COY LABORATORY PRODUCTS INC.

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Vinyl Hypoxic Glove Box Instruction Manual

Index

WARRANTY

This product is warranted against defects in material and workmanship during the first 12 months after original date of shipment.

The factory will, at its option, repair or replace defective material within this period at no charge for parts and labor.

All returns or exchanges must first be authorized by COY LABORATORY PRODUCTS, INC.

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COY LABORATORY PRODUCTS, INC.
14500 COY DRIVE
GRASS LAKE, MI 49240

The responsibility of COY LABORATORY PRODUCTS, INC. is limited to the purchase price of this product, and COY LABORATORY PRODUCTS, INC. will not be responsible for any consequential damages.

This warranty does not cover damage in shipment or damage as a result of improper use or maintenance of this product. This warranty does not cover damages caused by excessive line transients on the AC supply line.

1.0 WARNINGS

1. Never exceed 60% O_2 in the Chamber. The calibration of the sensor must be checked frequently as erroneous low readings can result from a degraded sensor.

2. Gas pressures into the back of the Oxygen Controller must not exceed 15 PSI.

3. The output of the O_2 Controller must be regulated using the controls on the front of the Controller, not to exceed a total of 40 SCFH. If the gas flow exceeds 40 SCFH, there is a danger the Sensor will not detect the rapid change, thus causing the gas level to exceed your set point value.

4. Never obstruct gas flow in or out of the Chamber Relief Valves.

5. Never attempt to service the O_2 Controller. Call COY LABORATORY PRODUCTS, INC. for assistance.

6. Never put an open flame or create a spark in a Chamber, especially under hyperoxic conditions.

7. The sensor cell membrane is delicate. Do not scratch, puncture, or permit sharp objects to touch the cell face. Sensor failure due to mishandling voids the sensor warranty.

8. **LATEX WARNING** Latex Gloves/cuffs with powder may be installed on this equipment. Some people are allergic to latex and/or the powder. COY Laboratory Products cannot account for the content of gloves bought from other vendors.

2.0 GENERAL OVERVIEW

2.1 This manual is designed to provide you with basic knowledge of a Coy Vinyl Hypoxic Chamber and the components supporting the system. It will provide insight on how to assemble, operate, and maintain the Chamber.

2.2 The Coy Hypoxic Chamber is available in three standard sizes Mini (30”/76 cm L) One Person (44”/111 cm L) and Two Person (78”/ 198 cm L) see Figure #2 for details and is constructed of flexible PVC. The seams are sealed together using a radio frequency welding technique. A tubular aluminum frame supports the Chamber on a 3/4" plywood base, covered with foam. The Chamber has two entry ports, one of which is used to install large equipment before the Chamber is initially purged. After large equipment has been installed, this entry port is sealed with a large plastic disc and taped in place. The other entry port is the airlock, and is used by the operator to enter the Chamber on a routine basis. Heated Fans are supplied with the Chamber to circulate chamber atmosphere and provide an area for the optional Desiccant Stak-Paks to be placed in the event of excess moisture.

2.3 *DO NOT THROW AWAY PACKING/CRATING MATERIAL* until all goods have been checked and received.

3.0 CHAMBER ASSEMBLY

This section describes how to assemble your Coy Vinyl Hypoxic Chamber. **MAKE SURE THE CHAMBER IS AT ROOM TEMPERATURE BEFORE YOU BEGIN ASSEMBLY PROCEDURE.** PVC (the plastic from which the Chamber is made) is very brittle at low temperatures. Prior to shipment the Chamber has been assembled and tested for leaks at the factory. Figure # 1 can be used as an aid to assemble the Chamber.

NOTE: Assume the Airlock is located on the right hand side of the chamber.

Figure #1: Vinyl Chamber Frame and Base

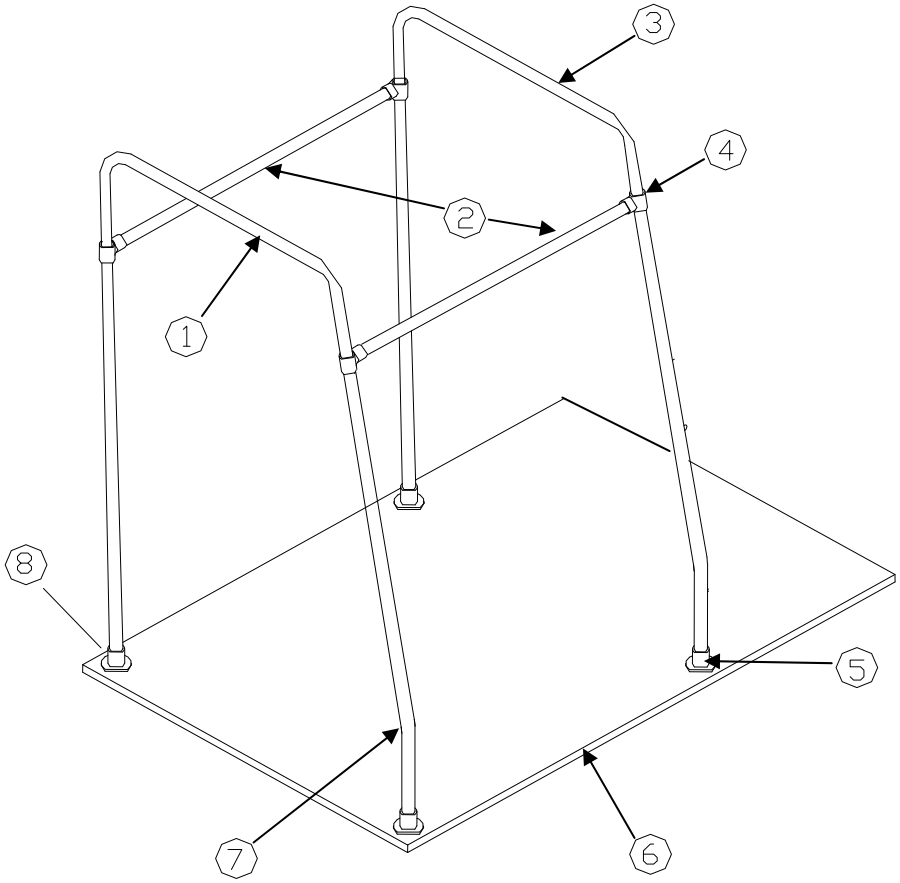


Figure #1 Legend

1. LEFT SIDE SUPPORT
2. STRAIGHT ALUMINUM POLE
3. RIGHT SIDE SUPPORT
4. COUPLING'S 3' (90 cm) ABOVE CHAMBER BASE
5. FRONT SIDE SUPPORT COUPLING
6. CHAMBER BRACE
7. SLIGHT ANGLE ON SIDE SUPPORT
8. REAR SIDE SUPPORT COUPLING

3.1 ASSEMBLY PROCEDURE

1. Locate the Chamber Care Kit supplied with the chamber. Inside the care kit you will find miscellaneous items needed to assemble and maintain the Chamber.

They are:

QUANTITY	DESCRIPTION
1	ROLL OF YELLOW TAPE (Used to seal equipment, entry port & gloves).
1 Pr.	NEOPRENE RUBBER GLOVES
1	PLASTIC CUFF
1 Pr.	CANVAS WORK GLOVES (Used to slip over rubber gloves for protection against punctures and cuts.)
1	VINYL REPAIR KIT
1	1/8” ALLEN WRENCH (For Chamber assembly)
1	5/32" ALLEN WRENCH (For Chamber assembly)

2. Once you have found a suitable place to set the Chamber, insert the side supports (#1, #3) in their cups (screwed to the Chamber base #5, #8). As you will notice in Figure # 1, and by inspection, the side supports are not symmetrical. At the bottom of each support, one side is bent at a slight angle (#7). This bend allows the Chamber to slant away from the user's face when it is fully assembled. The side that is bent is inserted in the cup closest to the front of the Chamber (#5). Side support couplings are located on all four corners of the Chamber. DO NOT TIGHTEN THE SETSCREWS THREADED IN THE COUPLINGS, YOU WILL DO THIS LATER.

3. Next, insert the two straight aluminum poles (#2) into the PVC hangers (The PVC hangers are actually sleeves parallel to the Chamber base and located near the top of the Chamber). One hanger is in front of the Chamber and the other is in back of the Chamber. DO NOT FORCE THE POLES THROUGH THE HANGERS. Rather, insert them with a rotating motion, which will help guide them. Then, with two people, lift one pole (either in front or back of the Chamber) and insert it in the coupling (#4) located about 3 feet (90 cm) above the Chamber base. You may have to spread the side supports slightly to insert the aluminum poles. One coupling is on the right side support (#3) and the other is on the left side support (#1). Now, install the other aluminum pole in the same manner as the first.

4. With the 5/32" Allen wrench tighten the setscrews located on ALL of the

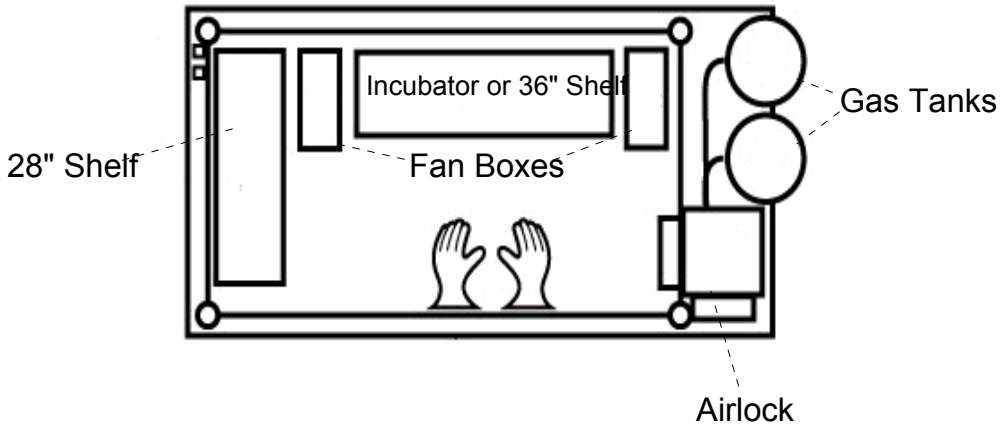
couplings. Tightening the setscrews will make the Chamber's frame ridged and self-supporting. The Chamber should now be standing and ready for large equipment installation.

3.2 COMPONENT PLACEMENT

3.4.1. This section contains information on placement of the Plug Strip, Fan Boxes, and Shelving Units. Figure 2 will illustrate a popular floor plan and may be used as a guide to help you choose a floor plan to maximize your work area.

Figure #2: Typical Chamber Floor Plans

One Person Unit



Mini Unit

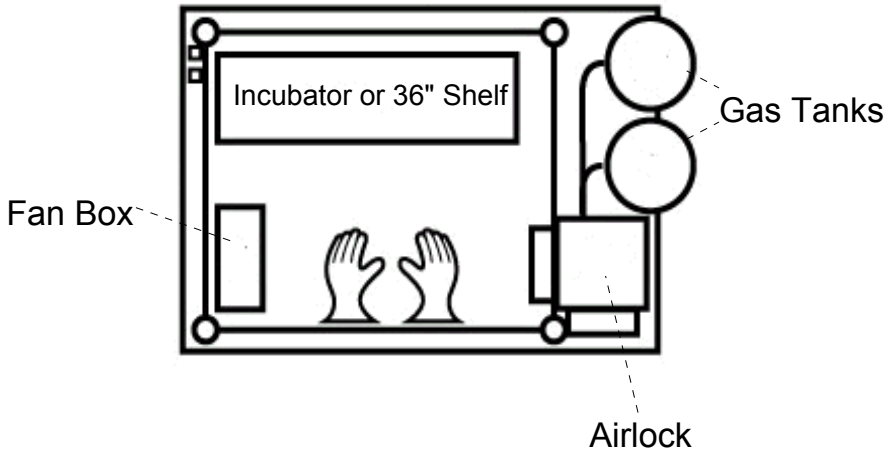
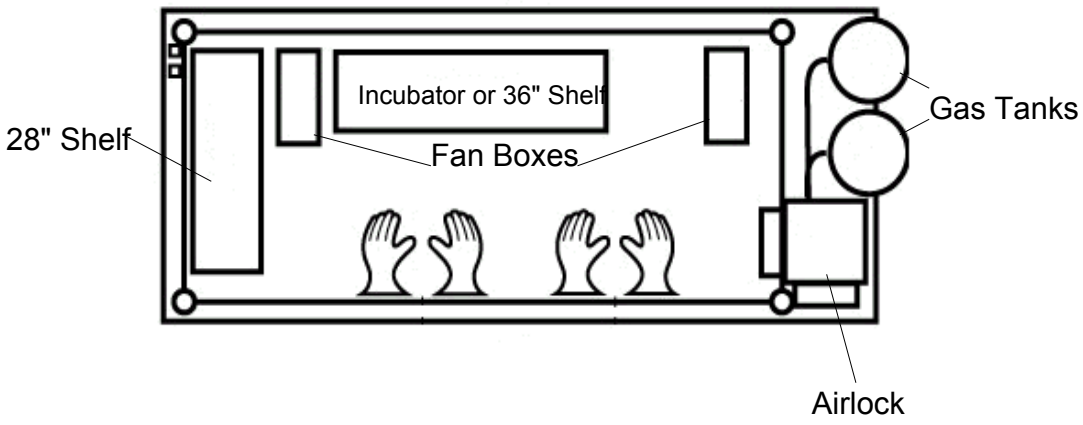


Figure #2: Typical Chamber Floor Plans continued

Two Person Unit



3.2.2 Oxygen Controller Placement

The Oxygen Controller is typically placed on top of the airlock or on a nearby shelf. Section 3.5.1 (page 11) will provide instruction for gas tubing connections.

3.2.3 COMPONENT PLACEMENT CONTINUED

Before you install large equipment, you must first insert the plug strip. Slide the plug strip through one of the nipples located at the bottom rear on either side of the Chamber, usually the side opposite the airlock. When the plug strip is completely inside the Chamber, forcefully push the plug strip's stopper into the nipple. Wetting the stopper with water will help you push it into the nipple. The best location for the plug strip is in back of the Chamber.

Now you are ready to install large equipment through the large equipment entry port. Large equipment is defined as anything too big to fit in the airlock, such as a Coy Incubator, Fan Boxes, Work Pads, Humidified Incubation Box and Shelving Units. Arrange the items to provide maximum work area. Coy recommends placing the work pads in front of the gloves and Fan Boxes at opposite ends of the Chamber.

Once large equipment has been installed, and arranged to your satisfaction, and plugged into the power strip, seal the large equipment entry port with the plastic disc. To do this, orientate the disc so that COY can be read from inside the Chamber. If you have the disc oriented correctly, COY will read backward when you stand outside the Chamber. Next, push the disc through the large opening and begin the sealing process by grasping the outside of the disc with both hands and pulling it through the hole until about 2" of PVC is resting on the sides of the disc. With one hand, hold the PVC in place; and with the other used as a lever,

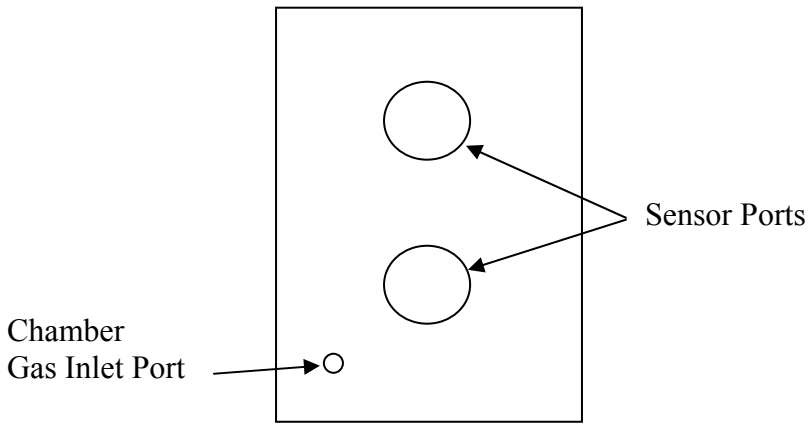
stretch the PVC over the disc. After the PVC has been stretched over the disc, seal the junction where the PVC and disc meet with yellow tape (supplied with your Chamber Care Kit). Taping the PVC and disc together is an important procedure and should be done with care so the tape does not wrinkle. Coy recommends 6 revolutions of tape to ensure an adequate seal. The first revolution of tape should be very flat and straddle the PVC and disc. This first revolution of tape actually seals the large equipment port. The remaining revolutions ensure a complete seal by overlapping the first revolution on both sides. When you complete this, there should be a band of yellow tape (created by the overlapping tape) about 3" wide.

3.3 O2 Control System Installation

1) Position the Chamber as desired then put the Oxygen Controller on top of the Airlock. Plug the Oxygen Sensor into the jack on the back of the Controller and insert the Sensor into the mounting port attached to the side of the Chamber(see Figure #3)

NOTE: may be located on the rear in some custom configurations.

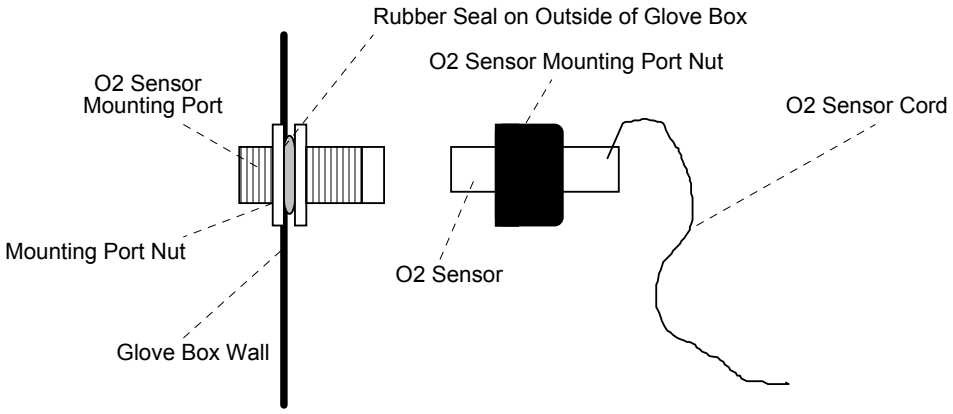
Figure #3 O2 Control System Mounting Port



2) Be sure the nut on the outside of the Sensor mounting port is unscrewed completely before inserting the sensor. Push the sensor into the port as far as possible and screw the nut down tightly (see Figure #4).

NOTE: If installing a CO2 Sensor you will notice the sensor is much thinner than the O2 Sensor but the port will still fit just tighten the port Sensor Mounting Port Nut further.

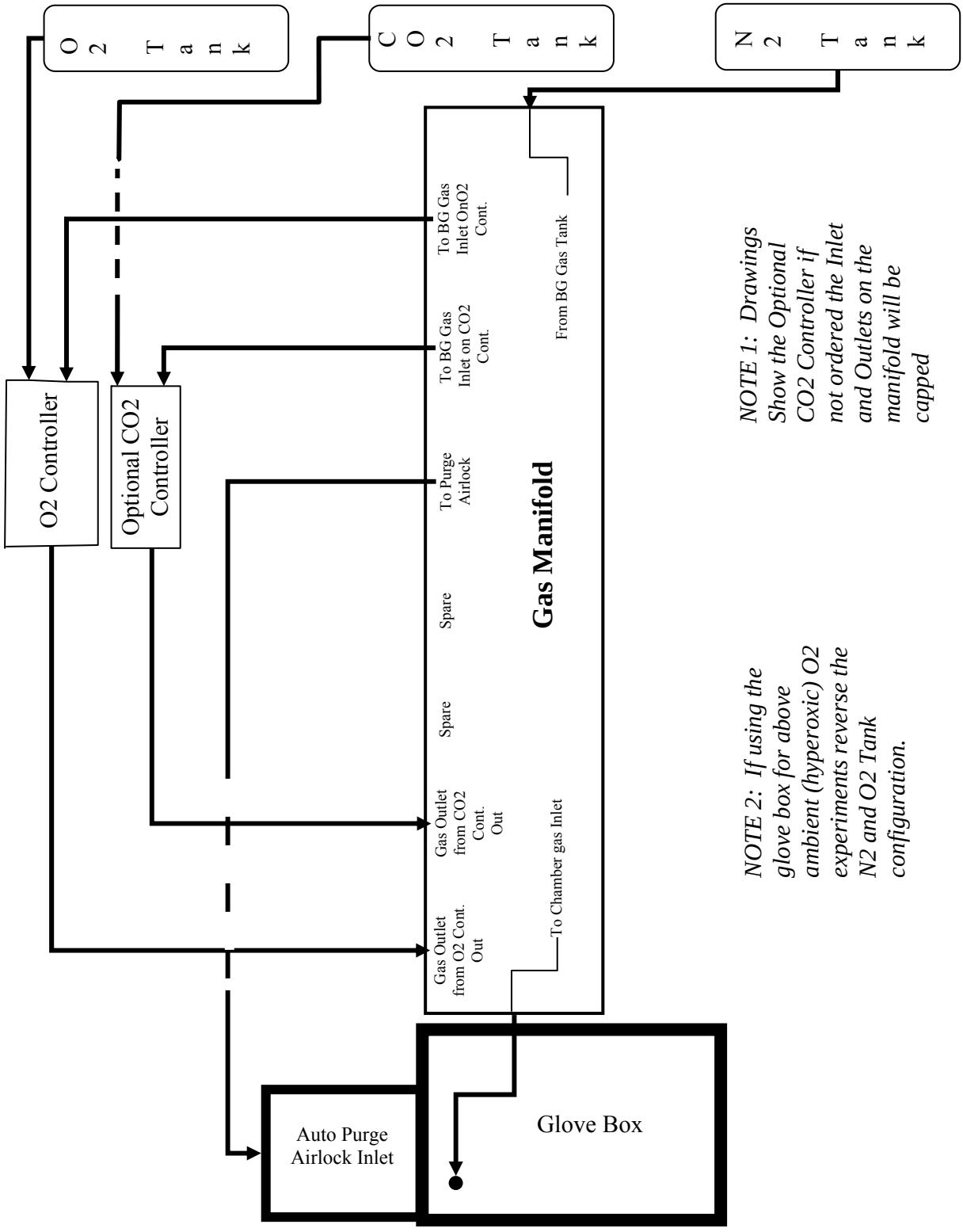
Figure #4 Mounting Port and Sensor Connection



3) Using the tubing provided, install the gas lines from the source tanks to the Glove Box, O2 Controller, and Airlock, optionally for the CO2 Controller if purchased, see figure #5 for connections. The T-fittings are supplied in a separate plastic bag. Tubing will have to be cut to fit.

NOTE ON ATTACHING TUBING TO QUICK DISCONNECT FITTINGS: Place a female fitting on each end of the tubing. Place 2 ty-wraps around the hose barb on the female fittings and pull them as tight as possible. Cut off excise ty-wrap.

Figure #5 Glove Box Gas Connections

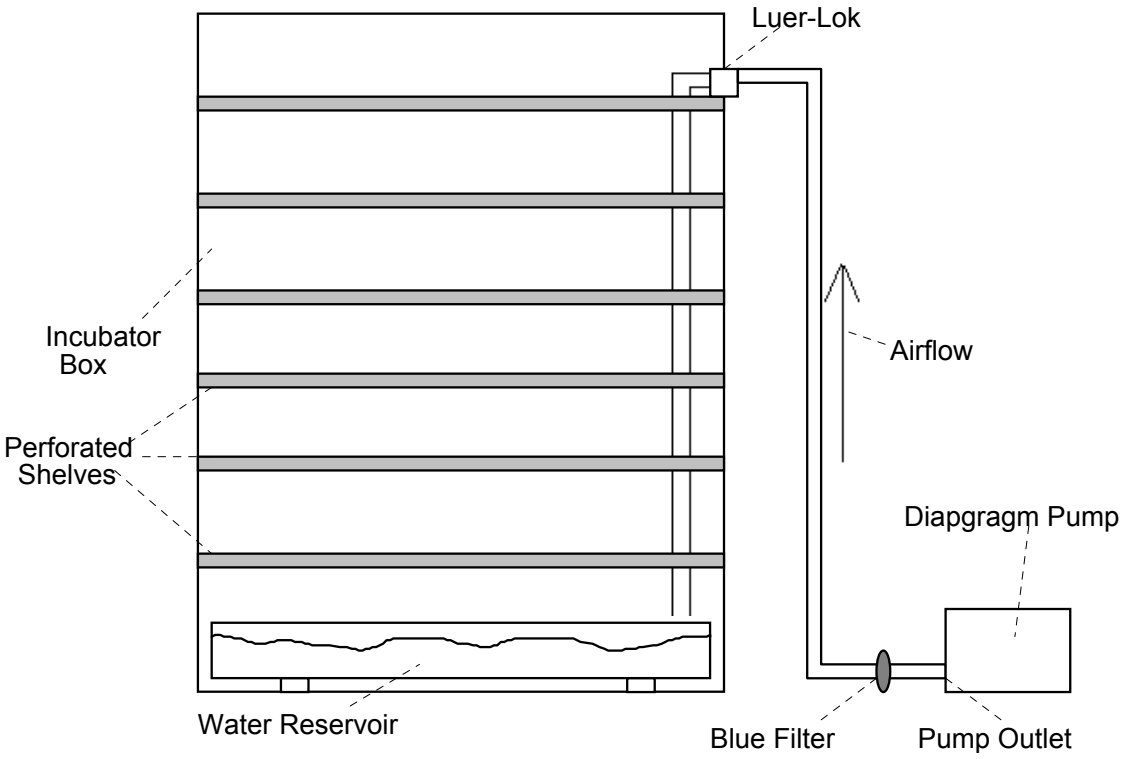


4) If using the COY Humidified Incubation box or Internal Incubator with a high moisture content, you may wish to purchase the COY Desiccant Stak-Paks (part # 6502-000) to maintain ambient humidity levels in the glove box. If you have purchased the desiccant at time of the hypoxic glove box order then place these desiccant Stak-Paks on each fan box now. These should be dried prior to installation for 2 hours at 120° C, and thereafter as necessary (see section 6.2.3/page 19 for more details on the desiccant Stak-Paks). As an alternative to the desiccant drying system you may choose a Large Capacity Dehumidifier which can be installed after initial purchase by modifying the Equipment Entry Port

Set Up for Humidified Incubation Box

5) Attach the small diaphragm pump to the blue filter using the flexible flexible tubing (use Figure #6 below for reference). Attach the filter using another piece of flexible tubing and luer-lok adapter to the fitting on the Incubation Box. For optimum performance, the Incubator Box should be level. We suggest you do all this now because of the easy access through the large side door (see section 6.2 page 21 for further details on the operation of the COY Humidified Incubation Box)

Figure #6 Humidified Incubation Box



6) The Pressure Relief Bubbler relieves the glove box of large internal pressure changes associated with gas purges. The system automatically allows gas to escape to the room atmosphere when the glove box's internal pressure rises.

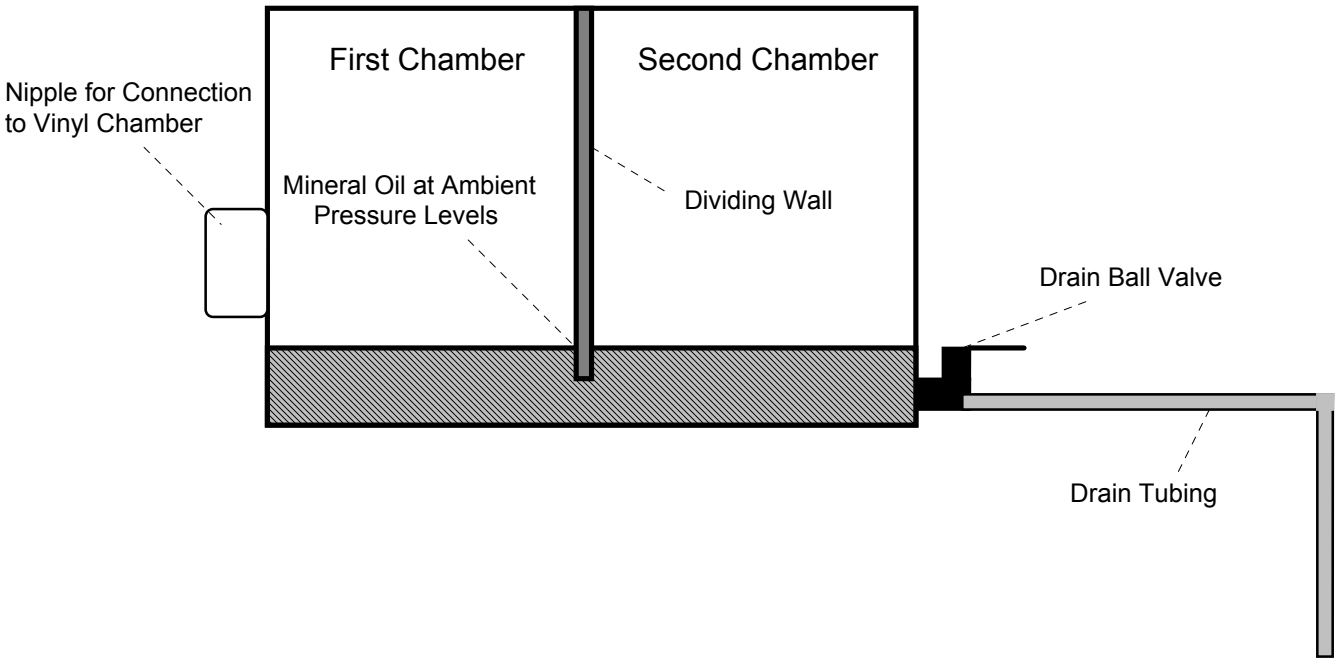
The Bubbler can handle up to 15 psi of gas flow. Larger flow rates may over pressurize and damage the glove box.

How It Works

The Bubbler is filled with of mineral oil (supplied) or alternatively water. The amount is up to the end user but enough to cover up to the inner dividing wall to form the initial barrier. Closer to the bottom edge the lower the internal pressure in the glove box the closer to the top the higher the internal pressure. The higher pressure translates into the difficulty the user will have in entering the gloves.

To adjust pressure levels add, more mineral oil through the hole in the top of the second chamber; or, using the ball valve and the supplied tubing, drain some of the oil out of the Bubbler. When draining the oil out, do not drop the mineral oil below the chamber dividing wall (see Figure #7).

Figure # 7 Pressure Relief Mechanism/Bubbler



4.0 GAS SUPPLY SETUP PROCEDURE

Passive CO2 Control

If optional CO2 Control System has been purchased skip this part and move on to Section 5.0 Establishing Initial Atmosphere.

To maintain the 5% CO_2 level required for certain buffers to function properly, use the following table to spike each one of the gases going into the Oxygen Controller with the appropriate level of CO2 based on desired O2 levels.

Desired Concentration of Oxygen	Air Displace to %	%CO2 Mix required	Desired % Co2	%CO2 Concentration in N2
20.80	100.00	0.00	5.00	N/A
15.00	72.12	27.88	5.00	17.9310
10.00	48.08	51.92	5.00	9.6296
5.00	24.04	75.96	5.00	6.5823
2.00	9.62	90.38	5.00	5.5319
1.00	4.81	95.19	5.00	5.2525
0.50	2.40	97.60	5.00	5.1232
0.10	0.48	99.52	5.00	5.0242

5.0 ESTABLISHING INITIAL CHAMBER ATMOSPHERE

1) Plug in and turn on the Oxygen Controller and choose a set point. At this point, it will be used only as a monitoring device. If using the Heated Fan Boxes, turn the heating elements on and adjust desired set point.

2) Disconnect the tank you desire (background tank if below ambient conditions, O2 tank if above ambient conditions) from the O2 controller and using that tubing connect directly to the Chamber Gas Inlet on the Chamber, thus bypassing the O2 controller for initial chamber purge. NOTE THIS IS ONLY DONE FOR RAPID PURGING WHEN LARGE O2 CHANGES ARE DESIRED.

3) Turn on the gas supply attached to the Chamber. Adjust the regulator so as not to exceed 15 psi (for the Chamber set up only). **Be sure to stay in attendance and monitor the filling operation.** If the Chamber starts to bulge, reduce the gas flow to allow the relief bubbler to catch up. The bubbler is designed to handle 15 psi input in a working glove box. As you are filling the glove box, monitor the oxygen level. The red digits are the measured O2 reading inside the glove box, and the green digits indicate the set point.

When you are within 1% of the desired O2 level, close the Ball Valve on the Gas Regulator disconnect the tubing and reassemble as described on page 12 Figure #5.

This process should take 5-30 minutes depending on the size of the glove box, flow rate, desired O2 level and additional content inside the glove box.

4) Now adjust the set point on the oxygen controller (see O2 controller manual for details on set point adjustment) and allow the controller to adjust to your desired O2 levels. As the controller is purging in gas you should adjust the flow rates on the flow meters on the front of the O2 controller. *NOTE: Gas has to be flowing through the system to make the adjustments, 40 scfh is recommended*.

5) Place the sensor(s) from the Heated Fan Box where you desire the most precise temperature control (center of a shelf or as described in #5 of the set up instructions above for the Humidified Incubation Box) and set the desired temperature. These modules are accurate to +/- 1° C.

6.0 SYSTEM FUNCTION IN CHAMBER

6.1 Standard Equipment

1. O_2 Controller
2. Fan Box (Heated or Unheated)
3. Plug Strip
4. Gas Leak Detector
5. Nitrogen (background) Gas Regulator
6. Purge Airlock (automatic or manual)
7. Feed-Thru Adaptors & Rubber Stoppers

1. O2 Controller

The Oxygen Controller is intended to maintain an oxygen level in the glove box by sensing the current concentration and then opening the appropriate solenoid valve to allow gas to flow and purge the glove box. The Oxygen set point (SP1) and background gas set point (SP2) are used to set the oxygen range desired in the Chamber. We suggest the range be adjusted to your desired level +/- 0.1%. It can be programmed to maintain a wider range, which may be of use when working with elevated oxygen levels. A call to the factory will help you to reprogram your Controller.
SP1 and SP2 should always be set at the desired oxygen level

When the solenoid is opened to allow gas flow, you will hear a metallic click and the flow meter of the appropriate gas will rise to the set level.

Under normal use, only one gas line will be open at a time. The flow must be adjusted using the flow meters on the front of the Controller. If the Controller has been incorrectly set, or there is a malfunction, it is possible to have both gases flowing at the same time. Gas inlet pressures must be regulated at the source to 10-15 psi. UNDER NO CIRCUMSTANCE SHOULD THE INLET PRESSURE EXCEED 15 PSI. See below for specifics on desired flow rates.

To obtain uniform O_2 concentration throughout the glove box, the Fan (es) must be on at ALL times when the Controller is operating.

The alarm (AL) and temperature (C°/F°) functions are not included with the Oxygen Controller, therefore these indicator lights will not work.

To check gross calibration of the oxygen sensor, remove it from the Glove Box Wall and measure ambient conditions. Allow 30 minutes for temperature and oxygen stabilization. The ambient condition should be 20.9%.

NOTE: Standard temperature operating range for the O2 Controller is 0-43□ C.

Below are recommended Flow Rates

Type/Size	Flow Rate (SCFH)	Flow Rate (SCFH)
Polymer and Aluminum, Chamber / Glove Box	O_2 Gas*	Background Gas
Mini Polymer	≤ 2	≤ 10
1 Person Polymer/Aluminum	≤ 2	≤ 10
2 Person Polymer	≤ 4	≤ 20
2 Person Aluminum	≤ 6	≤ 30
Flexible Vinyl Units		
One Person	≤ 8	≤ 50
Two Person	≤ 10	≤ 60
Mini	≤ 4	≤ 40
In-Vitro Cabinets		
Model 1	< 0.2	≤ 1
Model 2	< 0.2	≤ 1.5
Model 3	≤ 0.2	≤ 2.5
Model 4	≤ 0.4	≤ 4
In-Vivo Cabinets		
Model 15	≤ 1	≤ 6
Model 30	≤ 2	≤ 10
Model 60	≤ 4	≤ 20

*NOTE: The O2 Flow Rates assume a 100% tank of O2 is used. For forced air or other % of O2 rates will have to increase.

The gas flow on the Flow Meters can only be adjusted when gas is flowing through that particular Valve.

2. Fan Box (Heated or Unheated)

Unheated Fan Box

The unheated Fan Box consists of a fan that circulates the Chamber's atmosphere providing a homogeneous mixture of gases. When the unheated Catalyst Box is plugged into a suitable outlet, the fan will immediately turn on and run continuously; there is no "On/Off" switch. The unheated Catalyst Box is not equipped with heating devices, so it cannot control the Chambers temperature. All COY Fan Boxes are shaped to hold a COY Stak-Pak, which commonly contains desiccant, so the glove box atmosphere is circulated through the desiccant providing an efficient means of removing excess moisture. In an Unheated Hypoxic system that does not require desiccant, it is possible to remove the fan box from the system saving valuable floor space and replace it with a simple circulation fan available from COY (part # 2600-150).

Heated Fan Box

The heated Fan Box consists of a power switch, 2 heating cones, and digital display, temperature sensor, and controls. The Heated Catalyst Box can maintain the Glove Boxes temperature from ambient to about 40 degrees Celsius. Before operating the Fan Box, remove the heat shield, screw the heating cones into their sockets, and replace the heat shield. When the Fan Box is plugged in to a suitable outlet, the fan will turn on and the controller display will light up showing the ambient temperature. The thermostat will control the temperature by turning the heat cones "on" and "off". When the cones are "on", a dot appears to the left of the temperature reading. The fan runs continuously while the power switch is "on" regardless of temperature setting. You may adjust the set point by simply pressing set point, waiting for the display to change to SP, and then adjusting the desired set point up or down. The Sensor can be placed wherever the temperature is most critical, including inside the Humidified Incubation Box.

NOTE: For optimum performance of the heated Glove Box (around 37°C) the laboratory environment should not have wide (+/- 5°C) temperature swings. The ambient room temperature in the lab should not be below 18°C. The Glove Box should not be placed near a drafty window or near heating/cooling duct.

3. Plug Strip

The six receptacle plug strip supplies power to the interior of the Glove Box. The outlets are used for continuously operating equipment, such as Fan Boxes, Incubator, and miscellaneous lab equipment you may wish to use. The Plug Strip is sealed into the chamber through a feed through adapter with a rubber stopper. If the plug strip is adjusted for length, you may need to smear some fresh silicone around the outside portion of the feed through adapter to ensure the air tight seal.

4. Gas Leak Detector

The Gas Leak Detector senses hydrocarbons and will detect leaks as small as a pin hole in a Hypoxic Chamber that is pressurized and has a hydrocarbon source (alcohol) that has been placed on the interior of the Glove Box. To operate the Gas Leak Detector, turn the black dial on and turn it all the way to maximum. Let the Gas Leak Detector warm up for 5-10 minutes on the maximum setting. After the prescribed warm up, turn the dial down to minimum at which time the beeping tone will slow down. Rotating the black knob varies the speed of the beeping tone. To detect leaks, turn the black knob so that the beeping tone is the slowest. Then turn the knob in the opposite direction so the beeping tone is on the verge of speeding up. Now you are ready to detect leaks. The Gas Leak Detector is energized by a single size "C" battery. Periodically you will have to replace the battery. To do this, remove 4 slotted screws securing the front panel. The "D" battery is accessible for replacement. If you are having trouble getting the Gas Leak Detector to "settle down", try a fresh battery. See Gas Leak Detector Manual for additional Information.

5. Nitrogen (background) Gas Regulator

Gas Pressure Regulators decrease the pressure exiting from you gas supply (primary pressure) to a pressure suitable for the Airlock and Oxygen Controller (secondary pressure). The secondary pressure must **not exceed 15 psi** (4.2Kg/sq.cm), if the tanks are within approximately 10 feet (3 meters) of the airlock. If you extend the gas lines, you may need to set the regulators higher than the maximum 15 psi (1.4 Kg/sq. cm) to produce the same flow of gas. The Background Gas Regulator as supplied will fit a standard Nitrogen tank; however, if a CO2/N2 mix is used, then the supplied adapter will be needed.

Once the Gas Regulators are installed and all tubing connected properly, slowly open the supply tanks. The primary pressure gauge will now display the amount of gas remaining in the tank. Turn the pressure gauge valve to regulate the gas flow to the airlock (secondary pressure gauge) to read 15 psi.

6. Purge Airlock (automatic or manual)

This unit purges the airlock for a set amount of time at an adjustable flow rate by the customer. To reduce the amount of oxygen that is introduced to the Glove Box when bringing in materials and equipment.

1. Be sure both airlock doors are closed.

Automatic version only

2. Set the timer for the desired purge time in seconds.

   a. Each of the numbers on the keypad represents a digit in the display the number one (1) keypad represents the 1st digit from the right, the 2 controls

the second digit from the right and so on. The RST button is a reset button to stop a purge in progress.

b. To change each digit press and hold the button, the numbers will cycle 0-9. Release the button when the number you desire is reached. NOTE: the 4th digit does not display a 0 but is blank instead.

c. The timer will count down from the set time to zero, always displaying the time remaining on the purge.

Automatic Purge Airlock Times

Gas = Nitrogen 95% Carbon Dioxide 5%

All measurements taken with a starting oxygen concentration of 20.9% (ambient)

Flow Rate (SCFH) *Standard Cubic Feet per Hour*	Time *(Seconds)*	Final O2 Concentration in the Airlock *(%)*
90	120	3.0
90	160	1.0
90	210	.05

Suggested purge times for given flow rates are given below. Experiment with your system to find the optimum setting for your application.

Above Ambient Airlock Purge Times

Gas = Oxygen 95% Carbon Dioxide 5%

Automatic Purge Airlock Times

Flow Rate (SCFH) *Standard Cubic Feet per Hour*	Time *(Seconds)*	Final O2 Concentration in the Airlock *(%)*
20	18	35
20	30	55

3. Press the start button and be sure the gas flow rate is correct. Warning: you must check the flow rate as there is no warning light for lack of gas flow.

Manual Version Only

4. Open the ball valve and purge glove box while manually timing the length of purge to match the desired O2 levels (see chart below). Ball valve is open

when handle is parallel to the incoming gas line and closed when perpendicular.

Manual Airlock Purge Times

Flow Rate *PSI**	Time *(Seconds)*	Final O_2 Concentration in the Airlock *(%)*
15	25	10.0
15	40	5.0
15	60	2.0
15	100	0.0

**PSI is not a flow rate but for purposes of the manual airlock it can serve it's purpose with the COY supplied ¼" OD Flexible Tubing and Gas Regulator.*

Never purge the airlock at a rate higher than 15 psi with ¼ OD Tubing.

7. Feed-Thru Adaptors

A power supply is sealed into the glove box through the COY Feed-Thru Adaptor. Part of the Feed-Thru Adaptor is a rubber stopper that has been cut apart to accept the cord of the plug strip and resealed with silicone and placed in the Feed-Thru Adaptor. Push the rubber stopper as far in to the Feed-Thru Adaptor as possible to ensure an airtight fit.

NOTE: If you adjust the length of the plug strip cord you may want to place a small amount of silicone around the cord and the rubber stopper to ensure the airtight seal

Installing cords into the extra Feed-Thru Adaptor

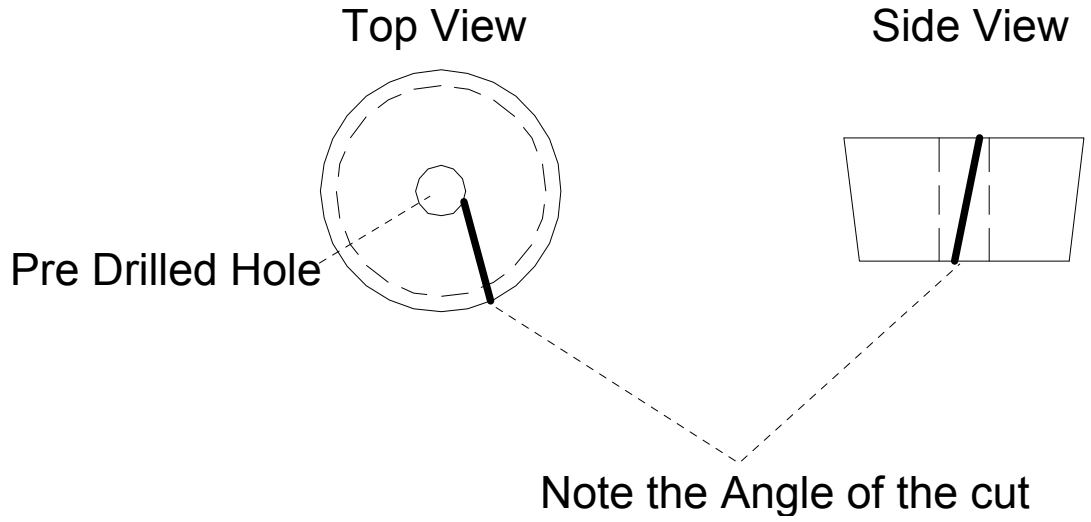
1. Measure the cord diameter being passed through the Chamber Wall.
2. Mark the center of the rubber stopper.
3. Use a drill bit slightly smaller in diameter then the cord diameter and drill a hole in the rubber stopper.

NOTE: The Rubber Stopper will get hot when drilling the hole.

4. Use a sharp Knife to cut the rubber stopper as shown on Figure #8
5. "Peel open" the rubber stopper and place a small amount of silicone in the drilled hole.

6. Place the cord into the hole, careful to position the cord to the ideal length inside and outside of the glove bag. Squeeze the rubber stopper over the cord and clean up the excess silicone with alcohol and paper towels.

Figure # 8 Rubber Stopper



6.2 Optional Equipment

*Note: Some of the following items (noted with a *) come with a complete manual which should be consulted for more detailed information prior to operation.*

1. Humidified Incubation Box
2. Internal Incubator (Large/Small)*
3. Stak-Paks (Desiccant and other materials)*
4. Atmosphere Filter*
5. CO2 Controller*
6. Animal Filtration System*

1. Humidified Incubation Box

This Cabinet, when placed in the interior of the chamber quickly takes on all characteristics of the glove box atmosphere and provides a high humidity atmosphere allowing cultures to be humidified while limiting the amount of humidity dispersed to the rest of the Glove Box. The small pump ensures uniform atmosphere inside the glove box. Temperature Uniformity will be +/- 1° C, or better with a stability in given location of +/- 0.5° C.

At 37° C with the pump running in the Incubation Box and two Stak-Paks in a 3 ft Chamber or four in a two person size unit, the humidity inside the Incubation Box stays over 80% Rh while the Chamber maintains 40% Rh or lower. Tests show that volume loss of aqueous fluid in plates in the Incubation Box kept under these conditions, changes by less than 2% over 48 hrs.

Hypoxic Units with the Automatic Humidity Control options should adjust controls to preferred levels and do not require desiccant.

Operation of the unit is simple, plug the pump in and allow it to bubble glove box atmosphere through the humidified Incubation Box. The air flow also agitates the water increasing humidity levels inside the box.

2. Internal Incubator (Model 2000/Model 2002)

These incubators provide an isolated area inside the glove box that can be heated up to 40° C. For added humidity a tray or beaker of water can easily be added. If concern arises regarding O2 uniformity inside the incubator COY Suggests purchasing a separate pump, drilling a small hole through the acrylic door and feeding tubing through that hole to bubble the chamber atmosphere to the interior of the incubator. If using water to add humidity to the interior of the incubator you should place the tube just above the water level.

The Temperature Set Point is adjusted by pressing the Set Point button and adjusting the desired set point with the UP or Down Arrows. The "Enter" button must be pressed for the controller to take the new set point (See separate incubator manual for further details).

Model 2002 Incubator

The Small Internal Incubator has 5 removable Drawers plus one extra (6 total). These drawers are designed with 4 aluminum racks to hold 100 mm plates. The drawers are sized to fit through the standard COY airlock door so that any samples that can be prepared on the lab bench can be placed directly on to the racks in the drawer and then placed back in the incubator. While the rack is out being prepared, the extra drawer should be used in place of the drawer that is being prepared. This will help prevent drastic swings in the incubator temperature. The Small Incubator capacity is 200 x 100mm plates.

Model 2000 Incubator

Complete with sliding doors and two interior shelves, this incubator will hold up to 475 100mm plates.

3. Stak-Paks General Info

Stak-Paks are constructed of a clear anodized aluminum frame and stainless steel screen. Their purpose is to provide users flexibility when adding chemicals to the chamber in order to produce a controlled environment. Typically, the Stak-Pak will be seated horizontally on the Catalyst Support Tray that is situated over the

Fan on the Atmosphere Control Cabinet. Up to 3 Stak-Paks can be vertically installed in a hypoxic glove box; more than 3 reduces the airflow and can disrupt temperature and moisture uniformity.

Examples of chemicals the user can place in a Stak-Pak are:

1. DESICCANT
2. ACTIVATED CHARCOAL
3. CARBOLYME (removal of CO2 in a glove box with live animals)

Figure #9 Stak-Pak Mounting

Stak-Pak

Desiccant Stak-Paks

The desiccant Stak-Paks are designed to maintain ambient humidity levels in the COY Hypoxic System when a Humidified Incubation Box or Internal Incubator is in operation and the Automatic Humidity Control option has not been purchased.

Since these equipment options are not sealed air tight from the rest of the glove box atmosphere, the trays of water will, in a short time, cause high humidity levels in the glove box. To prevent water condensation, the Desiccant Stak-Paks are used to maintain ambient humidity levels inside the glove box. The Desiccant will need to be rejuvenated once every 3-5 days in an oven at 200° C for 1 hour. If condensation and water build up begin to form on the chamber walls, it is a sign the desiccant needs rejuvenation. The Desiccant should be replaced once a year.

4. Atmosphere Filter, Recirculating (HEPA)

The Atmosphere Filter (HEPA) consists of a filter housing, small vacuum pump and tubing. The atmosphere is circulated through the pump then through the filter and back into the chamber. This removes 99.9% of airborne contamination with a size of 0.3 micron or larger. The pump cycles 30 cu. ft. per hour. Depending on the contamination present, you may have to run the filter 2-3 times a week or just a few times a month. Also, depending on contamination, the filter should be replaced 3-4 times a year or more.

5. CO2 Controller (Model #AC 100)

The Carbon Dioxide Controller is intended to maintain a CO_2 level in a chamber/glove box. It accomplishes this by sensing the current concentration, and then opening the appropriate solenoid valve to allow gas to flow and purge the system to the desired level. The set point is used to set the CO_2 level desired in the chamber. The controller has been programmed to maintain a level of CO_2 within +/- 0.1% of the set point, this is the **Control Tolerance**.

Starting in January 2004 all COY Hypoxic Glove Boxes are manufactured with 2 sensor ports and a Gas Manifold to enable the user to easily upgrade their units for CO2 control if not purchased with the original unit. See the CO2 Controller Instruction Manual for complete details on set-up and use of the CO2 Controller with the COY Hypoxic Glove Boxes.

In October of 2004 all CO2 Controllers are equipped to operate an Animal Filtration System (see below) to remove CO2 and other waste byproducts. The pump from this system is plugged into the rear of the CO2 Controller (labeled To A.F.S. Pump) and the switch is turned down to A.F.S. to operate. Set-Point is chosen (0% CO2) and the controller turns the pump on/off as needed based on the CO2 levels.

6. Animal Filtration System (Manual and Automatic Systems)

This system allows the user to maintain live animals in the glove box for long periods of time without worrying about toxic build up of CO2 or other waste by products. System comes equipped with 2 capsules, one filled with indicating Carbolime for CO2 removal. The other with activated charcoal for other various animal waste byproducts.

Manual System
The Pump Plugs into any suitable power supply outlet and is controlled by the ON/OFF switch attached to the supplied vacuum pumps power cord. There is no measuring device for this system, separate CO2 indicators can be used to tell the operators when to the turn the system ON/OFF or a protocol can be established

where the system operates on a set time interval (example: once a day for two hours, twice a day for one hour, etc.) The system can operate continuously but this will shorten the pumps life span (about 700 hours) and is generally not required for the amount of byproducts the animals give off.

HINT: System can be set up with store bought timers to automatically execute a set protocol, however it is recommended that the operation times be active when the user are there to see/hear the system working.

Automatic System
The COY CO2 Controller is included with a vacuum pump and capsules of Carbolime and activated charcoal. The Sensor is inserted into one of the two sensor ports (see page 6 for details on installing the Sensor). The pumps power cord is inserted into the rear of the CO2 Controller, the switch on the back of the CO2 Controller is set to A.F.S. (Animal Filtration System), and a set-point is chosen. The CO2 Controller turns the system ON/OFF based on the chosen set-point and the sensor readings.

In this configuration you do not have to be concerned with inlet and outlet ports on the CO2 Controller.

7.0 OXYGEN CONTROLLER OPERATION

This is merely to provide the user with a brief overview for operating the oxygen controller. For complete details, please consult the Oxygen Controller Manual.

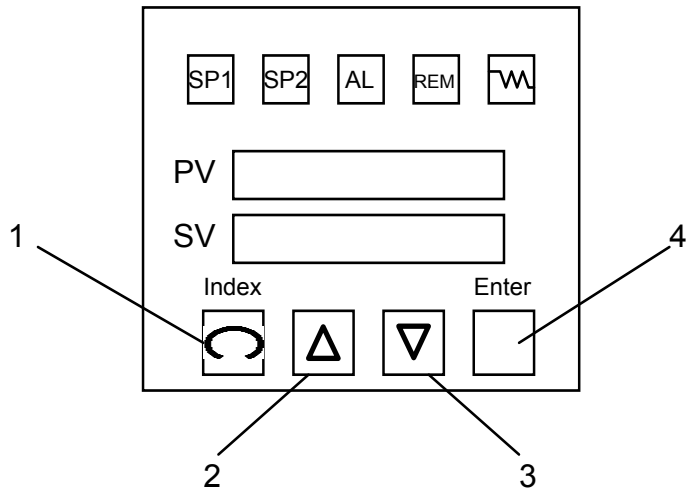
1. Plug the Controller into an electrical outlet.

2. Turn the unit on using the rocker switch on the back.

3. After going through a self-test routine, the display will show a red and green number. The red number (PV) is the measured O_2 concentration (PV=Present Value) and the green number is the oxygen set point value (SV).

4. Using Figure # 10, adjust the set points; Press the lower left key (1) until the desired set point, SP1 or SP2 appears. Press the arrow key (2 or 3) until the set point for the O_2 level is displayed in red on the top line. Press the right hand key (4) to set the value in memory. **If you do not do this, the new value will NOT be set, and the previous value will be restored.**

Figure #10 O2 Controller Display



SP 1 = Set Point 1
SP 2 = Set Point 2
AL = Alarm
REM = Not Used
PV = Present Value
SV = Set Value
= Not Used

5. Exit the adjustment mode by pressing the left hand key (1) until both numerical values described in (3) appear.

Calibration:
The sensor has an expected life of greater than 2 years. During that time, there may be a downward drift in the O_2 measurements. It is best to periodically (1/month) check the calibration. To do this, remove the sensor from the Chamber, plug the opening in the sensor fitting to maintain chamber integrity and allow the sensor to equilibrate to ambient conditions for 30 minutes. The ambient oxygen concentration should read 20.9% +/- 1%. If it does not, adjust the calibrate potentiometer (labeled "%") on the front until 20.9% +/- 1% is read. Contact Coy Laboratory Products, Inc. if the sensor requires frequent recalibration.

8.0 CARE AND REPLACEMENT OF GLOVES

Damaged gloves may be replaced without losing the atmosphere using the following instructions and Figure #11.

1. Obtain the extra glove and cuff, supplied with your chamber care kit. COY suggests the use of double sided tape to hold the new glove onto the cuff. A strip of the tape around the wide end of the cuff, and the glove stretched over it will hold the glove in place.

2. On the inside of the chamber remove the tape from the glove (figure #11 A).

3. Pull the glove through to the outside of the chamber (figure B) but do not remove it from the sleeve yet.

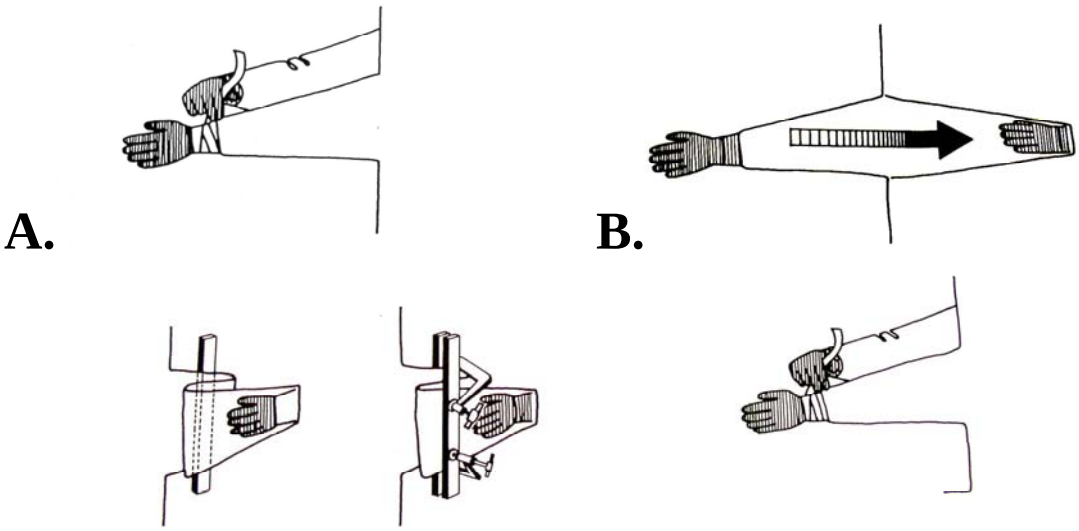
4. Carefully fold the sleeve in half and clamp the fold (figure C). The clamp can

be created by 2 smooth pieces of wood and 2 clamps.

5. With the clamp in place you may now pull the used glove out of the sleeve and insert the new glove. The vinyl should fit snuggly around the middle of the new cuff.

6. Remove the clamp and insert the glove into the interior of the chamber and tape it in place using the Vinyl Yellow Tape from COY. Start the first revolution of tape half on the vinyl sleeve and half on the neoprene glove. Overlap revolutions as you go around the cuff.

Figure #11 Glove Replacement Procedure



9.0 CARE OF PVC

9.1. PRECAUTIONS

Here are several precautions you can take to prolong the life of your chamber. Precautions you should carefully follow are:

1. *Do not set equipment with sharp edges on the chamber floor. Only shelving units and incubators supplied by COY should be placed on the chamber floor.*

2. *Always keep small instruments and sharp objects on the work mats. If you don't they may get lost under the mats and puncture the PVC.*

3. *Keep equipment and shelving units within easy reach so you do not stretch the chamber sleeves.*

4. Rings and jewelry should be removed prior to using the unit

9.2 CLEANING THE PVC

Any commercially available cleaner recommended for polyvinyl chloride (PVC) will be sufficient for cleaning dust, dirt and grease off the chamber and restoring its optical clarity. Coy Lab. Products uses "PLASTIC CLEANER MAGIC" (pt. no. 1600-480). It is also available at hardware stores or super markets. You should avoid cleaning the chamber with products containing ketones or other compounds that will damage PVC.

9.3 DETECTING LEAKS

1. Using the Gas Leak Detector supplied soak a rag in alcohol. Place the rag on the inside of the glove box. The alcohol is necessary as the gas leak detector only picks up hydrocarbons present in the alcohol.

2. With the inside door open inflate the chamber until the arms become extended. This stretches the vinyl and makes it easier to find small leaks.

3. Turn the Gas Leak Detector on and turn the sensitivity to the highest level and allow it to "warm" for 5-10 minutes. After the "warm-up" period turn the sensitivity down to the lowest point. If the "beeping" slows down then the Leak Detector is ready.

4. Turn the sensitivity up slightly so that you can audibly hear an increase in the beeping.

5. Now begin to go slowly over the entire glove box. Pay special attention to all the seals and seams, especially around the sleeves and gloves.

   NOTE: The interior of the sleeves and gloves will indicate a slight increase in the beeping. This may or may not be a leak as the gloves are more the most porous material on the glove bag, thus the slight increase in the beeping noise. A true leak in this area will have a large increase in the beeping noise. Call your local COY representative or the factory if you are unsure.

6. The gloves can easily be checked for leaks by simply pouring water into them. If water drips into the interior of the glove box then the glove should be replaced.

9.4 HOW TO PATCH PUNCTURE AND TEARS

Punctures and tears can be easily patched using the vinyl repair kit found in your chamber care kit. Before attempting to repair chamber leaks, release the pressure on the chamber by manually vacuuming it until gloves sag. If the leak is small, directly apply the liquid vinyl repair to the hole and surrounding area. Let it cure for about an hour and reapply. If the leak is large, cut a patch out of the extra vinyl supplied with the vinyl repair kit. Generously spread the liquid vinyl repair around the hole and apply the patch. You may have to temporarily hold the patch in place while the liquid vinyl repair is curing. In about an hour, apply the liquid vinyl repair to the edges of the patch, sealing the patch and PVC together.

After leaks are repaired, check them with your gas leak detector to verify correct repair. NOTE: SINCE LIQUID VINYL REPAIR CONTAINS HYDROCARBONS, WHICH ACTIVATE THE DETECTOR, IT MUST BE FULLY CURED BEFORE VERIFYING CORRECT REPAIR. The liquid vinyl repair works on the principle of softening the vinyl. When the vinyl is soft, it adheres to itself thus eliminating the leak.