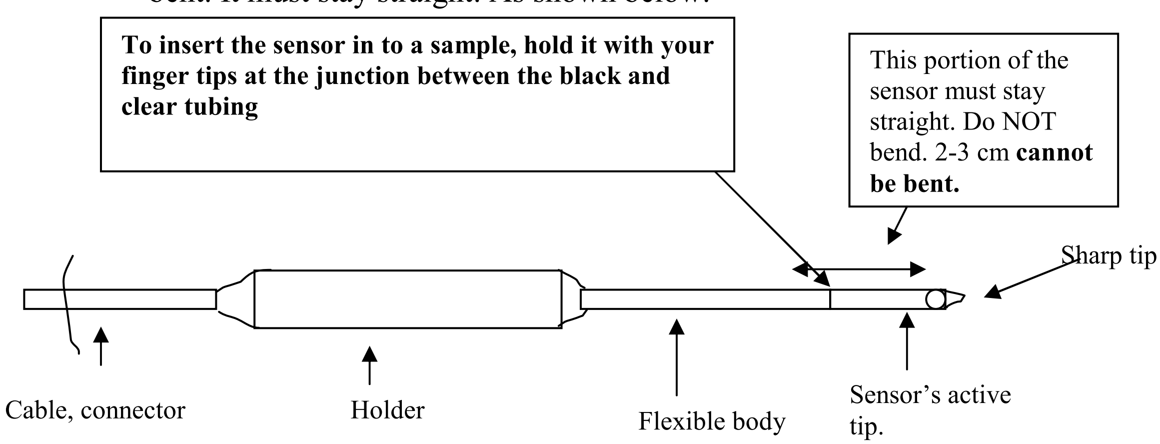


amiNO-2000

amiNO-IV Nitric Oxide Sensor

General Information and Instructions

For most of the applications, the sensor is used **straight without bending**. If the experimental setup requires bending the sensor into L-shape, 2-3 cm of the tip cannot be bent. It must stay straight. As shown below.



NOT DRAWN TO SCALE

Quick Tip: To get the optimum performance out of a newly received sensor, connect the sensor to the meter and immerse it in aqueous solution for few hours, preferably overnight.

Calibration of the Sensor:

Use the same procedures mentioned on page 8-10

Testing the Integrity of the Membrane:

Immerse the sensor in the calibration solution, sulfuric acid/potassium iodide. If the background rises immediately to a high value (over 500 nA and does not decay) and decreases immediately when the sensor is taken out of solution, then the membrane is broken.

Maintenance:

Other than occasional cleaning, amiNO-IV is maintenance-free.

Cleaning:

The sensor can be cleaned with many methods including immersion and wiping with alcohol or acetone, hot water, protease solution, or acid solution.

Storage:

For short term storage, up to seven days, the sensor can be kept connected to the meter and immersed in aqueous solution. For long term storage, the sensor can be disconnected and stored dry.

Sterilization:

The sensor can be immersed for few minutes in alcohol. Please consult us for the use of other methods.

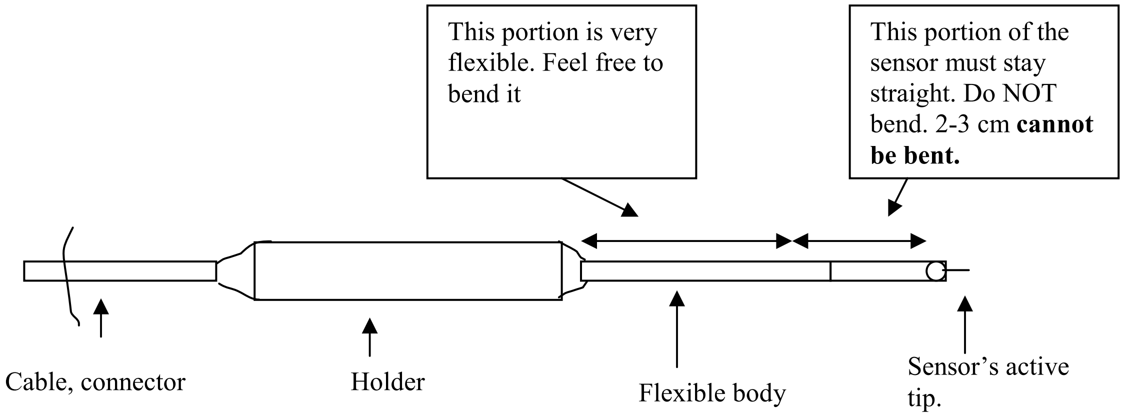
amiNO-100 Nitric Oxide Sensor

A100 micrometer tip sensor

General Information

Bending and Manipulating the Sensors

For most of the applications, the sensor is used **straight without bending**. If the experimental setup requires bending the sensor into L-shape, 3 cm of the tip cannot be bent. It must stay straight. As shown below.



NOT DRAWN TO SCALE

Quick Tip: To get the optimum performance out of a newly received sensor, connect the sensor to the meter and immerse it in aqueous solution for few hours, preferably overnight.

Calibration of the Sensor:

Use the same procedure as mentioned on page 8-10

Testing the Integrity of the Membrane:

Immerse the sensor in the calibration solution, sulfuric acid/potassium iodide. If the background rises immediately to a high value (over 500 nA and does not decay) and decreases immediately when the sensor is taken out of solution, then the membrane is broken.

Maintenance:

Other than occasional cleaning, amiNO-100 is maintenance-free.

Cleaning:

The sensor can be cleaned with many methods including immersion in alcohol or acetone, hot water, protease solution, or acid solution.

Storage:

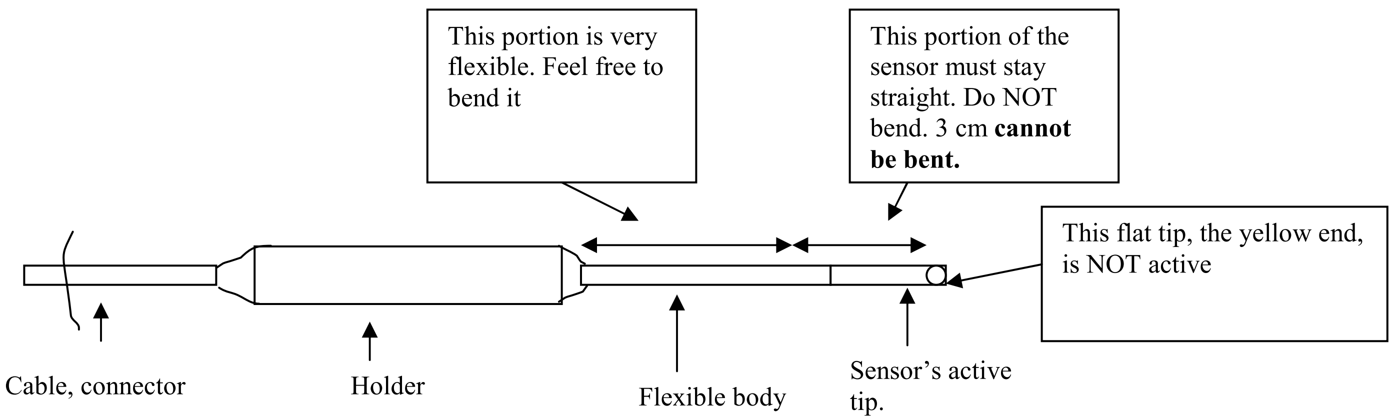
For short term storage, up to seven days, the sensor can be kept connected to the meter and immersed in aqueous solution. For long term storage, the sensor can be disconnected and stored dry.

amiNO-700 Nitric Oxide Sensor
A 700 micrometer tip sensor

General Information and Instructions

Bending and Manipulating the Sensors

For most of the applications, the sensor is used **straight without bending**. If the experimental setup requires bending the sensor into L-shape, 2-3 cm of the tip cannot be bent. It must stay straight. As shown below.



NOT DRAWN TO SCALE

> **Quick Tip**: To get the optimum performance out of a newly received sensor, connect the sensor to the meter and immerse it in aqueous solution for few hours, preferably overnight.

Calibration:

Use the same procedure mentioned on page 8-10.

Testing the Integrity of the Membrane:

Immerse the sensor in the calibration solution, sulfuric acid/potassium iodide. If the background rises immediately to a high value (over 500 nA and does not decay) and decreases immediately when the sensor is taken out of solution, then the membrane is broken.

Maintenance:

Other than occasional cleaning, amiNO-700 is maintenance-free.

Cleaning:

The sensor can be cleaned with many methods including immersion and wiping with alcohol or acetone, hot water, protease solution, or acid solution.

Storage:

For short term storage, up to seven days, the sensor can be kept connected to the meter and immersed in aqueous solution. For long term storage, the sensor can be disconnected and stored dry.

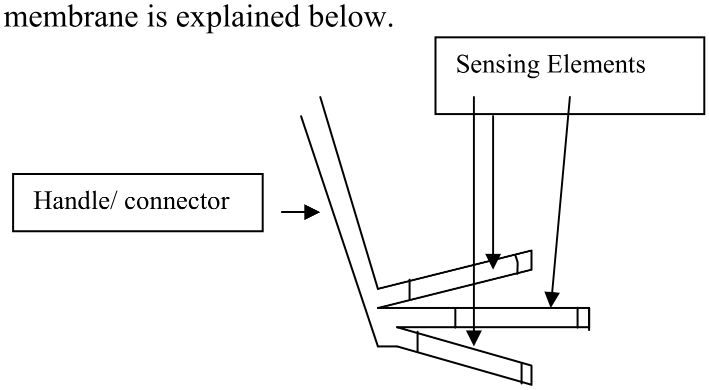
amiNO-FLAT Nitric Oxide Sensor

General Information and Instructions

The amiNO-FLAT nitric oxide (NO) sensor is very useful if used properly. Make sure to read the instructions and to familiarize yourself with the sensor and system.

amiNO-FLAT design was developed so the sensing elements cover a large area of cultured cells or flat tissue. The need to cover larger number of cells stemmed from the slow diffusion of NO. Having the sensing elements extended horizontally over the cells, reduces the diffusion path of NO from its point of release to the sensing elemens.

The sensor is integrated sensor, meaning that there **is no need for additional reference or counter electrode.** The sensing element, located at the tip of the sensor, is completely covered with a series of membranes including a gas permeable membrane to assure the selectivity of the measurements. The integrity of the sensor must be preserved. If any breakage or disintegration occurs, the sensor is not useable. Testing the integrity of the membrane is explained below.



The yellow plugs at the end of each sensing element is not active. NO can NOT pass through the plug. **NO can pass through the clear portion of the sensing elements only**. If you wish to activate the cells by an antagonist, add the antagonist between the sensing elements, below them, and around them (5 mm maximum distance). Cover the cells with a solution just enough to cover the sensing elements. Place the sensing elements as close as possible to the cells.

Quick Tip: To get the optimum performance out of a newly received sensor, connect the sensor to the meter and immerse it in aqueous solution (or water) for few hours, preferably overnight.

Calibration of the Sensor:

The amiNO-FLAT sensor can be calibrated by, at least, three different methods:

12. **The in situ generation of NO** is achieved by the addition of standard nitrite solution to an acidified solution in the presence of a reducing agent such as iodide ion according to the following equation:

    $$\mathbf{2\ NO_2^- + 2I^- + 4H^+ \rightarrow 2NO + I_2 + 2H_2O}$$

    We recommend using 0.05-0.1 M sulfuric acid solution containing 0.02-0.05 M, about 100 mg KI in 20 ml solution, potassium iodide (or sodium iodide). This solution can be used for many hours.
    A nitrite standard solution with a concentration of 50.0-100.00 micromolar is recommended.

    As shown in the chemical equation the mole ratio of nitrite to nitric oxide is 1:1 consequently, the amount of nitric oxide generated **equals** to the amount of nitrite added.

Using and Calibrating the Sensors and the System:

Before calibrating and using the sensor, the sensor should have been polarized for few hours, preferably overnight. The longer the polarization time, the more stable and reproducible the response of the sensor. Two procedures can be used for calibration: A. **Stirred Solution** and B. **Quiescent solution**

1. Immerse the sensor in a known volume-stirred calibration solution (sulfuric acid/potassium iodide) in a small beaker or Perti dish. If Perti dish is used, place the stirring bar in front of the sensing elements, but not touching them. The background might increase dramatically then decays (decreases). Wait for a stable background. The time needed to reach a stable background depends on how long the sensor was kept disconnected and dry (stored in air). As mentioned, keeping the sensor polarized and immersed in solution overnight is recommended. There is no danger of keeping the sensor immersed solution for many days.
2. Add standard amount of nitrite. We recommend using calibration concentrations in the range of the sample. For example, if the expected level of NO in the sample is under 500 nanomolar (nM), use calibration concentrations of 100, 200, 400 nM. To understand the response profile of the sensor, wait until the response decays before the next addition.
3. Calibrate the NO meter as recommended by the manufacturer or construct a calibration curve. Keep the sensor immersed in solution till the samples are ready.

Calibration using a quiescent solution:

Because it is not possible to stir a monolayer cells preparation, the user can calibrate the sensor under the same conditions of the sample. **a**. immerse the sensor in a Petri dish. Cover the sensing elements by the calibration solution

(sulfuric acid/iodide). **b**. add 20 microliters of 100.00 micromolar nitrite standard. Distribute the 20 microliter between and around the sensing elements. The user can use 40 microliters of 50.00 micromolar for better distribution. Since the final volume is NOT known, calculate the AMOUNT of NO generated> EXAMPLE: if 20.0 microliter of 100.00 micromolar nitrite standard were added then: moles = Molarity X Volume (L)
Then $100 \times 10^{-6} \times 20\ 10^{-6} = 2.0 \times 10^{-9}$ mole (2.0 nanomole). Make more additions.

NOTE: The calibration should be done at the same temperature of the sample to be tested.

Testing the Integrity of the Membrane:

Immerse the sensor in the calibration solution, sulfuric acid/potassium iodide. If the background rises immediately to a high value (over 500 nA and does not decay) and decreases immediately when the sensor is taken out of solution, then the membrane is broken.

Maintenance: Other than occasional cleaning, amiNO-FLAT is maintenance-free.

Cleaning:
The sensor can be cleaned with many methods including immersion and wiping with alcohol or acetone, hot water, protease solution, or acid solution. Do not keep the sensor immersed in pure organic solvents for more than 30 hours
Storage:
For short term storage, up to three days, the sensor can be kept connected to the meter and immersed in aqueous solution. For long term storage, the sensor can be disconnected and stored dry.
Sterilization: The sensor can be immersed in alcohol. Consult the manufacturer for other sterilization methods

amiNO-7 Nitric Oxide Sensor
(7 micrometer tip sensor)
and
amiNO-30 Nitric Oxide sensor
(30 micrometer tip sensor)

Both constructed from metal alloy fibers!

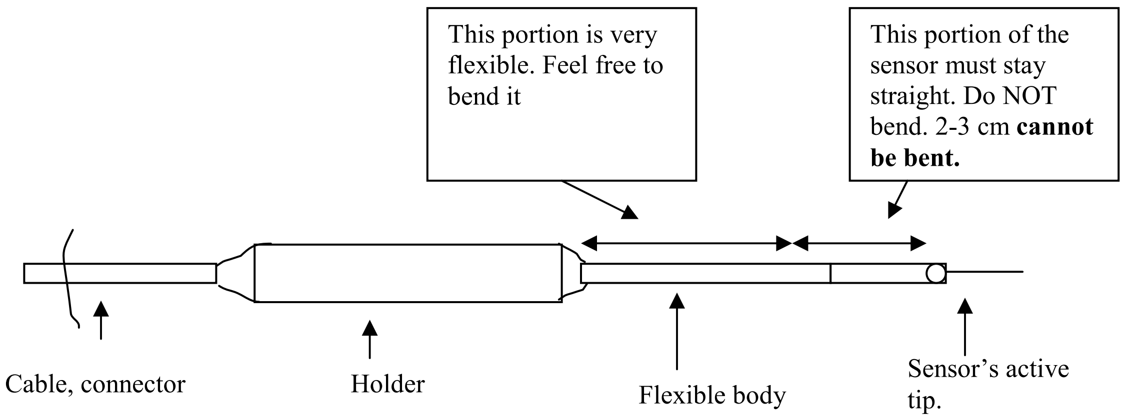
General Information

The amiNO-7 and amiNO-30 nitric oxide (NO) sensors are very useful if used properly. Make sure to read the instructions and to familiarize yourself with the sensor and system.

The sensor is integrated sensor, meaning that there **is no need for additional reference or counter electrode.** The sensing element, located at the tip of the sensor, is completely covered with a series of membranes including a gas permeable membrane to assure the selectivity of the measurements. The integrity of the sensor must be preserved. If any breakage or disintegration occurs, the sensor is not useable. Testing the integrity of the membrane is explained below.

Bending and Manipulating the Sensors

For most of the applications, the sensor is used **straight without bending**. If the experimental setup requires bending the sensor into L-shape, 2-3 cm of the tip cannot be bent. It must stay straight. As shown below.



NOT DRAWN TO SCALE

> **Quick Tip**: To get the optimum performance out of a newly received sensor, connect the sensor to the meter and immerse it in aqueous solution for few hours, preferably overnight.

Calibration of the Sensor:

1. Calibration using ascorbic acid-nitrite solution:

a. Connect the sensor to the meter. The sensor's tip should have been kept for more than one hour in distilled water for stable and optimum performance.
b. Prepare the calibration solution as follow: add 1.0 ml of 1.0 M sulfuric acid to 19.0 ml of distilled water. Mix with a stirring bar. To this solution add about 0.08 g (80 mg) of ascorbic acid . This will result in an ascorbic acid concentration of 0.02 M. The user can use in the range of 75-150 mg ascorbic acid without a problem. Moreover, the user can prepare 100 ml of this solution (water/sulfuric acid/ascorbic acid) and use as needed
c. Immerse the sensor in a 20.0 ml of the calibration solution (the user can use any volume). At least 5 mm of the sensor's tip should immersed in solution. Stir the solution using a small stirring bar.
d. Start recording and examine the stability of the background. When a stable background is reached, zero the meter and keep recording.

e. Add standard amount of potassium or sodium nitrite. We suggest the addition of 40 microliter of 100 micromolar nitrite standard solution to 20.0 ml calibration solution. This will result in a nitrite solution of 200 nM in the calibration solution. Wait for the response to stabilize. Make another nitrite standard additions such as 80, 100, 150, 200, etc. 2-3 additions are enough.

f. In this reaction the ascorbic acid reduces nitrite to NO at <u>about 50-55% efficiency</u>. For example, 200 nM nitrite solution will result in an NO concentration of 100 nM.

g. Calibrate the meter as recommended by the manufacturer.

2. **Calibration using NO saturated solution:** NO saturated solution has a concentration of 1.9 mM at room temperature.
3. **Calibration using NO releasing** agent such as S-nitroso-N-acetylpencillamine (SNAP). This reaction requires copper ions (10-100 micromolar). The efficiency of the reaction is 50% (1 mole SNAP decomposes to give 0.5 mole NO)
4. **Potassium iodide/sulfuric acid solution.** Consult the calibration procedure for amiNO-700.

Testing the Integrity of the Membrane

If the background of the sensor is more than 100 nA (100, 000 pA) in sulfuric acid/potassium iodide solution and not decreasing, then the membrane has pin holes. The sensor is still usable but should **not** be calibrated by the iodide/acid (procedure 4 above).

Maintenance:

Other than occasional cleaning, amiNO-7 and amiNO-30 are maintenance-free.

Cleaning:

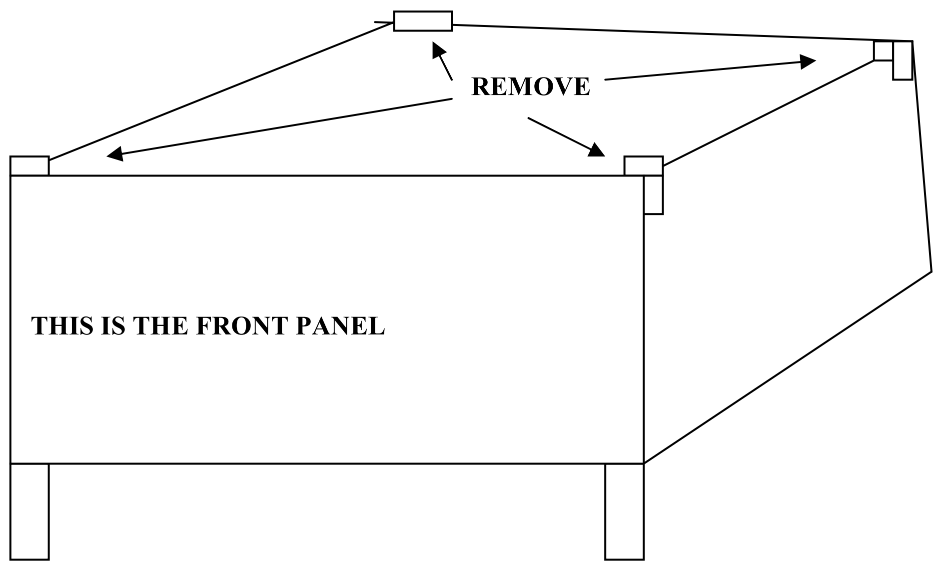
The sensor can be cleaned with many methods including immersion in alcohol or acetone, hot water, protease solution, or acid solution.

Storage:

For short -term storage, up to three days, the sensor can be kept connected to the meter and immersed in aqueous solution. For long term storage, the sensor can be disconnected and stored dry.

HOW TO CHANGE THE BATTERIRS

1. Remove the L-shaped corner covers as shown below
2. Open the cover by removing the screws found under these covers.
3. Change the batteries and reassemble the system.



Specifications of inNO

24 bit A/D converter	Current scale: +/- 2,500,000.0 pA
Resolution: 0.1 pA	Concentration scale: +/- 1,000,000.0 nM
Resolution: 0.01 nM	Computer 7.5 digit meter
Computer chart recorder	Digital filter (50/60 Hz notch filter)
Computer auto calibration	Computer auto zero
RS232 serial port interface	Run under windows 95, 98, 2000 and NT
Dimensions: 8x9x3 in.	Shipping weight: 1.5 lbs.
Power: battery powered. 6x 1.5 V AA	Battery life: more than 5 Years

Cables Accompanying the System

Cable	Connector type	Function
RS-232	Pins, RS-232	System to computer
Meter (system) Grounding Cable (MGC)	Banana plug to an alligator clip	Grounding the system
Sample Grounding Cable (SGC)	Needle to alligator	Sample grounding. Insert the needle in the sample and connect the alligator to ground
Potential Measuring Cable	Stereo to two alligator clips (red is working electrode)	Measuring the potential applied by the system on sensors. Connect to a multi-meter and read the DC voltage (850 mV). Change the potential using Vadj

How to Connect inNO to an Oscilloscope (Analog Output)

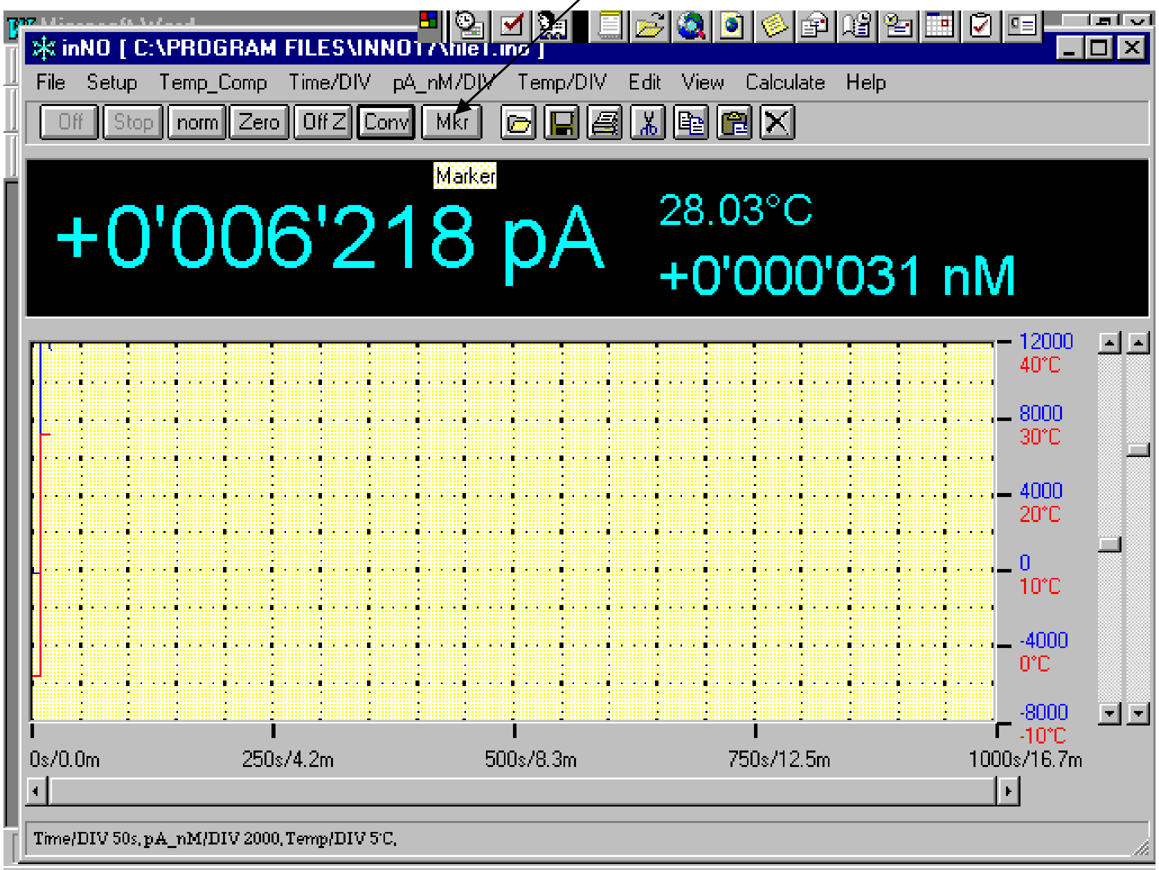
Insert the phone plug end of the potential measuring cable, Vw adj cable, (the one with a phone plug and 2 wires, red and black, ending with two alligator clips) to the EXT located on the back of the meter. Use the meter grounding cable to ground the meter. The ground port is located on the back of the meter. Then connect the red wire and ground wire to an oscilloscope to record the analog signal of NO change by pA. And/or connect the black wire and the ground wire to an oscilloscope to record the analog signal of temperature.

The use of this function is not recommended. It is only for those with special need in their research.

inNO-T, NO System

Additional Instructions:

1. Marker: The new software (version 1.9) offers an additional function similar to a foot switch to mark an experimental event. When clicking this button, a sharp and fast negative response is observed.
Please note that this function works only during recording.



2. To prints the notes, go to File then Print Notes.

3. To transfer data to Excel, click on the starting point, drag while holding the mouse button down, go to edit, choose copy, open Excel, and from Excel menu hit paste.

4. To transfer an image to Microsoft Word: Open the inNO file, hit PRINT SCREEN, open Word file, then for Word menu hit paste. This will be treated as a picture.

Warranty Information

*** Innovative Instruments, Inc. warrants the original purchaser that this equipment shall be free of defects in material and workmanship for one year from the date of receipt.**

- **The above warranty does not cover misuse and neglect.**

- **Sensors and other consumable are covered for one month from the date of receipt.**

*** This equipment is not intended for human use.**