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O2k-Manual B. www.bioblast.at/index.php/MiPNet08.04_POS-Service

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Service of the polarographic oxygen sensor OroboPOS

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Section Page

Summary: Service of the polarographic oxygen sensors (**OroboPOS, POS**) is the basis for signal stability, low noise and high time resolution. Sensor service may not be required for several months of operation. Performance of the OROBOROS Oxygraph-2k according to instrumental specifications is obtained only with oxygen sensors which are maintained in a good functional state.

POS service is required if (1) a new sensor is prepared, (2) the raw signal at air calibration declines, (3) signal noise is high, (4) the time response is prolonged (time constant >10 s) and biphasic, (5) the oxygen signal at zero calibration does not decline rapidly to a low level of zero current (0% to max. 5%). For each sensor, the frequency of POS service can be optimized on the basis of a long-term record of calibration values as a quality control (O2k-Calibration-List.xls).

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1. Accessories

Accessories for sensor service are provided in the OroboPOS-Service Kit (Fig. 1). In addition, you need distilled water and 25% ammonia solution (fresh). Store the OroboPOS in the dark.

Fig. 1. OroboPOS-Service Kit - Oxygraph-2k

①	26300-01	OroboPOS-Electrolyte powder, KCl
②	26200-01	OroboPOS-Membranes, FEP 25 µm; 40/Pck.
③	26520-01	OroboPOS-Polishing Powder for cathode cleaning
④	26510-01	OroboPOS-Polishing Cloth for cathode cleaning
⑤	26400-01	OroboPOS-Mounting Tool for membrane application
⑥	26800-01	Pipette\Plastic\1 ml ungraded for electrolyte
⑦	26600-01	O2-Zero Powder, dithionite ($Na_2S_2O_4$)
	26550-01	Pen-Contact Oil for stable low contact resistance

2. Cleaning

Prevent damage by electrostatic discharge (ESD) when handling the POS connectors or cable connections to the O2k (MiPNet14.01).

For sensor service, remove the black POS seal tip (1). It is normal to see many small bubbles in the electrolyte reservoir. This does not indicate that this caused a problem while the sensor was actually in use. Remove the PEEK membrane ring (2,3) and membrane (4; Fig. 2). Wash off the electrolyte with distilled water.

For cleaning of the anode and cathode, the sensor head is best removed from the OroboPOS-Connector

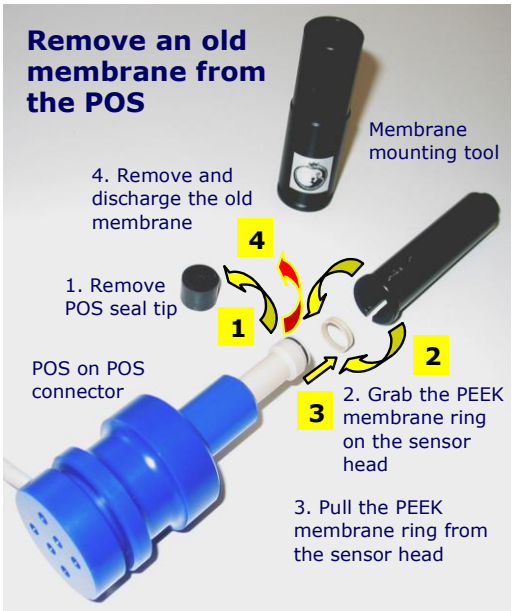

Figure 2. Removal of a used membrane from the POS.

and mounted to the blue base of the Perspex housing of the POS (OroboPOS-Service kit; page 1).

The cathode should be cleaned when its gold surface appears to be coated by a colored layer. The silver/silver chloride anode darkens after long-term operation, inadequate storage of the sensor or contact with hydrogen sulfide. This may cause high signal noise or reduces the signal output by >30%, reflected by the requirement to increase the gain. Such sensors can be improved by cleaning the cathode, anode and gold connections.

2.1. Cathode cleaning

The cathode must be treated with extreme care. It must not be touched with the fingers, nor exposed to detergents or greasy liquids.

Wash off the electrolyte from the POS with distilled water. Place the Petri dish with the polishing cloth supplied in the POS recharge kit (Fig. 1) on a flat surface, add some fine aluminum oxide powder (0.3 μm) with the tip of a spatula, and moisten it with a few drops of distilled water to obtain a thin paste (Fig. 3). Hold the sensor in a vertical position and polish the cathode for one minute in a figure-eight motion (Fig. 3). Wash the aluminum oxide powder carefully off the sensor with distilled water, and wash the cleaning tools.

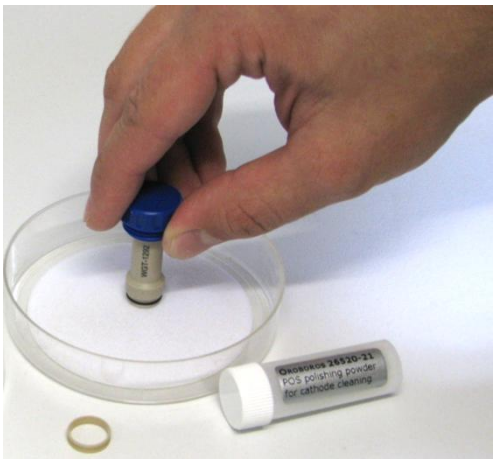

Figure 3. Cathode cleaning.

You may further clean the gold cathode with ultrasonic treatment at low power for up to 30 seconds while immersing the tip of the sensor head in distilled water. In extreme and rare cases, the cathode may be cleaned by adding a drop of 50-75% nitric acid onto the surface of the cathode for only 15 seconds (no longer) with care. Remove carefully any traces of nitric acid by washing with distilled water, and proceed as described above. After cleaning the cathode, the anode must be cleaned as well.

2.2. Anode cleaning

Fill the electrolyte reservoir of the sensor with concentrated (25%) ammonia solution. After up to 10 minutes the silver/silver chloride should appear bright gray. Wash the sensor carefully with distilled water. Repeat the application of ammonia solution twice. With severely aged sensors it might be necessary to prolong the exposure to ammonia up to several hours (overnight), sealing the ammonia under a membrane and under the POS cover slip in long-term applications. Protect the POS from light, since the silver/silver chloride anode is light sensitive.

The response time of the sensor signal may remain high with a biphasic pattern (exponential phase followed by a slow drift) even after polishing and cleaning with ammonia. Repeat the cathode/anode cleaning cycle several times.

2.3. Cleaning of the electrical connection

Unscrew the POS head and inspect both sides of the electrical connection (gold pin and threads). Remove any contamination such as grease and moisture with a fine paper cloth. If necessary, wash with distilled water and then with pure alcohol, and apply contact oil from the OroboPOS-Service kit to the connections. Before screwing the POS head onto the POS connector used for membrane application, clean the POS connector of moisture and any other contamination (particularly any salt crystals from the electrolyte). Similarly, clean the electrical cable connection to the O2k-Main Unit.

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3. Membrane mounting

For mounting a new membrane, the POS head is screwed on the sensor connector. Check the O-ring on the POS head to ensure that it fits properly and its surface is smooth and intact. You may apply a tiny amount of grease to the O-ring of the sensor head (Fig. 2). Rinse the mounting tool (OroboPOS service kit) with distilled water to wash off any electrolyte crystals (Fig. 4).

As electrolyte, use KCl solution (1 mol·dm^{-3}; 74.56 g potassium chloride per litre, in distilled water). Add distilled water to the electrolyte powder (Fig. 1) up to the 10 ml mark. Alternatively, dissolve 1.49 g KCl in distilled water with a total volume of 20 ml. Store at room temperature or 4 °C in a closed bottle. To prevent the formation of gas bubbles in the electrolyte, heat the electrolyte solution by shaking the sealed electrolyte container in hot water (40-70 °C) before filling the electrolyte reservoir of the POS.

POS membranes are contained in a small box (Fig. 1, item 6). They are fully transparent. Each membrane is separated by a non-transparent white paper sheet. Do not add the paper to the oxygen sensor. Separate the membrane from the paper sheets, carefully avoiding any mechanical damage of the transparent membrane. Do not touch the central area of the membrane with your fingers. The pair of forceps is used for separating the membrane and positioning a new membrane into the membrane guide of the POS mounting tool (Fig. 4 [1]).

Fix the PEEK membrane ring (which seales the membrane against the sensor body) to the membrane ring holder (Fig. 4 [2]). With the POS head filled with electrolyte, slide the membrane guide down the POS connector, and move the lower ring on the POS connector down to bring the membrane guide into a fixed position (Fig. 4 [3]). Then slide the membrane ring holder into the membrane guide and press firmly down to slide the PEEK membrane ring over the POS head (Fig. 4 [4]).

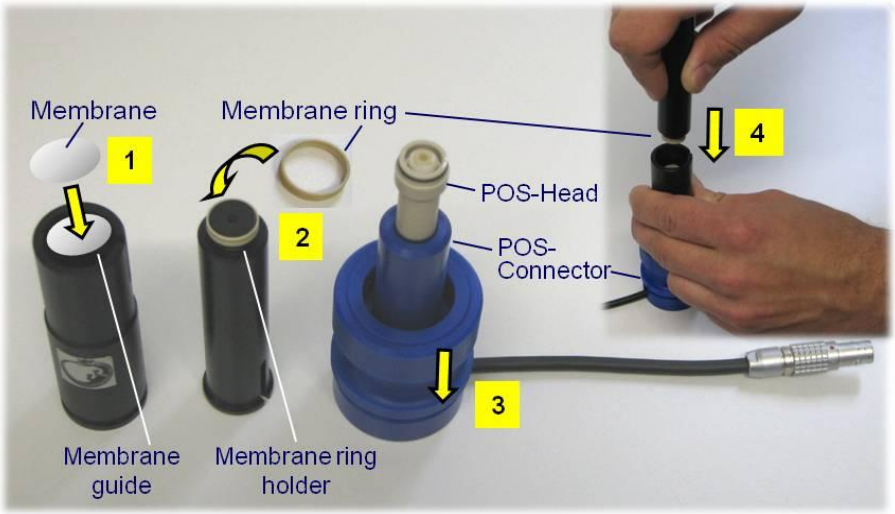

Figure 4. Mounting of a membrane onto the POS, using the membrane mounting tool which consists of two parts, the membrane guide and the membrane ring holder.

No gas bubbles should be trapped in the electrolyte reservoir after membrane application. As a check, you may inspect the electrolyte reservoir under a binocular. No folds should be visible in the membrane in the central area. Small folds in the membrane near the outer circumference have no influence, but large folds should be avoided. Wash excess electrolyte off the outside of the sensor and POS connector and wipe dry, before attaching the POS connector to the POS holder at the O2k-Main Unit.

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After sensor service and membrane mounting, the POS needs some time in operation to stabilize while the O2k is switched on. Such stabilization may require several hours. For this purpose the O2k may be left on overnight.

4. Electrical cable connection

Connect the POS cable to the O2k-Main Unit, avoiding bending and torsions of the cable. Insert the male plug of the POS cable into the female connector next to the control light of the stirrer. The red dot on the male plug has to face accurately upwards when inserting the plug.

See Supplement for O2k Series B to C.

5. Storage of the OroboPOS

5.1. Short-term storage in the Oxygraph-2k

For short periods of days or several weeks, the POS is stored in the Oxygraph-2k chamber. The chamber is washed with distilled water and completely filled with 70% ethanol for chemical sterilization. The stopper is inserted loosely without pushing it down beyond the point where the sealing ring is inserted into the glass chamber. This ensures a longer life time of the sealing rings. The receptacle of the stopper is completely filled with ethanol from the top, and is sealed with a black cover slip to avoid

evaporation of ethanol. Before an experiment, the ethanol is siphoned off and the chamber is washed with distilled water (MiPNet06.03).

5.2. Short-term shelf storage

For shelf storage, unplug the POS-Connector from the O2k-Main Unit. Clean the sealing tip and membrane with distilled water. The POS head is maintained moistened by applying a cup on the sensor head to **prevent the drying out of the electrolyte**. Store in the dark.

5.3. Long-term storage

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For storage of the POS for several months, the sealing tip and membrane are removed. Wash the electrolyte off the POS with distilled water. Even if the membrane is not damaged, remove it by gripping the membrane holding ring with the groove in the lower end of the membrane ring holder of the mounting tool (Fig. 2). The POS head is **stored dry and in the dark**. Check for any moisture and salt contamination in the electrical connector of the POS head. In this case, wash with distilled water and subsequently with pure methanol, dry at 60 °C for 24 h.

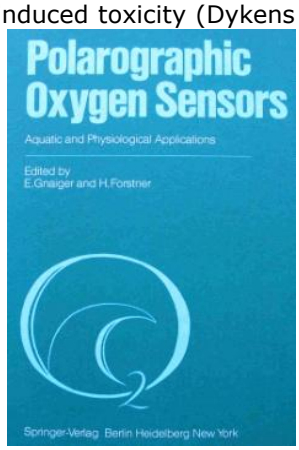
6. Replacement of the OroboPOS head

After cleaning and drying the gold cathode contact or drying the sensor body at 60 °C for a few days, the zero current should be reduced. In addition, the zero current of the bare cable, without sensor head connected, is tested for any leak currents. If the latter test excludes any sources of leak currents other than the POS, and POS service is not successful, the sensor head must be replaced.

A new sensor head can be screwed onto the sensor connector, if the old sensor head has been irreversibly damaged or should be replaced.

7. References

- Gnaiger E (2001) Bioenergetics at low oxygen: dependence of respiration and phosphorylation on oxygen and adenosine diphosphate supply. Respir Physiol 128: 277-291. »
- Gnaiger E (2008) Polarographic oxygen sensors, the oxygraph and high-resolution respirometry to assess mitochondrial function. In: Mitochondrial dysfunction in drug-induced toxicity (Dykens JA, Will Y eds) John Wiley: 327-352. »
- Gnaiger E, Forstner H, eds (1983) Polarographic Oxygen Sensors. Aquatic and Physiological Applications. Springer, Berlin, Heidelberg, New York: 370 pp. »
- Gnaiger E, Steinlechner-Maran R, Mendez G, Eberl T, Margreiter R (1995) Control of mitochondrial and cellular respiration by oxygen. J Bioenerg Biomembr 27: 583-596. »
- Hitchman ML (1983) Calibration and accuracy of polarographic oxygen sensors. In: Polarographic oxygen sensors, Gnaiger E and Forstner H, eds., Springer, Berlin, Heidelberg, New York: 18-30. »
- Hitchman ML, Gnaiger E (1983) A thermodynamic consideration of permeability coefficients of membranes. In: Polarographic oxygen sensors, Gnaiger E and Forstner H, eds., Springer, Berlin, Heidelberg, New York: 31-36. »



Full version » www.bioblast.at/index.php/MiPNet08.04_POS-Service
Next step – O2k-Manual D » MiPNet12.08 O2k-Calibration

Supplement: O2k-Series B to C

Electrical Cable Connection: O2k-Series B to C

Insert the male plug of the POS cable into the female plug positioned between the two control lights of the Oxygraph-2k (left chamber: between the MAINS and STIRRER lights; right chamber: between the STIRRER and COMM lights). The ridge on the male plug has to face accurately downwards when inserting the plug, before rotating the connector screw and fixing it finger tight.

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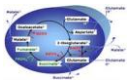
O2k-Manual

» MiPNet12.06 Oxygraph-2k: start high-resolution respirometry.
» MiPNet14.01 Electrostatic discharge (ESD): damage and protection.



O2k-Protocols

» MiPNet06.03 POS-Calibration-SOP.