



## Tech Note

## Operating the ISE-730 Dissolved Oxygen Electrode

### Items Provided with the ISE-730

- (1) Dissolved Oxygen Electrode with membrane housing
- (1) Additional membrane housing
- (1) Bottle of electrolyte solution
- (1) Filling tube
- (1) Set of operating instructions

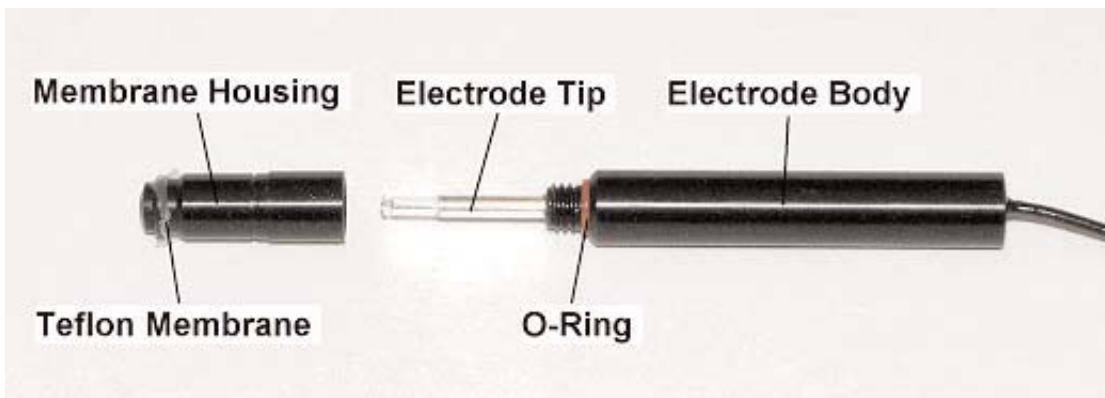


Figure 1: The ISE-730 dissolved oxygen electrode, disassembled to show its critical parts.

### Electrode Assembly and Preparation

1. The ISE-730 dissolved oxygen electrode (Figure 1) is shipped with a membrane housing covering the glass electrode tip. The end of the housing is covered with a Teflon™ membrane. If the electrode is unused, or has been stored for a long period according to the directions provided in this set of instructions, the membrane housing does not contain the electrolyte solution needed to make the electrode work.

**Warning: When removing and replacing a membrane, calibrating the electrode, or measuring oxygen concentrations, do not apply pressure against the internal electrode. Any excessive pressure against the internal electrode can cause the electrode to crack rendering it useless and unrepairable.**

2. To add electrolyte solution to the membrane housing, or to check on the level of electrolyte solution in the housing, carefully unscrew the membrane housing from the body of the oxygen electrode.

3. If the membrane housing contains electrolyte solution, check the level of the solution in this housing. The minimum height of electrolyte in the housing should be 6 mm, which will make the housing about one half full. When the housing is placed over glass element of the electrode, the solution will be high enough to make contact with the silver anode that surrounds the glass electrode tip.

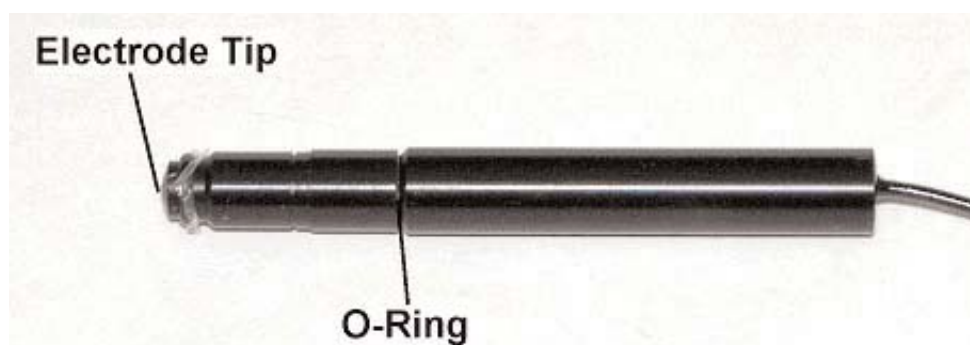
4. If electrolyte solution needs to be added to the membrane housing, push the filling tube provided in the kit into the opening in the nipple of the bottle of electrolyte solution. Then, fill the housing with electrolyte to the minimum height. To fill the membrane housing without adding bubbles, squeeze the bottle of electrolyte until there is no air in the filling tube; then, gently place the bubble-free end of the filling tube near the inside of the Teflon™ membrane

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and add electrolyte until its level reaches the minimum height. If an air bubble is trapped near the inside Teflon membrane, gently tap the side of the membrane housing to loosen the bubble.

5. Carefully insert the electrode tip into the membrane housing. Do not trap any air bubbles near the electrode tip and inside the Teflon membrane. Slowly screw the membrane housing onto the body of the electrode to prevent the creation of pressure that could stretch the Teflon membrane. Stop screwing the membrane housing onto the body of the electrode when the housing makes contact with O-ring above the threads on the body of the electrode. The O-ring should be visible in the gap between the membrane housing and the body of the electrode (Figure 2).

**Warning:** Screwing the membrane housing onto the body of the electrode too quickly or too far can stretch or break the membrane and prevent the electrode from working properly.



**Figure 2:** The ISE-730 dissolved oxygen electrode properly assembled. The membrane housing contacts the O-ring, leaving a gap between the housing and the electrode body. The electrode tip pushes the membrane out slightly.

6. Check the end of the assembled electrode to make sure the membrane housing is seated properly against the tip of the electrode. If the housing is seated properly, the tip of the glass electrode protrudes only slightly beyond the end of the housing (Figure 2).

### Electrode Setup

1. Obtain an assembled ISE-730 dissolved oxygen electrode (Figure 2), a DO2-100 current to voltage adapter (Figure 3), and a DIN-DIN cable (Figure 4).



**Figure 3:** The DO2-100 Current-to-Voltage Adapter.



2.

**Figure 4:** The Male DIN-DIN cable.

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2. Plug one end of the DIN-DIN cable into DIN input of the iWorx recording unit or amplifier (Figure 5). Plug the other end of this cable into the DIN connector on the DO2-100 current to voltage adapter. Attach the cable from the ISE-730 dissolved oxygen electrode to the BNC connector on the current to voltage adapter.



**Figure 5:** Dissolved oxygen electrode (ISE-730) and amplifier (DO2-100) connected to an IWX/214 with a male DIN-DIN cable.

### Calibration Procedure for the ISE-730 Electrode

The standard used for calibrating the dissolved oxygen electrode is the known concentration of oxygen in air-saturated deionized water. The amount of oxygen that is dissolved in water is known as its solubility ( $S$ ) and it is dependent upon the temperature, oxygen pressure in the air, and the concentrations of dissolved solutes in the water. Solubility ( $S$ ) can be determined by using the following equation:

$$S = (\alpha/22.414) ((P-p)/P) (r\%/100)$$

In the equation,  $\alpha$  is the absorption coefficient of  $O_2$  at the temperature;  $p$  is the vapor pressure of water at the temperature;  $P$  is the barometric pressure, and  $r\%$  is the percent oxygen in the air. For example, at  $26^\circ C$  and 760 mmHg and a concentration of oxygen in air of 21%,  $S$  equals:  $(0.02783/22.414L/mole)(734.91mmHg/760mmHg)(0.21) = 252 \mu MO_2$

1. Place the oxygen electrode in a 100 ml beaker containing room temperature deionized water, also known as 100% oxygen saturated water. There needs to be enough water in the beaker to submerge the tip of the oxygen electrode, and keep its tip away from the stir bar in the beaker. Place the beaker on a magnetic stirrer. Adjust the speed of the stirrer so the stir bar is rotating quickly and evenly.
2. Record the output of the electrode until the recording reaches a stable level near the top of the recording channel.
3. Mark the recording to identify the output of the electrode when placed in air saturated, room temperature deionized water. If you are recording data with LabScribe v1.8 or earlier, continue recording as the calibration solutions are changed. If you are recording data with LabScribe v2.0 or later, you can stop the recording while changing solutions.
4. Obtain a 100 ml beaker containing zero-percent oxygen calibration solution at room temperature, also known as 0% oxygen saturated water. Make sure there is enough solution in the beaker to keep the tip of the electrode clear of the stir bar.

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5. Turn off the magnetic stirrer. Remove the oxygen electrode from the beaker of deionized water and remove the beaker of deionized water from the stirrer.
6. Place the beaker containing zero-percent oxygen calibration solution on the stirrer. Turn on the stirrer and adjust the speed of the stirrer so the stir bar is rotating quickly and evenly. Place the oxygen electrode in the beaker with the zero-percent oxygen calibration solution.
7. Record the output of the electrode until the recording reaches a stable level near the top of the recording channel.
8. Mark the recording to identify the output of the electrode when placed in the oxygen depleted calibration solution. Stop the recording and save the calibration data in a data file.
9. Turn off the stirrer. Remove the electrode from the beaker of calibration solution. Hold the electrode over the beaker used for collecting waste liquid, and rinse the electrode with deionized water from a wash bottle. Blot any drops of solution from the electrode and place it in a beaker of deionized water.

### Units Conversion

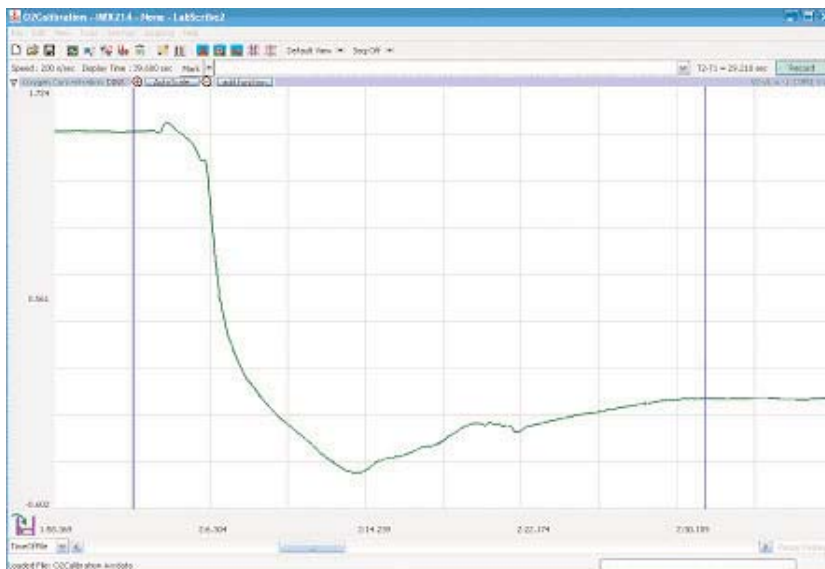
Measure the temperature (in °C) in the lab room. Assume the barometric pressure in the lab room is one atmosphere (760mmHg) and the concentration of oxygen in the air is 21%. From Table 1, find the dissolved oxygen concentration ([O<sub>2</sub>]) in deionized water at room temperature. This concentration will be used to calibrate the dissolved oxygen electrode.

**Table 1: Concentration of Oxygen in Air Saturated Deionized Water at 1 Atmosphere.**

Temp (°C)	O <sub>2</sub> Absorbance Coefficient (a)	Water Vapor Pressure (p in mmHg)	[O <sub>2</sub> ] (µM)
20	0.03102	17.54	284
21	0.03044	18.65	278
22	0.02988	19.83	273
23	0.02934	21.07	267
24	0.02881	22.38	262
25	0.02831	23.76	257
26	0.02783	25.09	252
27	0.02736	26.74	247
28	0.02691	28.35	243
29	0.02649	30.04	238
30	0.02608	31.82	234

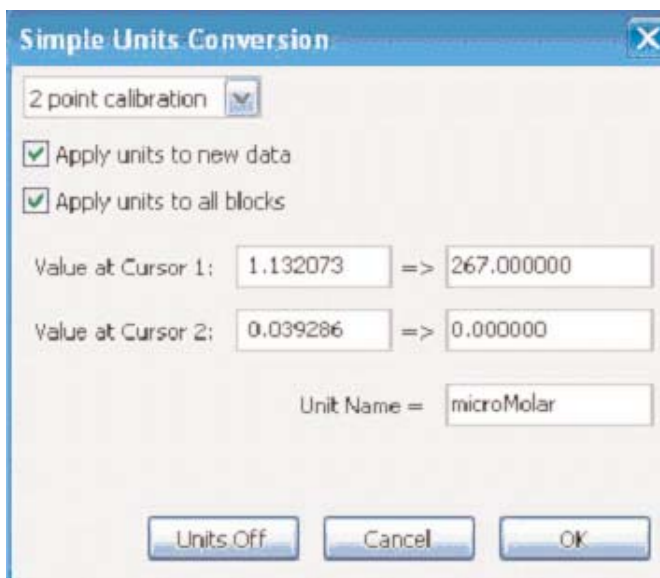
2. Scroll to the beginning of the calibration data for the ISE-730 dissolved oxygen electrode.
3. Adjust the display of the calibration data so that the stable output of the ISE-730 electrode at both the 100% and 0% oxygen saturation levels appear on the same display screen.
4. Place a measurement cursor on the stable section of data collected when the electrode was in the 100% oxygen saturated water (Figure 6). Place the second cursor on the stable section of data collected when the electrode was in the 0% oxygen saturated solution.

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**Figure 6:** Recording of the output of the ISE-730 dissolved oxygen electrode while it is in 100% air saturated water, and after the electrode was moved to 0% air saturated water.

5. To convert the output of the ISE-730 dissolved oxygen electrode from voltage to molarity of dissolved oxygen, open the simple units conversion dialogue window of the channel on which the calibration was recorded (Figure 7). Select the 2-point calibration technique from the menu on the window, and select to apply the units conversion to all the blocks of data in the data file.



**Figure 7:** The simple units conversion dialogue window used to convert the voltage output of the ISE-730 electrode to molarity.

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6. Notice that the voltages from the positions of the cursors on the recording are already entered into the appropriate spaces in the calibration equations. Find the concentration of dissolved oxygen in air saturated water at the room temperature From Table 1. Enter this concentration in the space that corresponds to the voltage output of the electrode when in 100% oxygen saturated water. Enter the value, zero, in the space that corresponds to the voltage output of the electrode when it is in 0% oxygen saturated water.

7. Enter the name of the units, mMolar, in the appropriate space, and click OK to activate the units conversion.

# Operating the ISE-730 Dissolved Oxygen Electrode

## Handling

When necessary, the membrane of the electrode can be replaced by following the assembly and preparation procedure described above.

## Cleaning

When using the electrode in solutions containing protein, the electrode should be soaked in an enzyme cleaning solution such as Terg-a-zyme (Alconox, Inc.) for a couple of minutes, after each use, to remove the protein from the surface of the membrane. This cleaning will prolong the useful life of the membrane.

## Storing

1. Always rinse or clean the electrode before storing.
2. For long-term storage (periods over 1 month):
  - o Remove the membrane housing from the electrode.
  - o Rinse the internal electrode with distilled water and pat dry.
  - o Place a new, unfilled membrane housing over the internal electrode and attach loosely (Do not seat completely). This membrane will serve to keep dust off of the electrode tip.
3. For short-term storage, the electrode can be left in room air with the filled membrane housing still attached.

## Problem Solving

1. When there is little or no response from the electrode:
  - o Remove the membrane housing from the electrode and inspect the internal electrode for cracks. If the internal electrode is cracked, it cannot be repaired and the complete electrode must be replaced.
  - o Examine the membrane housing and determine if it contains the appropriate amount of electrolyte solution. An insufficient amount of electrolyte solution in the housing can be caused by a hole in the Teflon membrane. Replace the membrane housing with a new one. Fill the housing with the appropriate amount of electrolyte and install the housing by following the assembly and preparation procedure described above.
2. When there is a sluggish response from electrode:
  - o Clean the surface of the membrane.
  - o Replace the membrane housing with a new one.
  - o Polish the face of the internal electrode and replace the membrane housing with a new one.
3. Repolishing the face of the internal electrode:
  - o Remove the membrane housing and rinse the internal electrode with deionized water
  - o Place a piece of polishing paper, or 3200-grit wet/dry sandpaper, on a flat surface.
  - o Place a couple of drops of deionized water on the polishing paper.
  - o Hold the glass portion of the internal electrode firmly between the thumb and forefinger, with the tip of the glass electrode perpendicular to the polishing paper.
  - o Polish the tip of the electrode in the wet area of the polishing paper using circular motions with very light pressure. About 10 circles are all that is required to polish the tip.
  - o Rinse the electrode thoroughly with deionized water.
  - o Assemble the electrode using a new membrane housing.

**Table 2: Zero-Percent Oxygen Calibration Solution**

Concentration (mMolar)	Salt	Grams/Liter in Deionized Water
15	Sodium Hydrosulfite	2.61

**Table 3: Electrode Filling Solution**

Concentration (mMolar)	Chemical	Grams/Liter of 95% Ethylene Glycol-5% Deionized Water
268	NaHCO <sub>3</sub>	22.5
10	KCl	0.75