



NOCHM

Nitric oxide chamber



World Precision Instruments, Inc.

USA

International Trade Center, 175 Sarasota Center Boulevard, Sarasota FL 34240-9258
Tel: 941-371-1003 • Fax: 941-377-5428 • E-mail: sales@wpiinc.com

UK

Astonbury Farm Business Centre • Aston, Stevenage, Hertfordshire SG2 7EG
Tel: 01438-880025 • Fax: 01438-880026 • E-mail: wpiuk@wpi-europe.com

Germany

Liegnitzer Str. 15, D-10999 Berlin
Tel: 030-6188845 • Fax: 030-6188670 • E-mail: wpide@wpi-europe.com

Japan

6-11, Uchikanda 2-Chome, Chiyoda-Ku, Tokyo 101-0047
Tel: 81-3-3258-1641 • Fax: 81-3-3258-1657 • E-mail: info@wpi-j.com

Australia

P.O. Box 1191, Glen Waverley, Victoria 3150
Tel: (03) 9887-6262 • Fax: (03) 9887-9585 • E-mail: wpi.au@ozemail.com.au

Internet

www.wpiinc.com • www.wpi-medical.com • www.nitricoxide.net • www.pipetter.com

www.wpiinc.com

INSTRUCTION MANUAL

Serial No. _____

051701

World Precision Instruments

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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 one year* 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 Service 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 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.*

Replacement Parts

G04	Gasket (1)
NOCHM-P	Side Fitting Solid Cap plus Gaskets (3)
33012	Top Fitting Cap

References

P. S. Brookes, E. P. Salinas, K. Darley-Usmar, J. Eiserich, B. A. Freeman, V. M. Darley-Usmar, P. G. Anderson. "Concentration dependent effects of nitric oxide on mitochondrial permeability transition and cytochrome c release". *J. Biol. Chem.* **275**, 20474-9 (2000).

NO Chamber Specifications

Sample Volume	1-3 mL
For Use With 2mm Electrodes	ISO-NOP, OXEL-1
Temperature range	4-40 °C
Water inlet and outlet	1/4-in. ID tubing

Introduction

The **NO Chamber** is a temperature stabilized closed chamber for measurements of nitric oxide, oxygen, and other species in cell culture. It is also ideal for the calibration of NO electrodes at controlled temperatures. The measurement of NO and other reactive gases will be underestimated in stirred solutions if the solution is allowed to equilibrate with air. In the case of NO, accelerated decomposition occurs as the result of diffusion of NO from the solution into the gas phase and the reaction of NO with oxygen. This reaction with oxygen makes a significant and variable contribution to NO decomposition, and hence accuracy of measurement, at concentrations of NO between 0.1-5 μ M. These problems can now be eliminated with the use of the NO closed chamber, which minimizes the headspace of the experimental solution used.

Features of the NO Chamber

- Closed chamber design greatly reduces the surface area of the experimental solution exposed to air.
- Matched to WPI's 2 mm tip diameter **ISO-NOP** and **OXEL-1** electrodes, the NO Chamber may fit, or be adapted to fit, other electrodes as well including **Kwik-Tip™** ion-selective electrodes and **DRIFEF-2** and **SDR2** reference electrodes for the measurement of Calcium, Potassium, Hydrogen and TPP.
- Optional Use Side Port which can be used for a second electrode permitting simultaneous measurements for NO and oxygen.
- Two openings on the Top Port for injection of reagents into the chamber.
- Operating temperature range of 4 - 40 °C controlled via an external circulating bath.
- Ideal for NO electrode calibration across a range of temperatures.

Instrument Description

The NOCHM consists of a Chamber (**A**) and a Top Fitting Cap (**B**), through which the ISO-NOP or OXEL-1 electrode can be inserted (see Fig. 1). An optional use Side Port (**C**) is also provided through which a second electrode can be inserted for the simultaneous measurement of NO and oxygen. The inner volume of the chamber (and hence sample volume) can be continuously adjusted in volume from 1.0 mL to 3.0 mL and is suitable for most cell suspension experiments and electrode calibrations.

The NO Chamber can be temperature-controlled by circulating water through the outer jacket of the chamber using an appropriate heating/cooling circulator. The circulator bath is connected to the NO Chamber through Inlet (**D**) and Outlet (**E**) Connectors.

The Top Fitting Cap (**B**) has 2 openings (**F**) for reagent addition to the chamber. WPI's FlexiFil™ microsyringes and MicroFil™ syringe needles are suitable for this purpose.

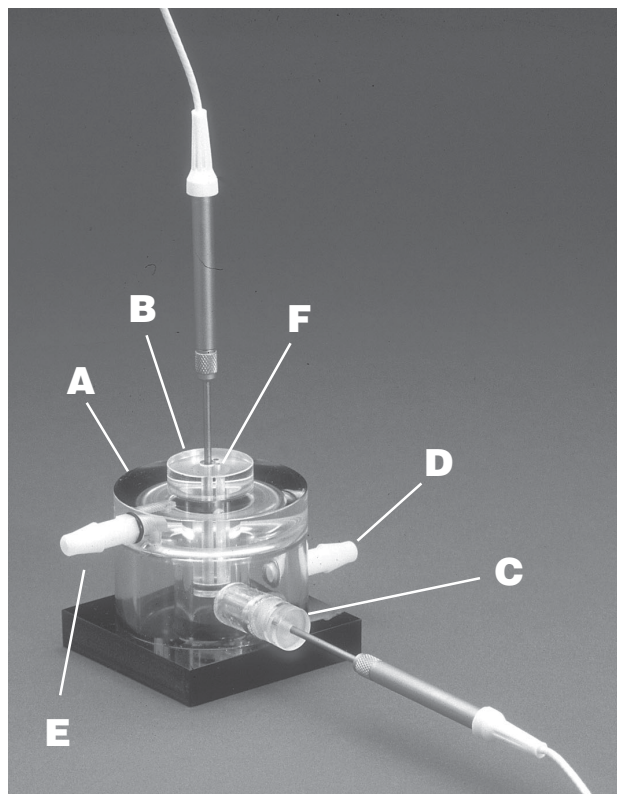


Fig.1

Instrument Maintenance

Cleaning

After use, rinse the chamber compartment and the Fitting Caps with water. To clean the compartment of protein residue use an enzymatic detergent, e.g. ENZOL (see Accessories for ordering information).

Sterilization

Caution: Do not autoclave the NO Chamber.

Ethylene oxide can be used to sterilize as well as Cidex Plus (see Accessories for ordering information). Although alcohol is acceptable, over time it may cause damage to the acrylamide unit.

Accessories

ISO-NOP	ISO-NOP electrode (2 mm tip dia)
OXCEL-1	Oxygen electrode (2 mm tip dia)
KWIKCAL-2	Holder and 3 Calcium electrodes
KWIKH-2	Holder and 3 Hydrogen electrodes
KWIKPOT-2	Holder and 3 Potassium electrodes
KWIKTPP-2	Holder and 3 TPP (tetraphenylphosphonium) electrodes
DRIREF-2	Dri-Ref, 2 mm diameter electrode
SDR2	SUPER-Dri-Ref, 2 mm diameter electrode
500787	Haake DC10-P5/U Circulating Water Bath (115v)
500788	Haake DC10-P5/U Circulating Water Bath (230v)
500789	Haake DC10-P5/U Circulating Water Bath (100v)
Enzol	Enzymatic Detergent
5000817	FlexiFil™ 10 mL microsyringe, blunt tip, 0.20 mm OD, 10 mm long
MF34G-5	MicroFil™ , 34 ga., 67 mm Long (pkg. of 5)
MF28G-5	MicroFil™ , 28 ga., 97 mm Long (pkg. of 5)
MF28G67-5	MicroFil™ , 28 ga., 67 mm Long (pkg. of 5)
7364	Cidex Plus
7357	Nitrite Standard Solution
SNAP50	SNAP, 50 mg
SNAP100	SNAP, 100 mg
SNAP500	SNAP, 500 mg
GSNO-50	GSNO, 50 mg
GSNO-100	GSNO, 100 mg
GSNO-500	GSNO, 500 mg

2. Refer to the manual of your circulator bath on how to adjust the temperature.
3. Once the circulating solution has reached the desired temperature, proceed with the experiment as described in the room temperature section above. **NOTE:** Once solution has been added to the chamber, additional temperature equilibration of the solution may be required, depending on its temperature at the time it was added.

NOTE: You will have to determine the correct temperature setting for the circulating bath in order to achieve the desired temperature of the sample in the NO Chamber. They may not be the same.

Set-Up

Parts List — The package should contain:

Part No.	Item
NOCHM	NO Chamber including Top Fitting Cap and Side Fitting Solid Cap
GO4	Gasket: package of 10
33013	Side Fitting Electrode Cap (1)

Unpacking

Upon receipt of this product, 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 loss or damage should be reported at once to the carrier and an inspection requested. Please read the section entitled “Claims and Returns” on the Warranty page of this manual.

Returns: Do not return any goods to WPI without obtaining prior approval and instructions from our Service Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100 mm (four inches) of shock absorbing material. Please read the section entitled “Claims and Returns” on the Warranty page of this manual.

Assembly

No assembly is required before use.

Operating Instructions

Caution: During all manipulations involving the nitric oxide or oxygen electrodes, avoid contact with the electrode tips. The membrane covering the tip is fragile and damage could occur upon contact with materials such as the rubber gasket or the plastic of the chamber.

See Instrument Maintenance for cleaning procedures. It is recommended that, at a minimum, the unit be flushed well with deionized water before use.

Room Temperature Use

Step 1: Side Port

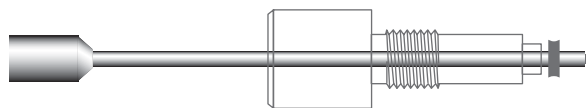


Fig. 2—Diagram of Side Fitting Electrode Cap with electrode and gasket in place.

Caution: The Side Fitting Solid Cap has a gasket at its inner tip. The gasket is required to effectively seal off the port. If the gasket should need to be replaced, use those provided.

1. If the Side Port is not to be used, ensure that the Side Fitting Solid Cap (including gasket) is in place.
2. To place an electrode in the Side Port, remove the solid Fitting Cap from the side port.
3. Insert the electrode through the opening in the Side Fitting Electrode Cap. Once inserted, install a gasket on the electrode sleeve, about 5 mm from the end (Fig. 2). **TIP:** wet the gasket first.
4. Carefully place the Side Fitting Cap, with the electrode, into the Side Port. Adjust the position of the electrode so that it extends into the chamber solution and tighten the Fitting Cap. *Moving the electrode in or out after tightening the Cap, although possible, may cause damage to the membrane at the electrode tip.*

Caution: Electrode tips are fragile. To avoid damaging the membrane tip, when disassembling the Side Fitting Cap and electrode from the NO chamber, first loosen and then remove the Cap with the electrode still inserted. Carefully remove the gasket from the electrode and then remove the electrode from the Cap.

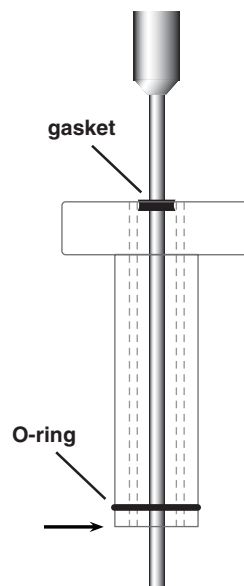


Fig. 3—Top Fitting Electrode Cap with electrode in place. Base of cap (see arrow) should touch the surface of the solution in the chamber.

Step 2: Top Port:

1. Slide the Top Fitting Cap (**B**) out of the Chamber (**A**).
2. Fill the chamber with the experimental solution (1-3 mL); add a stirrer bar if applicable.
3. Taking care to minimize contact with the electrode tip, fit the electrode through one of the gaskets provided. **TIP:** wet the gasket first.

Caution: To decrease the possibility of damaging the electrode membrane while reinstalling the gasket, it is recommended that the gasket be left on the top electrode between uses.

4. Slide the electrode into the opening on the outer side of the Top Fitting Cap just until it protrudes through the cap to the inner chamber side.
5. Replace the Fitting Cap into the top port.
6. Position the bottom of the Top Fitting Cap at the surface of the experimental solution. Ensure that at least one millimeter of the electrode is in the solution. Push the electrode further down into the Cap as necessary. The gasket holds the electrode in place and seals the chamber. (Fig. 3)
7. The two additional openings on the Top Fitting Cap are provided for injection of reagents into the chamber.

NOTE: although both the top and side ports can be used for either NO or oxygen measurement, the top port cannot be plugged and therefore must be used when only one electrode is employed.

Use with Circulating Water Bath

Caution: Exercise caution when connecting a heating/cooling circulator bath to the NO Chamber. Failure to properly secure the tubing to the inlet/outlet connectors of the NO Chamber may result in flooding.

1. To use the NO chamber at temperatures above or below room temperature but within the range of 4-40° C, connect an appropriate heating/cooling circulator bath to the inlet/outlet connectors (E, D) using 1/4-in tubing.