

PRICE: \$10.00

# YSI MODEL 5300

## BIOLOGICAL OXYGEN MONITOR

### INSTRUCTION MANUAL



**YSI Incorporated**

Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio 45387 USA  
Phone 513 767-7241 • 800 343-HELP • Fax 513 767-9353 • Telex 205437

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## GENERAL DESCRIPTION

The YSI Model 5300 Biological Oxygen Monitor is designed to measure oxygen uptake and evolution by biological systems. It utilizes Clark type polarographic oxygen probes immersed in magnetically stirred sample chambers, and produces oxygen uptake or evolution curves in 2 to 15 minutes. This two channel instrument offers continuous and simultaneous display and recorder outputs for monitoring the results of each evaluation of samples.

Two different sensor systems are available. The "Standard System," for the measurement of samples of 3 to 8 ml, consists of a 5300 Monitor, a 5301 Standard Bath Assembly, two 5331 Standard Oxygen Probes, and other supplies. The "Micro System," for the measurement of a fixed volume sample of 600 uL or for a continuous flowing sample, includes a 5300 Monitor, 1 or 2 5356 Micro Oxygen Chambers, 1 or 2 5357 Micro Oxygen Probes, and other supplies. The Micro Oxygen Chamber and the Micro Probe are manufactured for YSI by Instech Laboratories, Inc. Both sensor systems are briefly described in Installation and Initial Setup. (An extended discussion of system components will be found in Appendix II.)

## SPECIFICATIONS

Oxygen Consumption Rate Range: 3 to 250 uL O<sub>2</sub>/hr in air-saturated solutions; 15 to 1250 uL O<sub>2</sub>/hr in oxygen-saturated solutions.

Probe Linearity:  $\pm 1.0\%$  of full scale.

Oxygen Consumption of the 5331 Standard Oxygen Probe: Less than  $1 \times 10^{-3}$  grams O<sub>2</sub>/hr ( $< 0.1$  uL O<sub>2</sub>/hr) in air.

Oxygen Consumption of 5357 Micro Oxygen Probe: Less than  $6 \times 10^{-3}$  grams O<sub>2</sub>/hr ( $< 0.005$  uL O<sub>2</sub>/hr) in air.

System Stability: Typically 0.5%, maximum of 1% of full scale/hr.

Stabilization Time: 60 seconds maximum with probe and solution at operating temperature.

Response Time: 90% of final reading in approximately 10 seconds from N<sub>2</sub> to O<sub>2</sub>.

O<sub>2</sub> Leak Rate: Less than 1% of full scale in 15 minutes of operation, at 50% saturation or more.

Channels: Each of the two channels is independently operated, and has a separate display and recorder output. Each channel has two input jacks, one for the standard probe and one for the micro probe.

Displays: Two 3 1/2 digit LCDs, 1/2" high.

Instrument Size: 15.8 H x 28.5 W x 22 D cm, 2.4 kg max  
6.2 H x 11.2 W x 8.7 D in, 5.3 lb max

Power: 115 vac  $\pm 10\%$ , 60 hz, 0.12 amp max  
230 vac  $\pm 10\%$ , 50 hz, 0.06 amp max

Recorder Output: 0 to 2.000 volts corresponds to full scale. 20 K ohms minimum load impedance required. The display is tracked within  $\pm 2\%$  of full scale; the differential output capability (Channel 1 minus Channel 2) is accurate to  $\pm 4\%$  of full scale.

Sample Chamber Size: 600 uL with the 5357 Micro Oxygen Probe and 1 to 50 mL with 5331 Standard Oxygen Probe.

Temperature Stability: With suitable circulator,  $\pm 0.2^\circ\text{C}$  of circulator temperature in the sample chamber.

Stirring Speed: 480 RPM with 5301 Standard Bath Assembly and variable to 1600 RPM with 5356 Micro Oxygen Chamber

Sample Temperature Range: 5 to 40°C

## ACCESSORIES AND REPLACEMENT PARTS

These accessories and replacement parts for the YSI 5300 system are available from your YSI dealer.

### Accessories

5301 Standard Bath Assembly
5302 Macro Bath Assembly
5303 Macro Adaptor Kit
5304 Micro Adaptor Kit
5313 Magnetic Retriever
5331 Standard Oxygen Probe
5350 Membrane Mounting Kit
5356 Micro Oxygen Chamber
5357 Micro Oxygen Probe
5358 Cable Assembly
5360 Accessory Kit

Replacement Parts for	5301	5304
Nylon Hold Down Ring	5092	5092
Lucite Plunger	5093	5307
Chamber Pack	5215	5215
Magnetic Stirrer	5222	5309
Magnet Retriever	5225	

Replacement Parts for	5331	5357
O-Ring Applicator	5338	5360
O-Ring Kit	5945	5360
KCl and Standard Membrane Kit (30)	5775	5775
KCl and High Sensitivity Membrane Kit (30)	5776	
Standard Membranes (150)	5793	5793
High Sensitivity Membranes (150)	5794	

## INSTALLATION AND INITIAL SETUP

Except when operating at ambient temperature, a constant temperature circulator is necessary to control the temperature of the sample chambers. A recorder is necessary for a hard copy of experimental results. Your YSI dealer can recommend suitable circulators and recorders.

## STANDARD SYSTEM

The YSI 5300 Biological Oxygen Monitor Standard System consists of the following components:

- 1 YSI 5300 Biological Oxygen Monitor
- 1 YSI 5301 Standard Bath Assembly, includes 1 Model 5350 Membrane Mounting Kit
- 2 YSI 5331 Standard Oxygen Probes, each with a 5775 KCl and Standard Membrane Kit

The Standard Bath Assembly has four sample chambers with built-in magnetic stirrers, surrounded by a water bath. A constant temperature circulator is connected to the bath. Two standard oxygen probes are prepared for operation using the 5350 Membrane Mounting Kit to install the parts found in the 5775 KCl and Standard Membrane Kit. The probes are held in Lucite plungers which fit closely into the sample chambers. The probes plug into the back of the instrument.

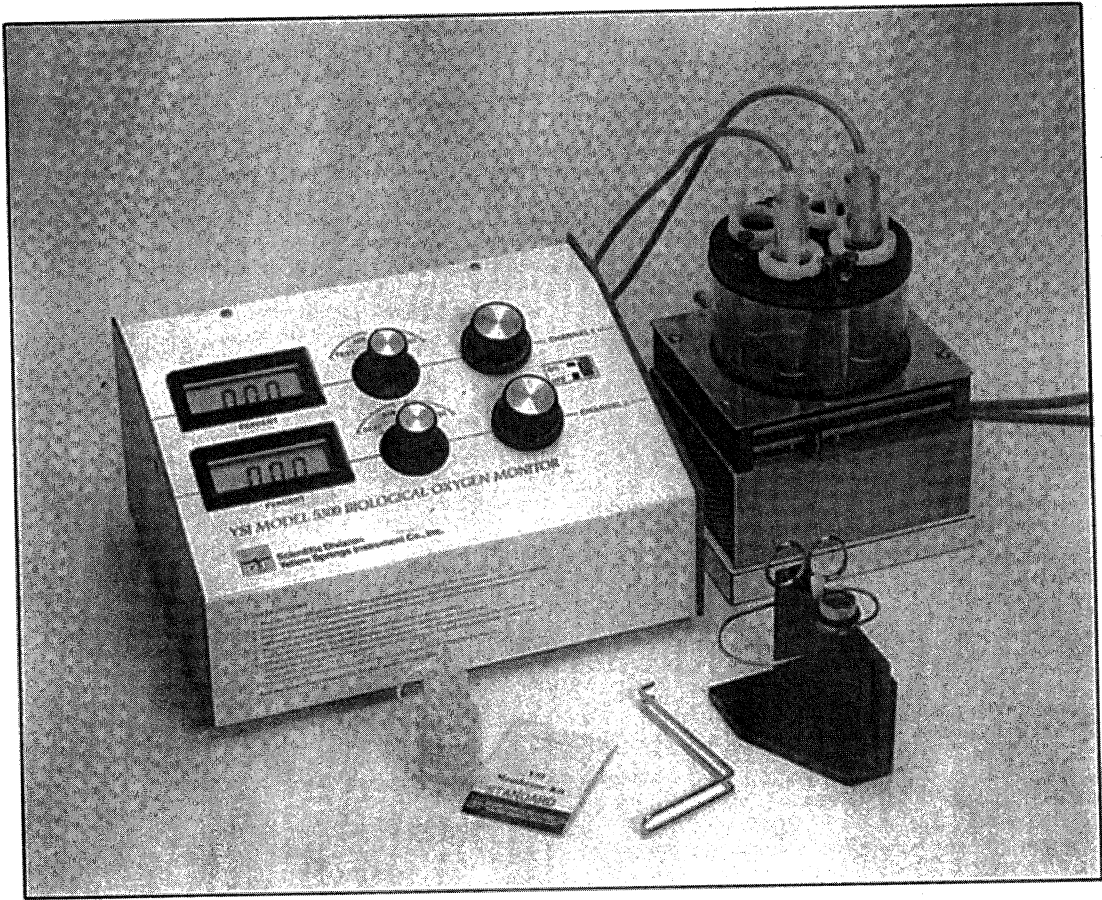


Figure 1. The Standard System

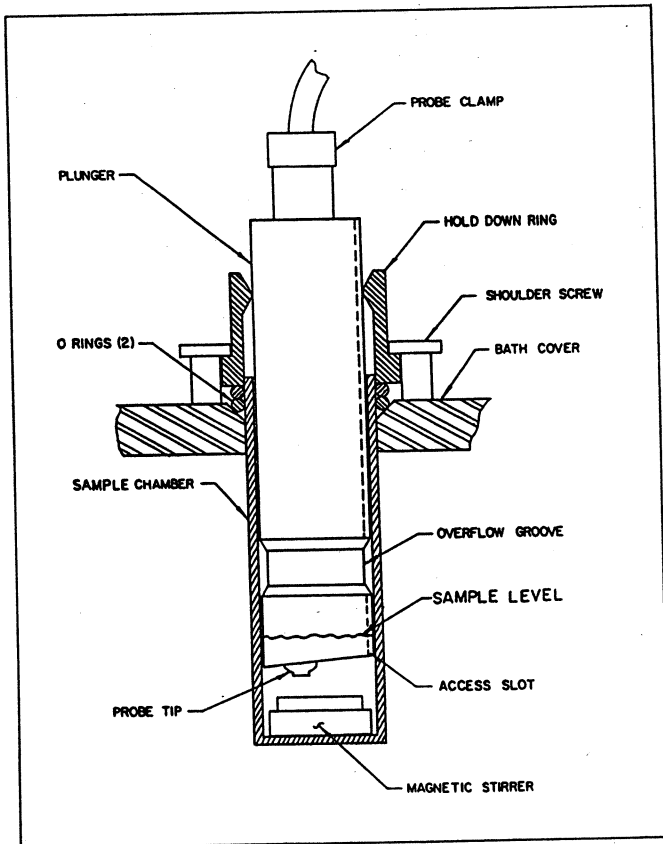


Figure 2. Probe, Plunger and Sample Chamber

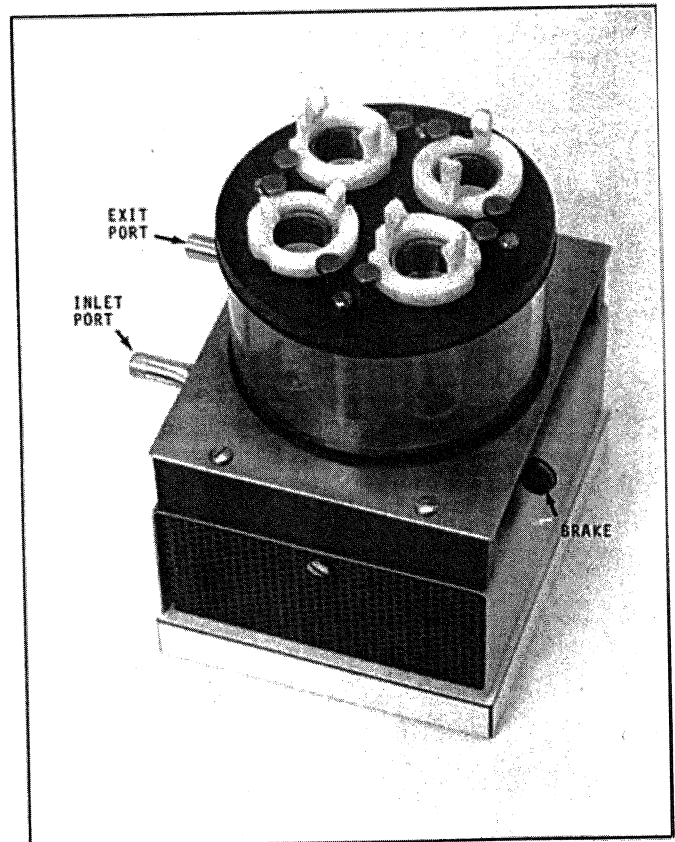


Figure 3. YSI 5301 Bath

Assemble the Standard System as follows:

1. Insert four glass sample chambers from the box marked 5215 from the Model 5301 into the holes in the bath cover.
2. With distilled or deionized water, wet eight of the large rubber o-rings supplied in the box marked 5215.
3. Place two rubber o-rings over each of the sample chambers.
4. Position each sample chamber in the bath so it rests on its stop in the correct vertical position while the hold-down ring is locked in place (next step).
5. Above the o-rings, place the nylon hold-down rings supplied in the box marked 5221 packed with the bath, pushing each one down and twisting clockwise to secure it under the stainless steel shoulder screws located beside each sample chamber hole.
6. Plug the line cords of the Model 5300 and Model 5301 into power receptacles and set the brake of the Model 5301 against the magnetic stirrer motor so as to prevent its rotation.
7. Connect the Model 5301 to a constant temperature circulator with the Tygon tubing supplied in the box marked 5221.
8. Fill the circulator with distilled water and set the controls to the desired temperature. Operate the circulator according to its own instructions.
9. Connect a recorder to the recorder binding posts on the back of the monitor. NOTE: The red binding post on the Model 5300 is positive and is at earth ground. The recorder should therefore be operated with its terminals ungrounded or with the positive terminal grounded. The Model 5300 has a self zeroing feature with no probes plugged into it, the recorder zero reference can be set with this zero output.

10. Prepare the oxygen probes for operation as described in the Probe Preparation section of this manual.
11. Plug the probes into the appropriate jacks on the rear of the Model 5300.

NOTE: When a Standard Oxygen Probe and a Micro Oxygen Probe are both plugged into the same channel's input jacks, the display and recorder values reflect the 5357 Micro Oxygen Probe response.

12. Numbered marking tape is supplied (box 5221) with the bath. Use it to identify each probe.

#### MICRO SYSTEM

The YSI 5300 Biological Oxygen Monitor Micro System consists of the following components:

- 1 YSI 5300 Biological Oxygen Monitor
- 1 or 2 YSI 5356 Micro Oxygen Chambers
- 1 or 2 YSI 5357 Micro Oxygen Probes
- 1 or 2 YSI 5358 Cable Assemblies
- 1 YSI 5775 KCl and Standard Membrane Kit

The 5356 Micro Oxygen Chamber can be used for fixed volume single samples or for continuous, flow-through monitoring. A constant temperature circulator, supplied by the user, is employed to control the block temperature. A remote magnetic stirrer controller keeps the sample chamber stirred when fixed volume samples are being measured.

The Micro Oxygen Probe is prepared for operation using the parts found in the 5775 KCl and Standard Membrane Kit. It is held in the sample chamber by a plastic, finger-tightened electrode sleeve.

The oxygen probe is connected to the monitor by the 5358 Cable Assembly. The monitor supplies a polarizing voltage and provides meter readout and recorder signal.

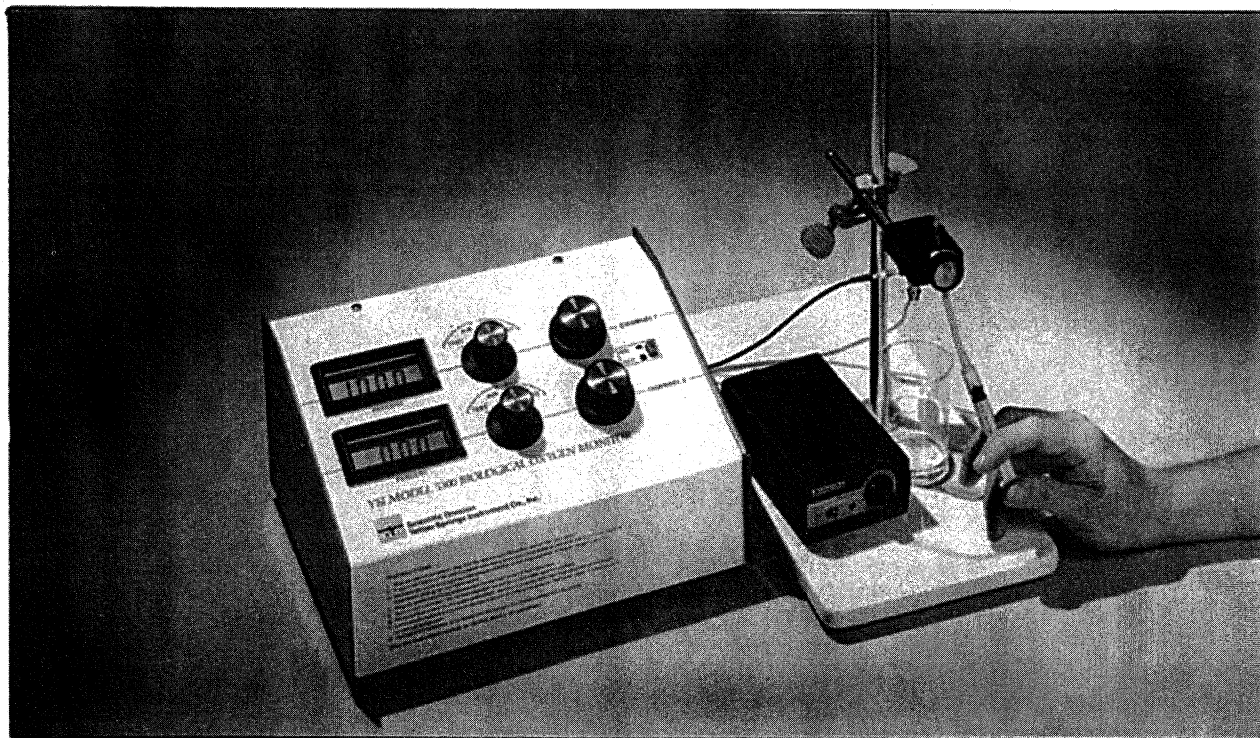


Figure 4. The Micro System

**Assemble the Micro System as follows:**

1. Assemble the 5356 Micro Oxygen Chamber as described in the Instech Laboratories Model 600A Instruction Manual provided.
2. Using Tygon tubing (not supplied), connect the Oxygen Chamber to a constant temperature circulator.
3. Fill the circulator with distilled or deionized water and set it to the desired temperature. Operate the circulator according to its own instruction manual.
4. Connect a recorder to the recorder binding posts on the rear of the monitor. NOTE: The red binding post on the Model 5300 is positive and is at earth ground. The recorder should therefore be operated with its terminals ungrounded or with the positive terminal grounded. The Model 5300 has a self zeroing feature with no probes plugged into it, the recorder zero reference can be set with this zero output.
5. Prepare the Micro Probe for operation as described in the Instech Laboratories Model 125/05 manual.
6. Thread the probe into the 5358 Cable Assembly.
7. With the monitor off, plug the Cable Assembly into the appropriate jack in the back.

**NOTE:** When a Standard Oxygen Probe and a Micro Oxygen Probe are both plugged into the same channel's input jacks, the display and recorder values reflect the Micro Probe's response.

8. Immerse the probe in distilled water up to 1" above the rubber o-ring until you are ready to insert it into the sample chamber.

**PROBE PREPARATION**

When preparing either the 5331 Standard Oxygen Probe or the 5357 Micro Oxygen Probe, certain precautions should be taken to ensure repeatable and reliable performance. Do not touch the membrane except at its edges where it will not come in contact with the probe's electrodes. The oils on your skin can cause contamination. With reasonably careful handling, a membrane can be expected to last a week or more. Because the membrane is water permeable, the probe tip should be kept immersed in distilled water after it has been prepared and until it is ready to be used. This will eliminate the need for frequent recharging of the probe with electrolyte.

When storing the probe for extended periods, clean it well and install a membrane (no KCl) to protect the electrodes.

The routine daily probe test is described in Operation.

**STANDARD PROBE**

The Standard Oxygen Probe comes with a YSI 5775 KCl and Standard Membrane Kit. It is designed to be used with the 5301 Standard Bath Assembly. The following instructions describe the probe's preparation for this device, and presumes the use of the 5350 Membrane Mounting Kit supplied with the bath. (The standard probe will also perform correctly in other setups, as the user may devise.)

The KCl and Standard Membrane Kit contains two 15 count packs of .001 inch FEP Teflon membranes and a squeeze bottle containing dry KCl for preparing the half-saturated electrolyte.

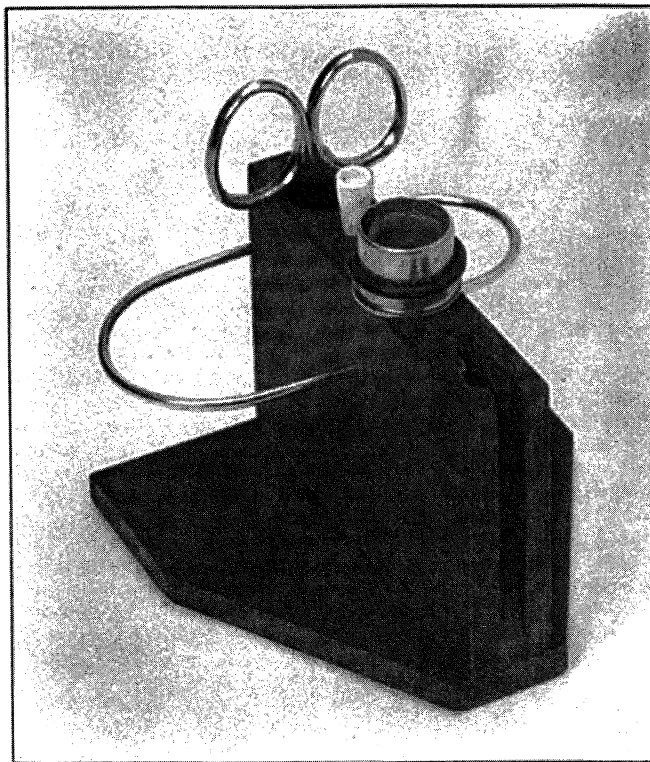


Figure 5. YSI 5350 Membrane Mounting Kit

Prepare the probe as follows (see Figures 5 and 6):

1. Fill the squeeze bottle with distilled water and ascertain that the KCl crystals are completely dissolved.
2. Remove the rubber o-ring and membrane from the probe.
3. Place a new membrane over the unflared end of the membrane holder.
4. Slip the large rubber o-ring from the Membrane Mounting Kit down over the membrane and membrane holder so as to form a drum.
5. Place the o-ring from the probe over the small end of the Teflon o-ring applicator and slide it to the large end.
6. Seat the probe (cable end down) firmly into the slot in the front face of the probe holder. The probe should rest in the bottom of the round hole.
7. Wet the whole tip of the probe, including the o-ring groove, with electrolyte.
8. Position the membrane holder (membrane up) directly over the probe. Pass the membrane holder down over the probe until it rests on the probe holder. The membrane should now be stretched over the end of the probe and be wrinkle free.
9. Keeping the membrane tight, place the large end of the Teflon o-ring applicator down against the end of the probe and push the rubber o-ring down off the applicator and onto the probe until it seats in the o-ring groove.
10. Remove the large rubber o-ring from the membrane holder and slip the holder off the probe by raising it straight up. Be careful not to disturb the membrane.

11. Inspect the probe carefully. The membrane should be free of wrinkles and without holes. No air bubbles should be present under the membrane. Use a loupe or microscope for this inspection.

12. Cut off the excess membrane material close to the rubber o-ring with the scissors supplied.

13. With distilled water, rinse the excess KCl solution from the outside of the probe.

14. Wet the rubber o-ring on the probe with distilled water and insert the probe into the Lucite plunger supplied in the box marked 5221 from the bath assembly.

15. By hand, screw the nylon probe clamp into the Lucite plunger until it is tight. (The rubber o-ring can be observed through the Lucite plunger). A black ring will appear and widen as the clamp is tightened. Inspect this black ring for continuity (a true seal is required). If the membrane has not been carefully trimmed, it could interfere with the seal.

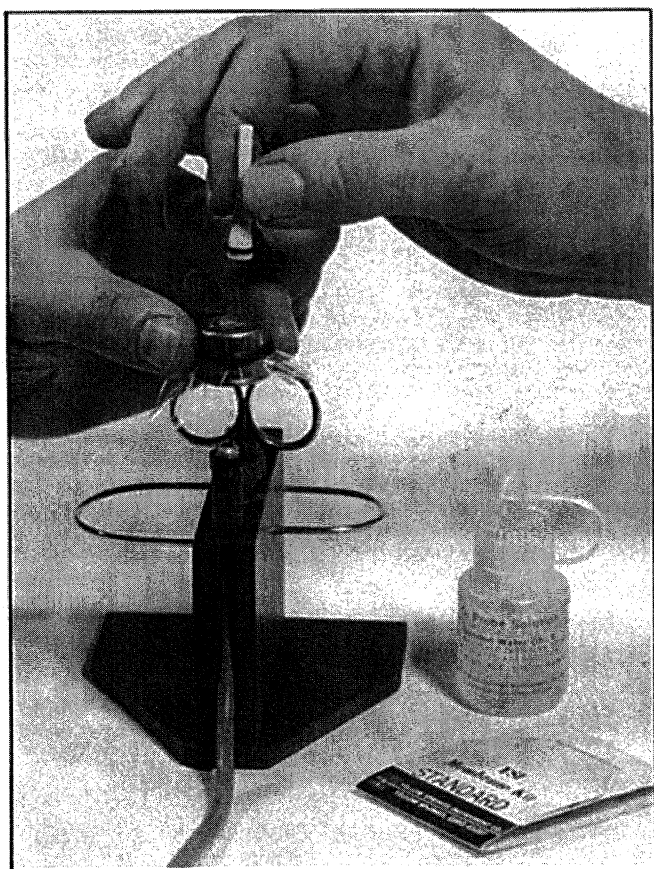


Figure 6. Mounting the Membrane on the Standard Probe

#### MICRO PROBE

The Micro Oxygen Probe is designed specifically for use with the 5356 Micro Oxygen Chamber. Follow probe preparation directions in the Instech Laboratories Model 125/05 Instruction Manual. To insure that the YSI system specifications are met, the probe should be prepared with the 5775 KCl and Standard Membrane Kit. High sensitivity membranes should not be used with this probe because they make the probe so sensitive to "noise," that oxygen readings are liable to be misleading.

#### OPERATION

##### Standard System

This section is intended as a general guide for use of the YSI 5300 with the "Standard System." It should be noted that this procedure can be generally followed for the "Micro System" as well. (See next section.)

1. Turn on the circulator and allow sufficient time for it to come to the desired temperature.

2. Turn on the monitor and release the brake on the bath assembly.

3. Into one of the sample chambers of the bath, place 3 mL of air-saturated distilled water, and the magnetic stirrer supplied with the bath.

4. Allow 3 minutes for temperature equilibration then reset brake.

5. Insert the prepared Channel 1 standard probe into the sample chamber. Remove all of the air from the sample chamber through the access slot on the Lucite plunger (a slight twisting of the plunger helps gather the bubbles at the access slot). The solution level in the access slot should be between the lower end of the Lucite plunger and its overflow groove.

6. Release the stirrer brake.

7. Rotate the Function Switch to AIR, and set the display to 100.0% with the Channel 1 CAL control. Turn the CAL control locking knob clockwise to lock the knob.

8. Set the recorder to full scale. Note that the monitor's recorder output for 100.0% is equal to 1.000 volt full scale.

9. Observe system stability as indicated by the recorder trace. The trace should be noise-free and have no more drift than 1/2% in 15 minutes.

10. Routine Daily Probe Test. (Does not need to be repeated for each measurement.)

a. Set the Function Switch to AIR and wait until the recorder traces a steady value.

b. Unlock the CAL control knob, set the reading to 90.0%, and relock the knob.

c. Turn the Function Switch counterclockwise to the TEST position and wait for a steady trace.

d. The TEST trace should be no lower than 87.0% of full scale after 2.5 minutes. If the probe does not meet this specification, the membrane should be replaced (see Probe Preparation). If performance still does not improve, the probe should be cleaned (see Probe Care and Maintenance).

11. After the Probe Test, unlock the CAL control knob and reset the display to 100.0% when you have determined that the probe is functioning correctly, then lock the CAL control knob.

12. Reset the brake and place a 3 mL sample and a magnetic stirrer into a sample chamber.

13. Release the brake and allow 3 minutes for temperature equilibration and then reset the brake.

14. Insert a prepared probe into this sample chamber as in Step 5.

15. Allow from 2 to 15 minutes for the recorder to produce a trace defining the oxygen uptake curve.

16. Determine the actual oxygen consumption rate as illustrated in the following example:

An air-saturated sample of Ringers solution at 1 atmosphere and 37°C. contains 5.02 uL O<sub>2</sub>/mL (see Table 1). Therefore, a 3 mL sample contains 5.02 uL O<sub>2</sub>/mL x 3 mL = 15.06 uL O<sub>2</sub>. If the oxygen concentration were to change from 82% to 64% in 5 minutes, the air saturation would have been reduced by 18%.

A change of 18.0% in saturation means 15.06 uL O<sub>2</sub> x 18.0% = 2.71 uL O<sub>2</sub>. On an hourly basis, 2.71 uL O<sub>2</sub> x 12/hr = 32.52 uL/hr.

17. To remove a magnetic stirrer from a sample chamber, use the magnet retriever supplied with the bath.

18. Consult Appendices I and II for a more specific and thorough treatment of a particular measurement situation, and for a detailed discussion of instrument principles and system components.

#### Micro System

A thorough discussion of measurement with the Micro System can be found in the Instech Laboratories Model 600A Instruction Manual provided with the 5356 Micro Oxygen Chamber.

#### Sequential Sampling with the Micro System

To run sequential samples on the micro system without having to disassemble the sample chamber for repeated disinfection, use the following procedure.

1. After measuring a sample, rotate the window-valve to the fill/rinse position and aspirate the sample with a syringe through the bottom port.
2. Inject 5 mL of a 5% Clorox solution into the bottom port, and allow it to flow out the top port with the rear port plugged.
3. After 15 to 30 seconds, aspirate the Clorox as in step 1.
4. Using the same procedure as in step 2, flush the sample chamber with 5 mL of distilled water or buffer.
5. Wait 30 seconds and repeat step 4. The display reading should be within 1% of the calibration value for the last sample.
6. Recalibrate for the next sample.

#### PROBE CARE AND MAINTENANCE

When properly used, the YSI 5331 Standard Oxygen Probe requires very little maintenance. It may occasionally be necessary to clean the silver anode to remove contamination. To clean the anode, immerse the probe in ammonia for 10 to 60 seconds, then wipe it with a cotton-tipped swab. The NH<sub>4</sub>OH reagent should be diluted 1:1 with distilled water and the probe cleaned only when necessary.

The platinum cathode requires servicing after long periods of use. The plateau within which the cathode is mounted must be kept flat and smooth. To re-establish this surface, the probe end should be rubbed in a circular motion on a frosted microscope slide, using special care to keep the probe body perpendicular to

the slide and to rotate the probe in only one direction--either clockwise or counterclockwise. Use distilled water on the frosted slide.

Consult the Instech Laboratories manual for service on the Micro Probe.

#### CALIBRATION

For practical purposes, calibration of 100.0% saturation in air-saturated water or in an experimental medium such as Ringers solution at known temperature and pressure and of known composition, will provide reliable, accurate, and repeatable results. The oxygen depletion assay described in the Operation section is based on a calibration in air-saturated water at the same pressure and temperature as the sample. Where comparative results are sought, the procedures described will provide reliable, accurate and repeatable results. However, if absolute values under specified conditions are needed, numerous factors affecting measurement must be carefully controlled.

The ability of a liquid to hold oxygen (or any gas) varies according to its constitution, its temperature and the ambient pressure. Oxygen solubility decreases as the salt content or temperature of a solution increases, or as atmospheric pressure decreases. Tables 1 through 6 in Appendix V provide data to help calculate oxygen solubility.

The probe membrane itself has a temperature coefficient of 2%/°C, while temperature changes in a sample chamber sealed by an inserted probe can cause another 2%/°C change in oxygen solubility. Barometric variation between the normal daily extremes (25 mm in a 760 mm scale) can cause 3% of change. (And a 343 meter elevation above sea level will cause as much as a 4% decrease in oxygen solubility. See Table 6.)

It is important for the experimenter to be aware that "100% oxygen saturation" can mean very different things--not only for different solutions, but for the same solution under different conditions.

#### WARRANTY

All YSI products are warranted for one year against defects in workmanship and materials when used for their intended purposes and maintained according to manufacturer's instructions. The YSI 5356 Micro Oxygen Chamber and the YSI 5357 Micro Oxygen Probe are manufactured by Instech Laboratories, Inc. and have a warranty of their own. Please see the warranty section of the instruction manual that came with each. Damage due to accidents, misuse, tampering, or failure to perform prescribed maintenance is not covered. This warranty is limited to repair or replacement at no charge.

#### If Service Is Required:

Contact the YSI dealer from whom you bought the instrument.

Report the date of purchase, model, serial number, and the nature of the failure. If the repair is not covered by warranty, you will be notified of the charge for repair or replacement.

When shipping any instrument, be sure that it is properly packaged and insured for complete protection.

In communications regarding this instrument or accessories please mention the model and serial numbers.



## APPENDIX

### I. Application Example

Preparation and evaluation of a mitochondrial sample is described below as an example of a normal sequence of procedural steps in using the YSI 5300 micro system. Presumably, users of the 5300 will develop their own techniques and procedures for their own particular purposes.

#### A. Cell Preparation

Liver biopsy samples (approx. 1 gm) are maintained in ice cold saline in the laboratory immediately following laparotomy. Each sample is placed into ice cold 0.25M sucrose in a small sterile plastic petri dish in a container of crushed ice. The sample is minced with scissors, chopped with a single edge razor blade, and rinsed twice with 0.25M sucrose to remove residual red cells. The liver mash is placed in 5 mL of 0.25M sucrose in a glass homogenizer tube. After 10-15 passes with a loose Teflon pestle, the homogenate is filtered through a #60 stainless steel (or nylon) mesh into a 50 mL polycarbonate tube. The filtrate is centrifuged at 500 g in a refrigerated centrifuge at 1°C for 5 minutes. The supernatant is carefully removed and saved. The tube is wiped clean with a Q-tip to remove excess lipid adhering to the walls, and the cells are then resuspended in 5 mL of a glutamate-maleate (GMC) medium of equal volumes of 0.005M K-phosphate buffer, 0.07M KCl, 0.005M glutamate, and 0.005M malate adjusted to pH 7.2 in 0.15M sucrose and 0.04M Tris. The cell suspension is mechanically stirred with a Vortex-Genie for 10 seconds before an appropriate volume is placed into the sample chamber of a YSI bath assembly. After a few seconds for equilibration, the initial oxygen uptake is measured at 37°C. (The probe is prepared as described by Lessler and Scoles<sup>1</sup>.) Either 1 uL of 0.5M succinate, or 1 uL of sterile blood serum is added with a micro-syringe to test for respiratory responses. Three to four runs are carried out with each sample, and a sub-sample of cell mash is saved for protein determination using the Biuret reaction<sup>2</sup>. All data are calculated in microliters of oxygen per hour per milligram of protein or in nanomoles of oxygen per hour per milligram.

#### B. Mitochondrial Preparation

The supernatant and remaining cell mash are resuspended in 5 mL of 0.25M sucrose in the glass tube of a homogenizer, and after 15 passes with a tight pestle, the resultant homogenate is poured into 50 mL polycarbonate tubes and centrifuged at 500 g at 1°C for 5 minutes to remove unbroken cells and cell debris. The supernatant is then pipetted into clean, dry 50 mL polycarbonate tubes, being careful not to disturb the sediment, and centrifuged at 12,000 g at 1°C for 10 minutes. After discarding the supernatant, a Q-tip is used to wipe the lipid residue from the sides of the tube. The grayish-black mitochondrial button is washed several times with 0.25M sucrose to remove the fluffy coat, and a glass needle is used to scrape off extraneous matter from the periphery of the mitochondrial button. The mitochondria are resuspended in 1-3 mL of 0.25M sucrose using a small Teflon pestle and a mechanical stirrer. A sub-sample of the mitochondrial suspension is saved for protein determination by the Biuret reaction. Mitochondrial oxidative phosphorylation is

<sup>1</sup>Lessler MA, Scoles PV. Respiratory activity of isolated chondrocytes with a miniaturized oxygen electrode system. "Ohio J Sci" 80:262-268, 1980.

<sup>2</sup>Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. "J Biol Chem" 177:751-766. 1949.

studied in GMC or other medium equilibrated with air at 30°C, with continuous stirring. Five runs are conducted with each mitochondrial sample at a dilution of 1.5 to 2.0 mg of protein per milliliter. Initial mitochondrial oxygen uptake is measured before 1 uL of 0.1M ADP is added. For mitochondrial coupling, 1 uL of biliary dog serum is added during the same run to test the respiratory response. Oxidative activity is recorded with a potentiometric recorder and expressed as nanomoles O<sub>2</sub>/minute/mg protein. ADP/O and Respiratory Control Ratios (RCR) were calculated and expressed as the mean of the 5 replicate runs with each sample. All data are reported as mean ± standard deviation and tested for significance by t test.

#### C. Mitochondrial Measurement Using the Micro System

1. Prepare the Micro Oxygen Probe as described in the Instech Laboratories 125/05 manual.
2. Prepare the Micro Oxygen Chamber as described in Instech Laboratories 600A manual.
3. Flush the sample chamber with air-equilibrated, distilled H<sub>2</sub>O. (Be sure to remove all bubbles).
4. Set the water circulator to the desired temperature and turn it on. (Isolated mitochondria are best measured at 25° or 30°C).
5. Check that the probe is ready for use, and screw it into the side port of the sample chamber. Then, attach the 5358 Cable Assembly, plugged into Channel #1 or #2 in the back of the monitor.
6. Check the operation of the monitor, the Model 5357, and the recorder by setting the Model 5300 to 100 % for air-equilibrated H<sub>2</sub>O.
7. Using a 5 mL plastic syringe attached to the bottom port of the sample chamber, flush with air-equilibrated reaction medium. (This is done by preparing the mitochondrial medium and equilibrating it with air by stirring it in a sample chamber of a 5301 Standard Bath Assembly or in a small Erlenmeyer flask placed on a stirring unit).
8. Important: the medium that fills the sample chamber must be air-equilibrated at the temperature at which the subsequent measurement is to be made.
9. Set the Function Switch to the AIR position, and calibrate to 100.0%. Set the recorder to full scale and lock the CAL control.
10. Rotate the sample chamber window so that only the top port is open. Remove the plug from the back port and slowly introduce the mitochondrial sample with a microburette or syringe. Sample volumes for mitochondrial studies are usually 1 to 10 uL and should be introduced at the same temperature as that maintained in the sample chamber (25° or 30°C) to eliminate a temperature differential to the probe.
11. After isolation, the protein content of the mitochondrial sample must be determined so that a correct dilution can be made. (Isolation of mitochondria is discussed in A and B, above.)
12. The mitochondrial sample (1 to 10 uL) is introduced into the equilibrated sample chamber which has a total volume of 0.6ml. The final mitochondrial concentration in the chamber should be no more than 2 or 3 mg protein/mL. (If the concentration is too high, the reaction will go too fast for adequate additions. If the mitochondrial concentration is too low, the measurement will be too slow and fewer replicate runs can be made).

13. Establish the base-line activity for the sample (see Figure 7) as shown by the expected unstimulated slope.

14. Add 1 to 3  $\mu\text{L}$  of 0.1M ADP with a microsyringe through the add port and allow the reaction to run until all the ADP is utilized. (This is shown by the return of the curve to its unprimed or basal rate.) This test for mitochondrial coupling and measurement of the  $\text{O}_2$  utilized per unit of time gives the ADP/O ratio and the control ratio (RCR).

15. Add 1 to 3  $\mu\text{L}$  of intermediates or other substances to be tested sequentially for activation or suppression of mitochondrial activity.

16. Terminate the run by addition of a small amount of 2,3 dinitrophenol (or other uncoupling agent) to get a record of the uncoupled mitochondrial activity.

Figure 7 shows the results of a similar experiment performed with YSI biological oxygen instrumentation. Obviously, the results of the user's experiments will not numerically match either the application example described above, or the record shown in Figure 7 below.

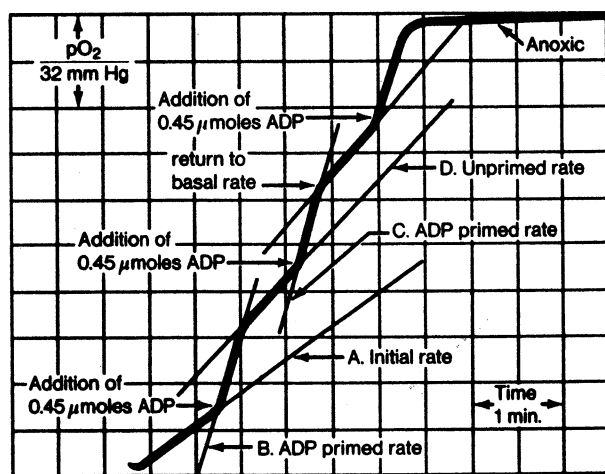


Figure 7. Record of oxidative activity of Mitochondria isolated from beef heart and primed with ADP.

## II. PRODUCT DISCUSSION

This appendix is intended to further acquaint users with the Model 5300 Biological Oxygen Monitor and the accessories that are available for use with it. Important tips for operation are also discussed.

### A. POLAROGRAPHIC OXYGEN PROBE

A Clark type oxygen probe is used in this complete polarographic system. A thin membrane stretched over the end of the probe isolates the electrodes from the environment. The membrane is permeable to gases and allows them come in contact with the probe face. When a suitable polarizing voltage is applied across the electrodes, oxygen will react at the cathode causing a current to flow. The current is proportional to the amount of oxygen which permeates the membrane. The probe actually measures oxygen pressure. Since oxygen is rapidly consumed at the cathode, it can be assumed that the oxygen pressure inside the membrane is zero. Thus, the force causing oxygen to diffuse through the membrane is proportional to the absolute pressure of oxygen outside the membrane. If the oxygen pressure

increases, more oxygen diffuses through the membrane and more current flows through the electrodes. A lower pressure results in less current. Diffusion through the membrane is directly proportional to pressure. The oxygen pressure/probe current relationship is stoichiometric. That is, the relationship between external oxygen pressure and probe current is linear.

The 5331 Standard Oxygen Probe contains a .025" diameter platinum cathode and a silver anode encased in an epoxy block. The 5357 Micro Oxygen Probe contains a epoxy body covered with a silver anode sleeve. These configurations facilitate cleaning, minimize the volume of filling solution required, and insure membrane tension. A Teflon membrane is secured with a rubber o-ring.

The characteristics of the polarographic probe should be understood by the experimenter.

### 1. Electrical Characteristics

The current output of the probe is dependent upon the cathode area and the permeability of the membrane directly above the cathode. Individual probe variations can be attributed to differences in membrane characteristics; both film irregularities and variations of installation methods contribute to such differences. Variations of  $\pm 10\%$  may be experienced with membranes from the same package. Wider variations should be suspect. Look for gross membrane imperfections, membrane rupture or damage during installation.

### 2. Voltage Plateau

When the system is operating correctly, the current output of the probe is nearly flat between 0.6 and 0.8 volts input. A long, flat "plateau" region permits current to be relatively independent of applied voltage, and results in linearity of output signal for a wide range of oxygen pressures.

The probes are operated with a polarizing voltage of 0.8 VDC. The plateau specification is that the output signal shall change less than 3% when the polarizing voltage is lowered to 0.7 VDC. In terms of system performance, this translates to a departure from linearity of less than 0.2%, at worst case conditions.

The TEST function of the 5300 provides a means for checking the plateau.

The plateau test should show a change of less than 3% for new and freshly cleaned probes. Determine this by comparing the displayed values at AIR (0.8 volts) and TEST (0.7 volts). Probes showing slopes of 5 to 8% (older probes or those needing cleaning) may prove entirely serviceable. Check the probe recovery time when subjected to a step change in the polarizing voltage (make the observation when changing from the AIR to the TEST position). The signal should be within 3% of the AIR value 2 1/2 minutes after switching (see Operation section).

### 3. Noise

Noise can be attributed to many parts of the system, to poor grounding, to pick-up from high voltage machines, etc., but two kinds of noise can originate in the probe.

a. The occasional burst or spike occurring frequently but randomly. Check a second probe; if both probes behave in the same way, the trouble is probably elsewhere.

If the noise originates in the probe, a damaged membrane may be the cause (folds or creases in the

membrane are always suspect). Examination of the probe under a low power microscope or jewelers loupe can be useful. Check for holes, creases, KCl growths, or drying out under the membrane.

b. Continual noise of several percent of full scale which may increase in magnitude with time. The membrane may be in perfect condition. The silver anode may not be making good reference contact with the solution. Cleaning with ammonia is recommended (see Probe Care and Maintenance).

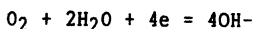
#### 4. Temperature

The current from the probe is highly dependent on the temperature, and in particular, the temperature of the membrane adjacent to the cathode as well as the sample chamber temperature. The permeability of the membrane is temperature sensitive. The FEP Teflon membrane YSI uses has a temperature coefficient of permeability of 2%/°C. Accurate temperature control is therefore required, and temperature equilibration time must be considered when making changes in the setup. An additional 2 %/°C change occurs in a sealed sample chamber because of the change in oxygen solubility. So, it can be seen that a 1°C change in temperature can cause a change of as much as 4% from the 100.0% calibration value.

When the nature of the experimental samples necessitates measurement at temperatures from 5°C to 15°C, it is recommended that the Model 5301 Standard Bath Assembly be used. At these lower temperatures it will be necessary to use a more sensitive membrane due to the lower oxygen content in your sample. It is recommended that the YSI 5776 KCl and High Sensitivity Membrane Kit be used. The membranes that come in this kit are .0005" thick and allow twice the sensitivity that the standard experimental results accordingly.

#### 5. Oxygen Consumption by the Probe

The polarographic probe consumes oxygen.



The rate of oxygen consumption by the probe is in direct proportion to the current produced by the electrodes. The most practical way to minimize the errors due to oxygen consumption by the probe is to use a small area cathode.

A probe current of 1 microamp is equivalent to 8.3 x 10<sup>-11</sup> grams of oxygen per second consumed by the probe.

The 5331 Standard Oxygen Probe has a probe current of about .33 microamps in air at 37°C. For a biological system containing 19 micrograms of oxygen (3 milliliters at 37°C), about .13% error would accrue per 15 minutes of operation. The 5357 Micro Oxygen Probe has a probe current of about .018 microamps in air at 37°C. For biological systems containing 3.8 micrograms of oxygen (.6 milliliters at 37°C) about .04% error would accrue per 15 minutes of operation.

For most work, the errors described above can be neglected, but when using very small samples or conducting experiments for extended periods, significant offsets may accumulate.

#### 6. Electrolyte Saturation and Bubbles in Electrolyte

The oxygen probe must be considered a part of the total system under study. For example, the 1/2 saturated KCl electrolyte which fills or covers the probe has a definite volume and contains dissolved gases including oxygen (only the electrolyte covering the platinum

cathode is free of oxygen). The amount of oxygen "stored" in the electrolyte depends on the volume of the electrolyte and its immediate history.

When the oxygen level of the sample is decreased during a run, oxygen is induced to leave the electrolyte, pass through the membrane and enter the sample, thus introducing possibly significant error.

The 5331 Standard Oxygen Probe has an electrolyte volume of 2 to 3 microliters while the Model 5357 Micro Oxygen Probe has less than 1 microliter. Errors caused by oxygen in the electrolyte in either probe, therefore, may be disregarded except for very small samples.

More serious is the presence of gas bubbles under the membrane. Volume for volume, an air bubble contains 20 times more oxygen than air-saturated water. Thus, if a large gas bubble is present, significant error can occur with normal sized samples. Furthermore, since equilibrium must be attained by diffusion through the membrane, the system may be sluggish and exhibit slow drifts.

#### 7. Drift of Calibration

A probe in good condition operating in a well controlled environment will typically exhibit drifts of less than 5% per hour. A drift rate of only 1% per hour is not uncommon, but external factors influence the test and some of them are difficult to identify or control.

Factors that may influence the drift rate of the system are changes in air composition in the laboratory (barometric pressure changes, sudden changes in humidity), mold or bacteria breeding in a solution that has been left in a sample chamber, or organic material picked up by the probe (as from hands, or from contact with the bench).

#### B. SAMPLE CHAMBER AND BATH-STIRRER UNITS

The following discussion is based on use of the YSI 5301 Standard Bath Assembly (see Figure 3), but it applies generally to any of the bath assemblies offered by YSI.

##### 1. Warm-up and Equilibration

When first turned on, most circulators are slow to reach the desired temperature. Allow sufficient time for the bath assembly to reach temperature before starting measurements.

To minimize the equilibration time, the substrate may be brought to temperature before introducing the sample, and the probe and plunger can be kept at temperature in one of the spare sample chambers. In this way, meaningful data can be gathered within a minute after sample introduction.

##### 2. Saturation of Solution

As an air-saturated solution is heated to the operating temperature of the system, the solution is automatically kept at saturation by the constant evolution of gas. In such a situation, it is only necessary to wait 2 or 3 minutes for temperature equilibration.

The appearance of gas bubbles in the sample chamber after the insertion of the Lucite plunger is an indication that the solution has not completely equilibrated.

Saturation of the solution with oxygen mixtures other than air in the Model 5301 can be achieved. First, the

Lucite plunger is withdrawn part way up the sample tube. Then the saturating gas can be bubbled through the solution and into the space between the Lucite plunger and the solution (a 0.05" diameter plastic tube will fit down the Lucite plunger through the access slot). With the stirrer turned on, the solution will saturate in a few minutes. After the solution has been saturated, the plastic tubing can be withdrawn back into the overflow groove. Flushing the overflow groove with the saturating gas will prevent the introduction of air into the sample chamber during the insertion of the Lucite plunger into the solution.

To prevent excessive evaporation, the saturating gas can be bubbled through water before being introduced into the chamber.

### 3. Position of the Lucite Plunger

The Lucite plunger should be inserted into the sample chamber so as to expel all gas through the access slot. Bubbles that tend to stick to the Lucite plunger surface can be removed by raising the plunger to let enough air (or saturating gas) into the sample chamber to gather all the bubbles, and then reinserting the plunger into the solution to expel the gas.

Solution level in the access slot should be between the lower end of the Lucite plunger and the overflow groove. (See Figure 2.) It is important that no solution be in the overflow groove during a measurement. Solution in the overflow groove reduces the amount of material being observed in the sample chamber and increases the liquid surface in contact with the air. A larger liquid surface increases air diffusion into the solution.

With materials that foam when stirred, twisting the Lucite plunger slightly helps gather small bubbles at the access slot.

### 4. Small Samples, Inhibitors, and Activators

Additions of a few tenths of a milliliter can be made to the sample chamber without removing the Lucite plunger by using a hypodermic needle inserted down the access slot.

The needle is inserted well into the sample chamber and the injection is made. After the introduction is completed, the solution level in the sample chamber is lowered so that it is once again between the overflow groove and the lower end of the Lucite plunger.

When the injection is made, the sample solution in both the needle and the syringe should be free of air bubbles.

A short length of 0.05" diameter plastic tubing can serve as a flexible extension to the needle. Teflon tubing is recommended because it is chemically inert and remains stiff at 37°C.

## C. BATH ASSEMBLIES

A variety of bath assemblies are available for use with the Model 5300 Biological Oxygen Monitor. Each is unique and should be considered for the user's particular need.

### 1. YSI 5301 Standard Bath Assembly

The bath provides relatively air-tight sample chambers which are stirred magnetically and controlled in temperature when connected to a constant temperature circulator.

The four sample chambers are held in place with locking nylon hold down rings and sealed in the bath with two rubber o-rings.

The nylon hold down ring also serves as a tensioning device to hold the Lucite plunger in position while allowing a very fine adjustment regardless of sample size.

The Lucite plunger (see Figure 3) has a slanting face and an access slot along the side for removal of gas bubbles from the sample. The removal of bubbles is of the greatest importance because of the 20 to 1 increase in the amount of oxygen per unit volume in the gas phase over that in the saturated solution.

The solution level in the access slot should be between the overflow groove and the lower end of the Lucite plunger. The small amount of unstirred solution in the access slot and around the Lucite plunger then serves as a barrier to oxygen diffusion into or out of the stirred solution in the sample chamber. The error caused by diffusion through this path is no more than 1/2% per 15 minutes for sample chamber oxygen pressures between 50 and 100% of the outside oxygen pressure. Errors due to oxygen leakage can be minimized by operating with the smallest possible oxygen atmosphere, and by limiting the running time of the sample.

If saturating gases other than air are being used, leakage errors may be minimized by flushing the overflow groove with the saturating gas.

The access slot can also be used for the introduction of samples, inhibitors and activators into the sample chamber with the aid of a hypodermic syringe.

Stirring is necessary to prevent oxygen depletion by the probe of the solution directly adjacent to the cathode. The stirring constantly renews the solution in front of the cathode and results in a steady reading.

In situations where more or less sample agitation is desired other stirrers can be substituted. If other stirrers are used, the probe operation should be checked with stirrers interchanged to see that readings are not changed. (See Standard System in Installation and Initial Setup to check probe operation.)

### 2. YSI 5302 Macro Bath Assembly

This unit operates in the same way as the 5301 bath, except that it has only a single sample chamber and can accommodate substrate volumes from 20 to 50 milliliters, which could contain such specimens as small aquatic plants, animals or large tissue slices. It is also useful where increased oxygen availability is required. The bath assembly incorporates magnetic stirring and provides for constant bath temperature when used with a circulator. The Lucite plunger in this unit may be drilled to accommodate more than the one Standard Oxygen Probe it is manufactured to hold.

### 3. YSI 5303 Macro Adaptor Kit

This accessory kit enables the user of a Standard Bath Assembly to quickly change it into a Model 5302 Macro Bath Assembly for accommodating larger samples. With this kit, the user has the choice of a 3 to 8 milliliter or a 20 to 50 milliliter measurement capability.

### 4. YSI 5304 Micro Adaptor Kit

This kit gives the Standard Bath Assembly greater sensitivity for measuring small sample volumes (1 milliliter) in experiments where limited sample is available, or where the uptake rate is low in relation to

the total oxygen in the system. Two specially modified Lucite plungers and magnetic stirrers are included to use with the Model 5331 Standard Oxygen Probes. A specially designed YSI 5313 Magnetic Retriever also comes with this kit; it facilitates the removal of the magnetic stirrers from the sample chambers.

#### 5. YSI 5356 Micro Oxygen Chamber

The 5356 Micro Oxygen Chamber is designed for a 600 microliter sample. The chamber provides magnetic stirring (to 1600 rpm) and, when connected to a circulating water bath, controls the temperature of the Micro Oxygen Probe and the sample under test. It can either run single samples in the batch mode, or make in-line measurements in the flow-through mode. A low pressure flow-through cell is included for high flow rates. An Eppendorf-type filling port in the back of the sample chamber allows for convenient sample additions. This unit is made for YSI by Instech Laboratories, Inc. (See Micro System in Installation and Initial Setup.)

#### D. ELECTRONIC UNIT

The Model 5300 Biological Oxygen Monitor provides the polarizing voltage for the oxygen probes used and in turn measures the probe current. Each channel operates independently and has separate controls, liquid crystal displays and analog recorder outputs. The Function Switch provides for measurements in AIR or in O<sub>2</sub>-saturated environments by switching the gain of the amplifier by a factor of 5:1. Switching to the TEST positions reduces the polarizing voltage from 0.8 VDC to 0.7 VDC for checking probe current output. The CAL controls enable the user to adjust the calibration value to 100.0% with input currents on the 5357 Micro Oxygen Probe input jacks from 5.9 na to 140.0 na, and on the 5331 Standard Oxygen Probe input jacks from 120.0 na to 2.8 ua.

The instrument will operate in environments of up to 90% relative humidity and will maintain  $\pm 1\%$  linearity at temperatures up to 40°C.

The recorder output red binding post is positive and at earth ground. When attaching a recorder, its terminals should be ungrounded or with the positive terminal grounded. The 5300 has a self-zeroing feature when no probes are plugged into it, so the recorder zero reference can be set against this zero output. This output has a 0 to 2.000 VDC full scale correlation with the display. When the display reads 100.0%, 1.000 VDC  $\pm .002$  will be present at the recorder binding posts. A recorder with 20K ohm minimum impedance is required. A differential output is available across the black recorder output binding post and is equal to (Channel 1 minus Channel 2)  $\pm .4\%$ .

The monitor should be powered from a well-grounded receptacle that is isolated from large power consuming devices. The excellent stability of this instrument can be compromised if high voltage sags occur.

#### III. CIRCUIT DESCRIPTION

Channel 1 and Channel 2 are controlled by separate pc boards. Each channel has its own amplifier and display sections; the Channel 1 pc board supplies power for both channels. (See Schematic and PC Board Layout.)

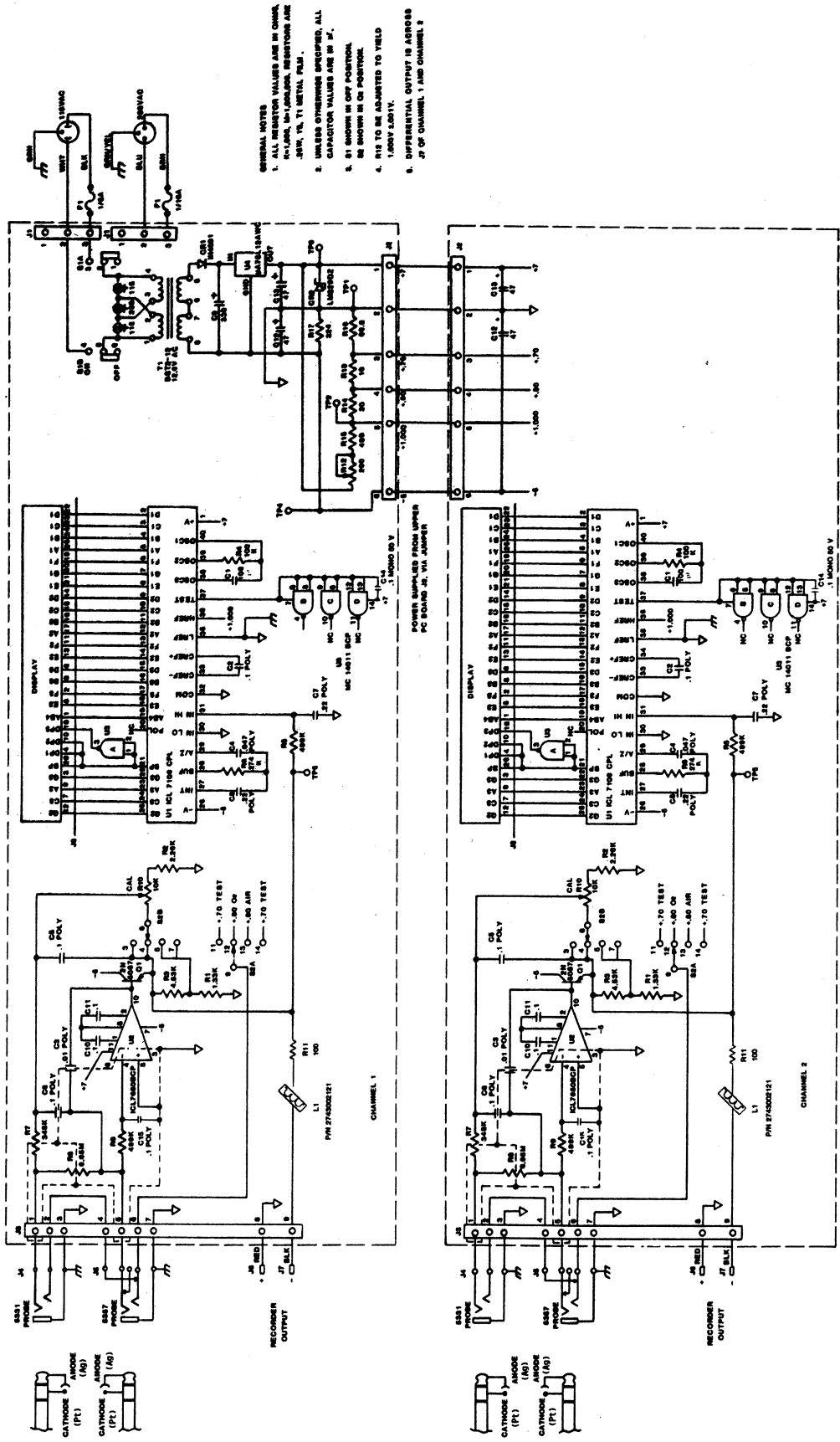
The power supply circuit consists of a conventional transformer, half-wave diode rectifier and filter. One three-terminal regulator (U4) is configured to provide +12 VDC. The +12 VDC supply in turn feeds a Zener diode (CR2) and a voltage divider network to generate +7 VDC

and -5 VDC. The +0.7 VDC and +0.8 VDC supplies are used as polarizing voltages for the probes; the +1.000 VDC is for the A/D reference. To ensure the accuracy of the +1.000 VDC supply, R12 is variable. TP1, TP2, TP3 and TP4 offer test points. All of these DC voltages should be ripple-free.

The amplifier circuit is supplied by S2A with a polarizing voltage which runs through J4 and J5. If J5 has a probe plugged in, J4 will have no voltage available via the now open switch in J5. The current that the probe produces is compared to grounded pin 5 of U2, and U2 in turn provides an output voltage on pin 10. With no probe input, the output goes positive and Q1 keeps the circuit turned off (zero reference). When current flows through the probe, (from pin 6 to pin 5 of J5) this current must flow through R7 and R8. Q1 provides a current sink via the voltage dividers R1/R3 and R2/R10. U2 drives Q1 to maintain a virtual zero between its input pins 4 and 5, thus maintaining a constant polarizing voltage on the probe. S2B selects the gain ratio for the O<sub>2</sub> vs. AIR circuit. When in the AIR mode, R2 and R10 are paralleled by R1, and when in O<sub>2</sub>, R2 and R10 are paralleled by R1 and R3. This relationship causes a 1:2094 voltage gain. (When calibrated to 100.0% in the AIR mode and then switched to the O<sub>2</sub> mode, the display should change to 20.9%.) The CAL potentiometer permits display value adjustment. The voltage from the R10 slider returns to the probe input jack via R7 for the 5331 probe, and via R7 and R8 for the 5357 probe. R7 and R8 determine the U2 gain, so the approximately 20:1 value difference compensates the approximately 20:1 current difference between the two probes. C5 and C6 provide display stability; C3, C10, C11 and R9 provide zero reference and zero stability. The output of this circuit goes to the recorder output and display driver circuit. It can be tested at TP5.

The display circuit receives the analog input via R5 and C7, and compares it to the +1.000 VDC reference. The A/D converter (U1) decodes this input and drives the LCD via an elastomeric connector. U3 pin 3 drives the decimal point on the LCD. C1 and R4 form an oscillator circuit for U1. C4, C8 and R6 form U1's auto zero and integrator circuits. C14 provides EMI noise suppression.

IV. SCHEMATIC

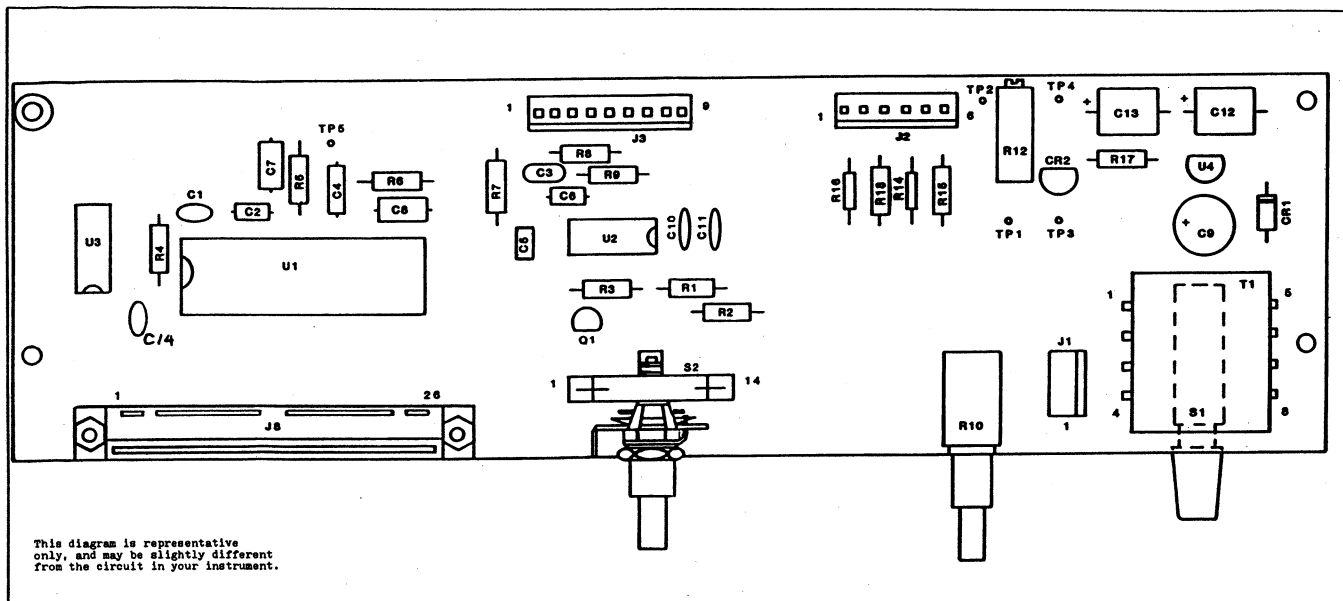


- GENERAL NOTES
1. ALL RESISTOR VALUES ARE IN OHMS UNLESS OTHERWISE SPECIFIED. ALL CAPACITOR VALUES ARE IN P.F.
  2. UNLESS OTHERWISE SPECIFIED, ALL RESISTOR VALUES ARE IN OHMS UNLESS OTHERWISE SPECIFIED. ALL CAPACITOR VALUES ARE IN P.F.
  3. R1 TO BE ADJUSTED TO YIELD 1.000V ±0.01V.
  4. DIFFERENTIAL OUTPUT IS ACROSS J7 OF CHANNEL 1 AND CHANNEL 2.

This schematic is representative only. It is subject to change without notice.

DSB10 D SCHEMATIC

## V. PC BOARD LAYOUT



## VI. TABLES

The following tables are provided as aids to the users of this manual in the computations of their experimental results.

Table 1. Volume of Oxygen Dissolved in Aqueous Medium (microliters of oxygen per milliliter at 1 atmosphere)

Temp. °C	Equilibrated with 100% O <sub>2</sub>		Equilibrated with Air (21% O <sub>2</sub> )	
	H <sub>2</sub> O <sup>1</sup>	Ringers Solution <sup>2</sup>	H <sub>2</sub> O <sup>2</sup>	Ringers <sub>2</sub> Solution <sup>2</sup>
15	34.2	34.0	7.18	7.14
20	31.0	31.0	6.51	6.51
25	28.5	28.2	5.98	5.92
28	26.9	26.5	5.65	5.56
30	26.1	26.0	5.48	5.46
35	24.5	24.5	5.14	5.14
37	23.9	23.9	5.02	5.02
40	23.1	23.0	4.85	4.83

<sup>1</sup>From "Handbook of Chemistry and Physics" 40th Ed., Chemical Rubber Pub. Co., Cleveland, 1958-1959.

<sup>2</sup>Recalculated from Umbriet et al (1964). "Manometric Methods", 4th Ed. Burgess Pub. Co.

Table 2. Solubility of O<sub>2</sub> in Buffered Mitochondrial Medium Equilibrated with Air (20.9 % O<sub>2</sub>)

Temp. °C	ug atoms O <sub>2</sub> /mL <sup>1</sup>	uMoles/mL (mM)
15	0.575	0.288
20	0.510	0.255
25	0.474	0.237
30	0.445	0.223
35	0.410	0.205
37	0.398	0.199
40	0.380	0.190

<sup>1</sup>Solubility of O<sub>2</sub> experimentally determined by Chappell (1964) "Biochem." J. 90,225., in a buffered mitochondrial medium containing NADH<sub>2</sub> inorganic phosphate, and isolated mitochondria.

Table 3. Bunsen Coefficients for Solubility of Oxygen in Plasma and Blood<sup>1</sup>

Temp. °C	Plasma	Blood Hb g/100ml			
		5g	10g	15g	20g
15	0.0302	0.0310	0.0312	0.0316	0.0323
20	0.0277	0.0282	0.0284	0.0287	0.0293
25	0.0257	0.0261	0.0263	0.0265	0.0271
28	0.0246	0.0249	0.0251	0.0253	0.0259
30	0.0238	0.0241	0.0243	0.0245	0.0251
35	0.0220	0.0226	0.0227	0.0229	0.0234
37	0.0214	0.0220	0.0221	0.0223	0.0228
40	0.0208	0.0211	0.0212	0.0214	0.0219

<sup>1</sup>From Christofordes et al "J. Appl. Physiol." 26:56, 1969 and "J. Appl. Physiol." 27:592, 1969.

Table 4. Solubility of Oxygen in Water and Various Solvents

Relative Solubility	Effect of Certain Electrolytes on Solubility = Solubility Concentration Per Unit M Concentration		
Solvent	Solubility at 20°C		
1M NaCl	0.724	NaCl	0.0073
1M KCl	0.737	KCl	0.0069
1M HCl	0.932	KF	0.0078
1/2M H <sub>2</sub> SO <sub>4</sub>	0.894	NaNO <sub>2</sub>	0.0053
1M KOH	0.670	1/3 x K <sub>3</sub> Fe(CN) <sub>6</sub>	0.0060
1MNaOH	0.662	1/2 x K <sub>2</sub> C <sub>2</sub> O <sub>4</sub>	0.0071
1M Sucrose	0.554	NaH <sub>2</sub> CO <sub>3</sub>	0.0081
Methyl Alchl	7.670	1/2 Na <sub>2</sub> CO <sub>3</sub>	0.0085
Ethyl Alchl	7.700	NaOH	0.0090
Acetone	6.910	Lactic Acid	0.0003

Sources:

Sendroy, Dillion & Van Slyke, "J. Biol. Chem." 105:597, 1939.

Van Slyke et al, "J. Biol. Chem." 78:765, 1928.

Handbook of Respiration, Wright Air Development Center Report, 58-352, Page 6.

**Table 5. Gas Solubility Relationships**

ug/mL = 0.031 uMole/mL	
ug/mL = 0.700 uL/mL at 0°C	ug - micrograms
uM/mL = 32.000 ug/mL	uM - micromoles
uM/mL = 22.400 uL/mL at 0°C	uL - microliter
luL/mL = 1.430 ug/mL at 0°C	mL - milliliter
luL/mL = 0.045 uMole/mL at 0°C	

To correct for temperatures other than 0°C when using values in microliters:  $uL \text{ at } 0^\circ C \times [(273 + T^\circ C) / 273]$   
= uL at T°C

**Sources:**

Sendroy, Dillion & Van Slyke, op cit.  
Van Slyke et al, op cit.  
Handbook of Respiration, op cit.

**Table 6. Atmospheric Pressure Vs. Altitude Correction Factors<sup>1</sup>**

Atmospheric Pressure (mm Hg)	Altitude		Correction Factor
	(Ft)	(m)	
768	-276	-84	101
760	0	0	100
752	278	85	99
745	558	170	98
737	841	256	97
730	1126	343	96
722	1413	431	95
714	1703	519	94
707	1995	608	93
699	2290	698	92
692	2587	789	91
684	2887	880	90
676	3190	972	89
669	3496	1066	88
661	3804	1160	87
654	4115	1254	86
646	4430	1350	85
638	4747	1447	84
631	5067	1544	83
623	5391	1643	82
616	5717	1743	81
608	6047	1843	80
600	6381	1945	79
593	6717	2047	78
585	7058	2151	77
578	7401	2256	76
570	7749	2362	75
562	8100	2469	74
555	8455	2577	73
547	8815	2687	72
540	9187	2797	71

<sup>1</sup>Derived from 16th Edition of "Standard Methods for the Examination of Water and Wastewater".

**Table 7. Interfering Gases**

H<sub>2</sub>S, SO<sub>2</sub>, halogens, nitrous oxide and CO are interfering gasses. If you suspect erroneous readings, it may be necessary to determine if these are the cause. The following gases have been tested for response.

100% Carbon Monoxide	Less than 1 %
100% Carbon Dioxide	Less than 1 %
100% Hydrogen	Less than 1 %
100% Chlorine	2/3 O <sub>2</sub> response
100% Helium	None
100% Nitrous Oxide	1/3 O <sub>2</sub> response
100% Ethylene	None
100% Nitric Oxide	1/3 O <sub>2</sub> response

**Required Notice:**

(The Federal Communications Commission defines this product as a Computing Device and requires the following notice):

This equipment generates and uses radio frequency energy and if not installed and used properly, may cause interference to radio and television reception. It has been type tested and found to comply with the limits for a Class A or Class B computing device in accordance with the specifications in Subpart J of Part 15 of FCC Rules, which are designed to provide reasonable protection against such interference in a residential installation. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- \* reorient the receiving antenna
- \* relocate the computer with respect to the receiver
- \* move the computer away from the receiver
- \* Plug the computer into a different outlet so that the computer and receiver are on different branch circuits

If necessary, the user should consult the dealer or an experienced radio/television technician for additional suggestions. The user may find the following booklet prepared by the Federal Communications Commission helpful: "How to Identify and Resolve Radio-TV Interference Problems". This booklet is available from the U.S. Government Printing Office, Washington, DC 20402, Stock No. 004-000-00345-4.



**YSI Incorporated**

Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio 45387 USA  
Phone 513 767-7241 • 800 343-HELP • Fax 513 767-9353 • Telex 205437