

Instech Oxygen Consumption Chamber Setup FOXY and FOSPOR

Manual Version 3.8 with Instech Viewer 2.60

Set Up Hardware and Install Software

1. Unpack all parts,
2. Load Viewer ver. 2.60 from flash drive provided or use a Dropbox link by running “setup.exe., *before* attaching any USB connections but DO NOT start the Viewer yet.
3. If you have ordered more than one channel, you will receive a multi-port USB hub to connect all NeoFox control units to your PC. More channels can be added without changing the hub. The small end of the USB should be connected to the rear of the hub. The other end to an unused USB input on your PC. It will probably not be necessary to attach the hub power supply for only 2 channels. The computer should recognize the hub and automatically install it.
4. For single channel units, no hub is required and the USB cable should be connected directly from the NeoFox to the PC.
5. For more than one channel, attach each Neo to the hub with cables provided and power on the Neos by plugging in the power cable..
6. New hardware message should come up and accept the Neos and install the drivers.
7. Once the units have been recognized as USB ports, it is best to Check the Device Manager in My Computer—Properties--Hardware for the presence of Ocean Optics NeoFox in the USB list. Once verified, then it is OK to start the viewer.
8. When the Viewer is started, tabs corresponding to the serial numbers of the Neos should be seen above the display as it cycles through and detects them.
9. You switch between them by clicking the top tabs (active one is the lighter color).
10. Instech will have installed the latest NeoFox Image bin file-latest firmware for the NeoFoxes.
11. Note-If not all the drivers are found, shut down the program, remove the USB from the hub. Reconnect the USB hub and drivers should load. Restart the Viewer.

Set NeoFox Parameters

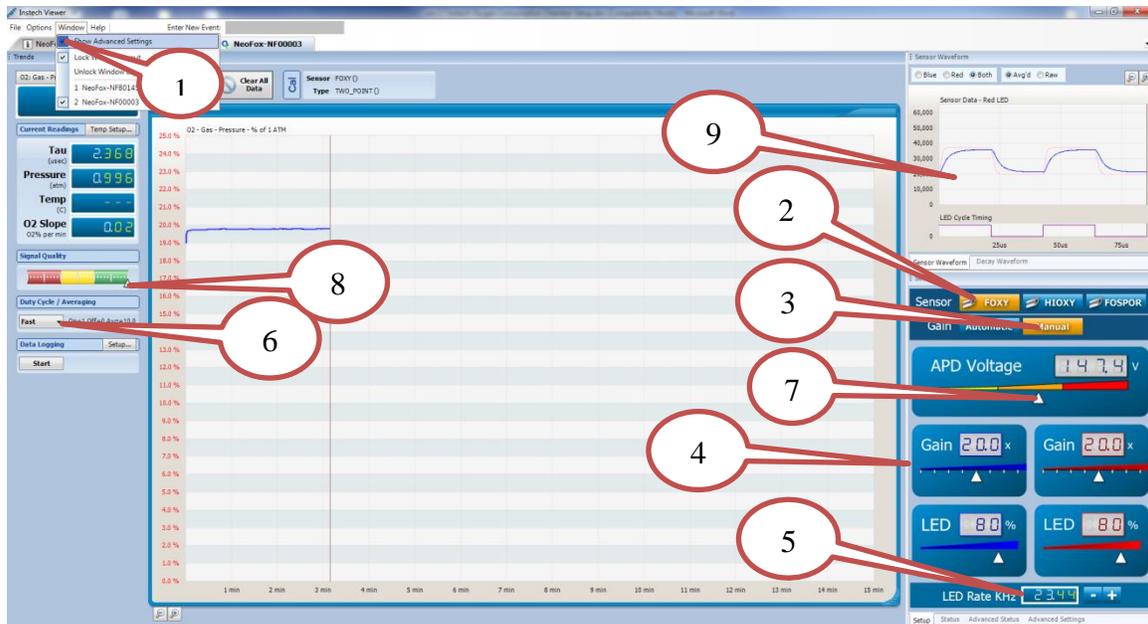
The Instech chambers presently in use, come with FOXY material patch installed.

100% alcohol cannot be used in the chamber without seriously degrading the FOXY patch coating. Compounds diluted in organic solvents are permissible as long as the organic solvent concentrations are not high. Damage can be detected by observing the amplitude of the fluorescence signal (blue waveform).

Newest versions are also being made with FOSPOR material which is more stable, has a slightly higher sensitivity and is resistant to brief exposure to 100% alcohol. The main

difference in Setup is the lower value of LED rate, typically 5.86 KHz instead of 23.44 KHz. Illustrations may show either rate and material.

INITIAL NEOFOX SETUP



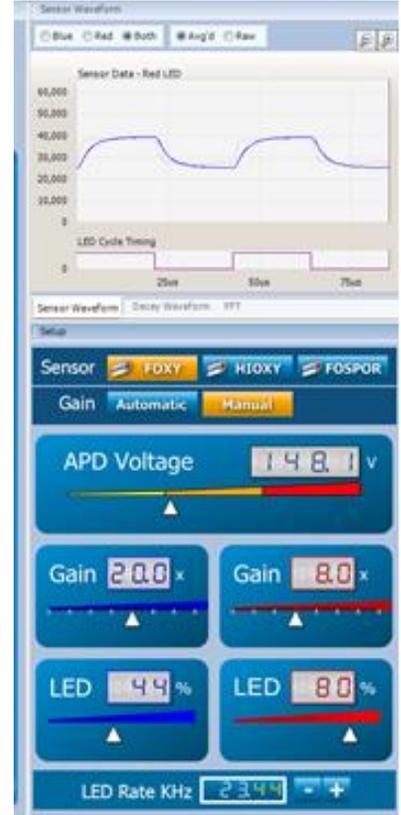
1. In the top line WINDOS tab pull down select (check) to Show Advanced Settings.
2. Select FOXY for the sensor or FOSPOR depending upon the model. See setup sheet provided.
3. Select Manual Gain. This is required to be able to change parameters and should be left in this mode. **Automatic will bring in default values that are incorrect and will have to be reset.**
4. Use the checkout sheet provided and set the values to match the sheet for initial operation.
5. Set the LED rate down to 23.44 KHz by hitting the (-) button for LED Rate KHz.
6. Set Duty Cycle/Averaging to Fast or 5.86 KHz for FOSPOR.
7. APD may not be variable and has been previously set to optimum value. Under special conditions it can be unlocked and adjusted-contact Instech Labs.
8. The small arrow on the Signal level bar graph should *always* be in the green area indicating adequate signal level to determine the rise time (tau). This can be affected by blue gain, LED rate, and LED intensity (typically set to 80% for both blue and red LED traces). The red LED trace is the internal timing reference.
9. The Sensor waveform is an excellent way to determine the quality of your settings. See examples below.



Too High



Too Low

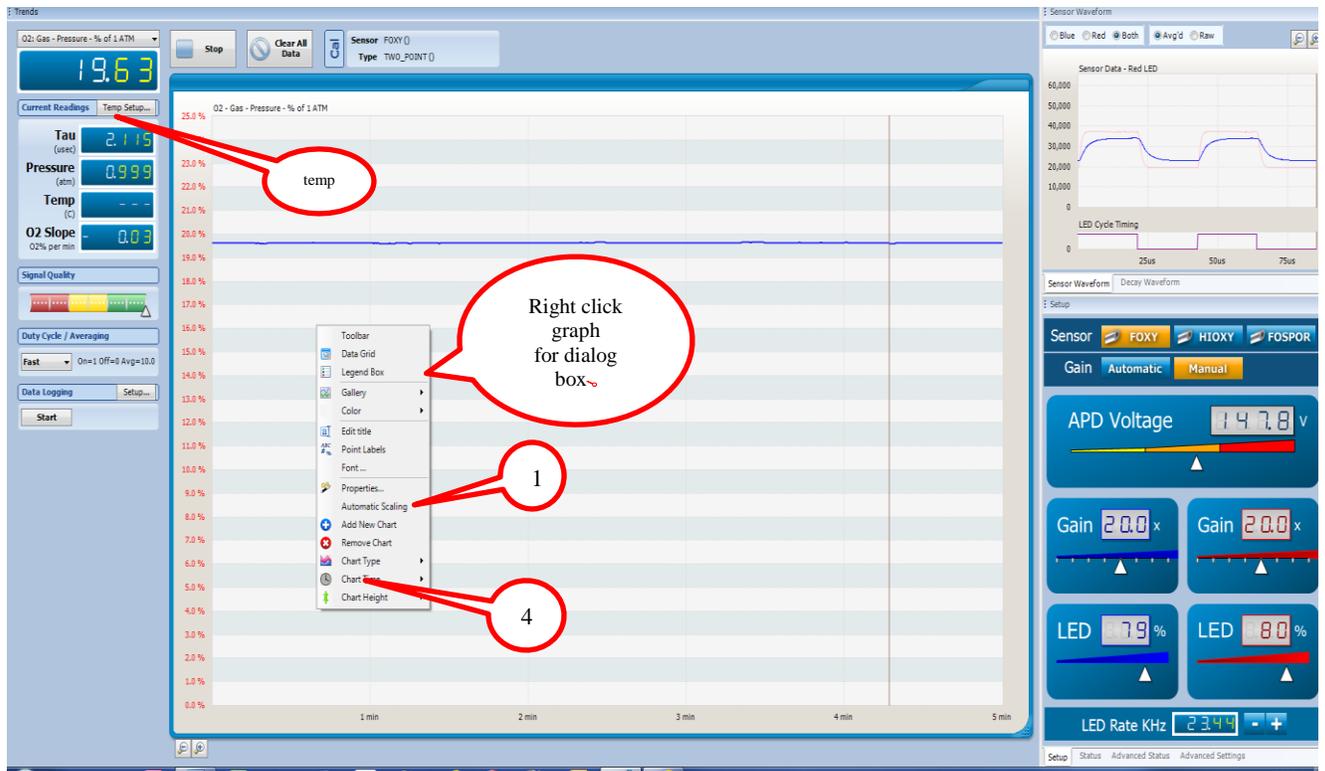


Good

Temperature Probe

If you have an older temperature probe, insert it into the block and attach the cable to the rear of the NeoFox mini-phono input. Newer units will have a screw in bolt type sensor. It is not necessary to have more than 1 probe, even for multiple chambers as long as they are in series from a single circulating water bath.

Select from options on Temp Setup. You can apply the Temperature from the NeoFox that has the probe plugged into it and apply it to all others.



Oxygen Display Graph--Right click in display area for choices

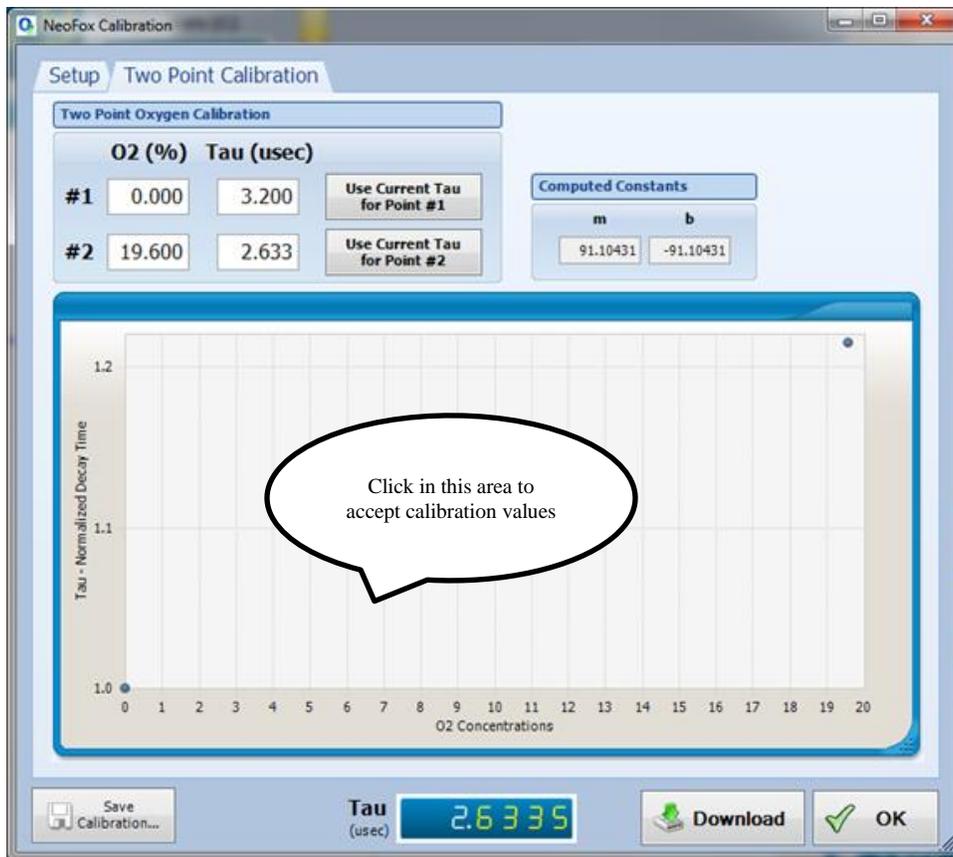
1. Deselect Autoscaling and always leave in this condition. Y-axis legends will turn red.
2. Y axis adjustment. Click on Y-axis to set minimum to 0 and maximum to 25 for % readout.
3. Tau graph can be removed for cleaner display.
4. Time base can also be adjusted for your experiment –select chart time and set appropriately.
5. Data can be logged to disk for post processing.
6. Save graphical setup and Hardware settings. This will automatically restore your graphics when restarting the Viewer.
7. Powering down the NeoFox will cause its defaults to be recalled which may not match your saved settings. You can leave the power to the NeoFox on when not in use. When the Viewer is stopped, the excitation will be turned off to reduce photobleaching of the sensing dye.

Calibrating Probes

Meaningful Oxygen values will be displayed only after a Two Point Calibration has been performed for each channel. Initially work in percent. Once a calibration has been performed, you may switch to other display values, e.g. Torr or concentration units. Calibrations should always be performed at your final operating temperature which is the reason the circulating water bath connections. Oxygen values are temperature sensitive. Start with the chamber that has the temperature probe installed and run temp setup to transfer the known value to remaining chambers. This will result in the proper conversion to umoles/liter.

Rough calibrations for check out purposes only

1. View the waveforms to establish proper gain settings.
2. Start with chamber empty and exposed to air.
3. Select Options/calibration/Two Point.
4. Press "Use Current Tau for Point #2"
5. This will enter the Tau for air.
6. We will skip the tau for zero oxygen at this point and enter manually a value about 1.5x the #2 value since we do not really have the sensor exposed to the zero oxygen solution. Do not use the current value for #1 button for the rough setup. When actually running samples, use solution depleted of oxygen by adding sodium dithionite and recording the tau for that solution.
7. **Click the mouse in the graph area** and the two tau values will be used for this calibration. Blue dots will move indicating that they have been accepted.
8. Click Download and accept. Clicking OK does nothing but get you out of this screen.
9. Verify that trace and wave forms look good.
10. You can now move on to actual calibration for your solutions.
11. Do not select no temperature (none), either use probe or transferred values.



Final calibrations

- Remove the plug and place air equilibrated buffer into the chamber, making sure it is at temperature. Take care with this step. Place buffer in a vial in the water bath with an air space above the fluid and shake frequently. **Remember you will start in % Oxygen units. The percent oxygen is *not directly* 20.9%. This is only true when measuring gaseous oxygen. The partial pressure in mmHg is $.209 * (760 \text{ mmHg} - \text{Vapor pressure of water at your operating temperature}^*$ see table in Appendix 1). Percent will be this value divided by 760. For example, at 37C partial pressure should be 148.9 mmHg and 19.6%. Use this value as your ambient sample calibration value.**
12. Click the button to enter the current tau reading in point #2.
 13. Add sodium dithionite crystals into the chamber, while stirring, until the tau stops increasing. Titrate for maximum tau. Remember this amount. Excess is permissible. Use that value for point #1. Remember to **click in the graph area** before downloading the calibration values to each Neo unit. Two blue dots will update. Calibrations are stored there in the NeoFox, not in your computer, and will be recalled for the next use. If you do not click the graph first, the calibration will **not** update.
 14. Rinse well and repeat for other chambers.
 15. It is good practice to record the tau values for future comparisons.

16. Temperature probes are for information and do not compensate any of the readings. Again, only one sensor is needed for the string of chambers. Temperature probes may need to be checked for any offset that may exist. There is no way to alter the temperature probe calibration.

Operating the titanium chambers

Please try to minimize spills to prevent damage to the stirring motors and to facilitate cup removal.

1. Remove the glass plug/collar assembly by lifting straight up.
2. Add liquid to the chamber-slightly in excess of stated volume.
3. Orient the plug and collar assembly so that the high side of the bevel aligns with the angled fill port hole (leftmost illustration). Set screw should indicate the high side.
4. Slowly lower the plug while checking for bubbles and allow the excess to spill out into the cup
5. Seal the chamber by rotating the plug assembly 180 degrees (rightmost illustration).
6. To minimize air leakage, leave the small column of fluid in the angled fill port. Do not wick out excess in the fill port hole.
7. Additions may be made by rotating the plug to the fill position (center). Use a needle or narrow tipped pipette that allows excess fluid to pass by and spill into the cup ring. The long needle or tip is preferred to make the additions at the bottom of the chamber and the overflow is at the top.
8. Check that no air has been injected. Eppendorf pipettes will finish with an air bubble and are not recommended to be used in their normal mode.
9. For longer term measurements, the center-fill plugs are recommended for the best sealing.
10. The larger two chambers require the use center-fill acrylic plugs.
11. . O-rings may need occasional wiping with silicone grease supplied to facilitate insertion.

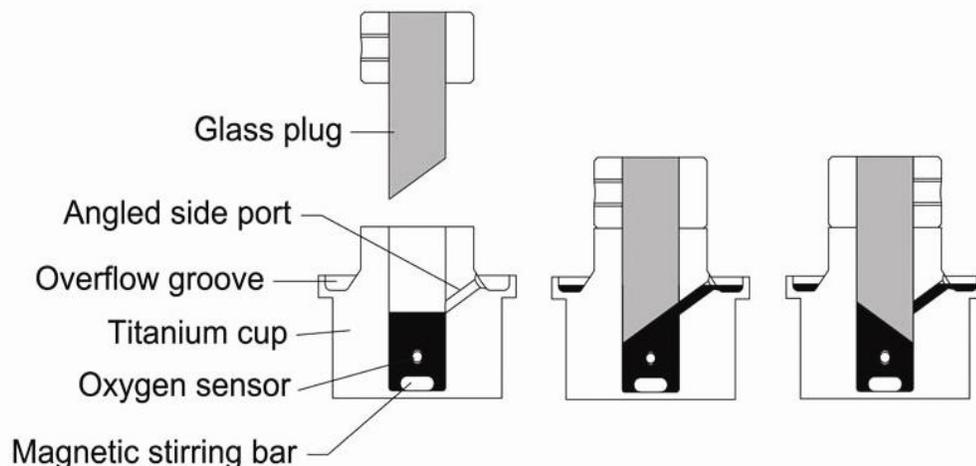
The chamber volume is approximate and should be checked by the user.

Maintenance

No maintenance is required for the long life sensing patch as it is part of the chamber. If damaged, the cup will need to be returned for repair.

Cup Removal or Replacement

1. Remove the glass plug.
2. Remove the SMA fiber connection from the block coupler by unscrewing the hex nut of the SMA.
3. Remove the fiber from the block.
4. Loosen the setscrew across from the fiber attachment which will unlock the chamber cup from the aluminum block.
5. Twist and pull the cup vertically out and remove the stir bar.
6. Smear the underside of the new cup flange with silicone grease to prevent spilled liquid from running under the cup lip and entering the stirring motor cavity. New chambers now have an O-ring to prevent spillage from entering the motor cavity that will not allow it to slip out easily. Even with the O-ring, a smear of grease under the cup is suggested.
7. Press the cup into the block and twist to visually align the sensor hole with the coupling sleeve hole. This will distribute the silicone grease as well.
8. Replace the SMA coupler into the sleeve.
9. When properly aligned, the tip of the SMA connector will engage the recess in the titanium cup. Gently rotate the cup back and forth until rotation is stopped by this engagement. This assures final alignment is correct. Use the blue tubular wrench or 5/16" open end wrench in this process.
10. Run the software for this channel to assure signal is maximized. When properly positioned retighten the cup hold set screw (front top of block).
11. Once parameters have been set for this patch material, switch view to show



Advanced Setup and scroll down to Blue Intensity. It should be ~ 2000 to 3000 range. If it is low, either the alignment is incorrect or parameters (blue gain or LED intensity) need to be adjusted.

12. Replace stir bar and glass plug.

Replacing Glass Plug/Valve

Chips may make it difficult to clear air bubbles.

1. Remove the plug.
2. Loosen the setscrew and remove the glass.
3. The correct position of the plug within the black collar is when the glass is flush with the top edge of the collar.
4. Align the setscrew with the highest point of the bevel to permit clearance of air bubbles.
5. Retighten set screw.

Installing Firmware Updates

These are seldom required as Viewer works with most previous firmware versions.

1. Make to new Image file available down loaded from Ocean Optics.com e.g. on the desktop.
2. Stop data collection.
3. Select Options, then Firmware Update and follow prompts
4. Do not disconnect power during the update.
5. Repeat for each unit to be updated.

Specifications

Chamber with internal FOXY patch sensor

Materials	Titanium cup, Glass or acrylic plugs, glass covered stir bar and possibly acrylic caps.
Stirring	Integral miniature motor driven stirrer and speed controller, power supply provided. 100-240 VAC input
Volume approx.	175 µL standard, 500 µL and 1000 µL optional. User interchangeable.

Patch material FOXY coating for use with aqueous samples only—**no organic solvents.**

Readout

Ocean Optics NeoFox phase measurement unit.

% O₂ range 0-20%

DO range 0-10ppm

Temperature 0-60C

O₂ resolution 100-500 ppm

DO resolution 4-20 ppb at room temperature

O₂ accuracy 5% of reading

Min detectable level 100-500 ppm

Response time <1 sec in gas
~45 seconds in water

NeoFox Power supply (provided) Input 100-240 VAC, Output 5VDC @ 1 amp

Configurations

OXYGEN CONSUMPTION MEASUREMENT SYSTEM



One channel system
FOL/C1T15P

This system is based on a fiber optic fluorescence lifetime measurement of oxygen concentration, the most advanced, efficient and stable technique available. The system reads the oxygen concentration in the medium surrounding the test specimen and, when sealed, yields a accurate measure of the oxygen consumption rate (OCR).

The top loading chamber is constructed of titanium with a glass plug/valve and glass covered stirring bar to eliminate any chemical reaction with the medium as well as oxygen absorption and re-release as the pO_2 falls. Titanium provides a totally inert environment with excellent thermal equilibration characteristics. The chamber is designed to permit analysis of small samples, with volumes low as 17.5 μ l.

The fluorescence lifetime sensor is integral to the chamber and is capable of thousands of determinations without replacement or maintenance. The technique provides unparalleled stability with virtual immunity to fluorescence amplitude variations over time. Below the chamber is a miniature magnetic stirrer, with speed control, capable of low speed operation to keep the cells in suspension while minimizing damage. Stirring is not a requirement of the sensor.



The control software graphically displays oxygen levels and permits exporting the data to a text file for further analysis using a spreadsheet. Use a circulating water bath to maintain constant temperature control of the chamber and ensure accurate measurements. The system is equipped

with a sensor to monitor temperature. It is available in one, two, three and four channel configurations.

This is a preliminary information sheet. Please call for more information.



Chamber Operation: (A) Push the glass plug slowly into the chamber to expel air through the angled side port. (B) Add reagents through the side port. (C) Rotate the cap 180 degrees to seal the chamber.



Four channel system
FOL/C4T15P

Part No.	Description	Unit
FOL/C1T15P	FOL system, 1 channel, 17.5 μ l. chamber	ea
FOL/C2T15P	FOL system, 2 channels, 17.5 μ l. chambers	ea
FOL/C3T15P	FOL system, 3 channels, 17.5 μ l. chambers	ea
FOL/C4T15P	FOL system, 4 channels, 17.5 μ l. chambers	ea
FOL/C1T50P	FOL system, 1 channel, 50 μ l. chamber	ea
FOL/C2T50P	FOL system, 2 channels, 50 μ l. chambers	ea
FOL/C3T50P	FOL system, 3 channels, 50 μ l. chambers	ea
FOL/C4T50P	FOL system, 4 channels, 50 μ l. chambers	ea

<http://www.instrondata.com/OxygenConsumption.html>

Cellular uptake system for cells growing on glass cover slip

This system forms a closed volume by virtue of a glass bottomed cavity and cover slip to form the top surface with a trapped volume of approximately 200 microliters. The sensing patch is located on the inner surface of the glass bottom and is interrogated from below fiber optically. Readout uses the fluorescence lifetime method. Two side ports are provided to fill and make additions.

The chamber can be held in a ring stand mount and rotated 90 degrees to facilitate clearing of any air bubbles. A small amount of silicone vacuum grease can be used to hold cover slip in place and form a seal.

REV	ECO	DESCRIPTION	DWN BY	APPROVED/DATE

ITEM NO.	PART NUMBER	DESCRIPTION	Default/ QTY.
1	rect. base block		1
2	7020-05-0006		1
3	7020-05-0008	SHORT WATER TUBE	1
4	7020-05-0143	SMA CONNECTOR TOOL	1
5	bifboro		1
6	187 patch		1
7	12.5 window	WINDOW BOROFIL 12.5 DIA	1
8	7020-05-1012	THEMISTOR SENSOR BOLT & WIRE	1
9	1 dia cover slip		1
10	7020-05-0026	EPPENDORF FILL PORT PLUG	2
11	97395A397	.0625x .1875 DOWEL PIN	2
12	SMA nut		1
13	92185A564		1

Location: U:\7020_05\cover slip\rect assy

UNLESS OTHERWISE SPECIFIED: DIMENSIONS ARE IN INCHES TOLERANCES FRACTIONAL ANGULAR MATCHES* BEND & TWO PLACE DECIMAL & .005 THREE PLACE DECIMAL & .010	DRAWN M LOUGHANE 5/29/2013	NAME DATE
PROPERTY AND CONFIDENTIAL THE INFORMATION CONTAINED IN THIS DRAWING IS THE SOLE PROPERTY OF NORTH STAR. ANY REPRODUCTION IN PART OR AS A WHOLE WITHOUT THE WRITTEN PERMISSION OF NORTH STAR IS PROHIBITED.	CHECKED ENG APPR. MFG APPR. Q.A. COMMENTS:	<p>INSTECH S209 Millia Hill Road Plymouth Meeting, PA 19462</p> <p>TITLE: rect assy</p> <p>SIZE B DWG. NO. REV SCALE: 1:2 WEIGHT: g SHEET 1 OF 1</p>

Saturated Vapor Pressure, Density for Water

Temp (°C)	Temp (°F)	Saturated Vapor Pressure (mmHg)	Saturated Vapor Density (gm/m ³)	Temp (°C)	Temp (°F)	Saturated Vapor Pressure (mmHg)	Saturated Vapor Density (gm/m ³)
-10	14	2.15	2.36	40	104	55.3	51.1
0	32	4.58	4.85	60	140	149.4	130.5
5	41	6.54	6.8	80	176	355.1	293.8
10	50	9.21	9.4	95	203	634	505
11	51.8	9.84	10.01	96	205	658	523
12	53.6	10.52	10.66	97	207	682	541
13	55.4	11.23	11.35	98	208	707	560
14	57.2	11.99	12.07	99	210	733	579
15	59	12.79	12.83	100	212	760	598
20	68	17.54	17.3	101	214	788	618
25	77	23.76	23	110	230	1074.6	...
30	86	31.8	30.4	120	248	1489	...
37	98.6	47.07	44	200	392	11659	7840

Appendix 1

Courtesy of hyperphysics.phy-astr.gsu.edu/HBASE/kinetic/watvap.html