




Service of the polarographic oxygen sensor OroboPOS

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Section		Page
	1. OroboPOS-Service Kit.....	18
	2. Cleaning	18
	3. Membrane mounting	20
	4. Electrical cable connection	21
	5. Storage of the OroboPOS	21
	6. Replacement of the OroboPOS head	22
	7. References.....	22



*Links are functional on the OROBOROS USB flash drive.

Summary Service of the polarographic oxygen sensors (**OroboPOS, POS**) is the basis of signal stability, low noise and high time resolution. Performance specifications of the OROBOROS O2k can be met only with oxygen sensors which are maintained in a well monitored functional state.



POS service may not be required for several months of operation. POS service is necessary if (1) a new sensor is prepared, (2) the signal at air calibration is not stable over time, (3) signal noise is high, (4) the time response is prolonged and biphasic (time constant >10 s), (5)

the oxygen signal at zero calibration does not decline rapidly to 0% to max. 5% of the signal at air saturation. For each sensor, the frequency of POS service is optimized on the basis of long-term calibration records for quality control (O2k-Calibration-List.xls).

1. OroboPOS-Service Kit



Clip B1

20610-02 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 O ₂ -Zero Powder, dithionite (Na ₂ S ₂ O ₄)
⑧	26550-01 Pen-Contact Oil for stable low contact resistance

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



Removal of a used membrane from the OroboPOS.

2. Cleaning

Prevent damage by electrostatic discharge (ESD) when handling the [OroboPOS-Connector](#) 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 the bubbles caused a problem while the sensor was actually in use. Remove the PEEK membrane ring [2,3] and membrane [4]. Wash off electrolyte with distilled water.

Clip B2

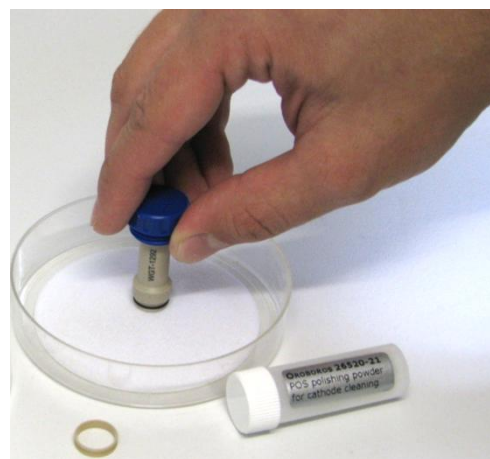
For cleaning the anode and cathode, the sensor head is removed from the [OroboPOS-Connector](#) and mounted onto the blue base of the Perspex housing of the POS (OroboPOS-Service kit).

The cathode is 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 reduce 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.

➡ [Clip B2](#) Mount the OroboPOS onto the blue storage base. Wash off any electrolyte from the POS with distilled water. Place the Petri dish with the polishing cloth, supplied in the OroboPOS-service kit, on a flat surface. Moisten it with a few drops of distilled water. Add some of the [OroboPOS-Polishing Powder](#) (aluminum oxide, 0.3 μm) with the tip of a spatula. Hold the sensor in a vertical position and polish the cathode in the thin paste for one minute in a figure-eight motion. After cleaning the cathode, wash the polishing powder carefully off the sensor with distilled water. Subsequently, the anode is always cleaned as well (Section 2.2).



Cathode cleaning on the polishing cloth. ➡ [Clip B3](#)

If noise or the response time of the sensor signal remains high with a biphasic pattern (exponential phase followed by a slow drift) even after polishing the cathode and anode cleaning with ammonia, repeat the cathode/anode cleaning cycle several times. Finally, wash the polishing cloth.

In rare cases 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 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 (Section 2.1).

2.2. Anode cleaning

➡ [Clip B4](#)

Fill the electrolyte reservoir of the sensor with fresh concentrated (25%) ammonia solution. After up to 10 minutes the silver/silver chloride should appear bright gray. Wash the sensor with distilled water. Repeat the application of ammonia solution twice. With severely aged sensors it is

necessary to prolong the exposure to ammonia up to several hours (overnight), sealing the ammonia under a membrane and under the POS cover slip. Protect the POS from light, since the silver/silver chloride anode is light sensitive.

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 salt crystals, grease and moisture with a fine paper cloth. If necessary, wash with distilled water and then with pure alcohol. Apply contact oil from the OroboPOS-Service Kit to the connections only if necessary. Before screwing the POS head onto the [OroboPOS-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.

3. Membrane mounting

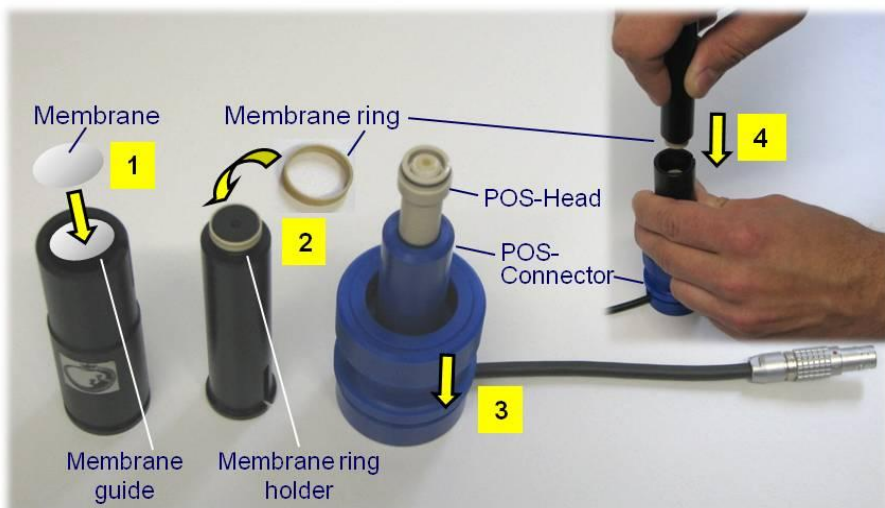
 [Clip B5](#)

The POS head is screwed on the [OroboPOS-Connector](#). Check the O-ring on the POS head to ensure that its surface is smooth and intact. In exceptional cases, apply a tiny amount of grease to the O-ring of the sensor head. Rinse the [OroboPOS-Mounting Tool](#) ([OroboPOS-Service Kit](#)) with distilled water to wash off any electrolyte and salt crystals.

As electrolyte, use KCl solution ($1 \text{ mol}\cdot\text{dm}^{-3}$; 74.56 g potassium chloride per litre, in distilled water) provided in the OroboPOS-Service Kit. Add distilled water to the [electrolyte powder](#) 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 $4 \text{ }^{\circ}\text{C}$ in a closed vial. To prevent the formation of gas bubbles in the electrolyte, heat the electrolyte solution by shaking the sealed electrolyte vial in hot water ($40\text{-}70 \text{ }^{\circ}\text{C}$) before addition of electrolyte to reservoir of the POS.

[POS membranes](#) are contained in small boxes in the OroboPOS-Service Kit. 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.

[1] Position a new membrane into the membrane guide of the [OroboPOS-Mounting Tool](#) using the [pair of forceps](#). **[2]** Fix the [OroboPOS-Membrane Ring](#) (which seals the membrane against the sensor body) to the [membrane ring holder](#). Fill the POS head with electrolyte. **[3]** Push the lower ring on the [OroboPOS-Connector](#) strongly downwards, slide the [membrane guide](#) downwards across the POS head and fix the membrane guide to the [POS connector](#) by releasing its lower ring. **[4]** Slide the membrane ring holder into the membrane guide and press firmly down to slide the OroboPOS-Membrane Ring over the POS head.



Mounting a membrane onto the OroboPOS, using the OroboPOS-Mounting Tool which consists of two parts, the membrane guide and the membrane ring holder.

influence, but large folds should be avoided. Wash excess electrolyte off the POS and POS connector. Apply a [OroboPOS-Seal Tip](#) and attach the [OroboPOS-Connector](#) to the [OroboPOS-Holder](#) (O2k-Main Unit, Chapter A).

No gas bubbles should be trapped in the electrolyte reservoir after membrane application. No folds should be visible in the membrane in the central area. Inspect the electrolyte reservoir under a binocular. Small folds in the membrane near the outer circumference have no

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4. Electrical cable connection



Insert the male plug of the cable of the [OroboPOS-Connector](#) into the female connector next to the control light of the stirrer (O2k-Main Unit). The red dot on the male plug has to face accurately upwards when inserting the plug. Avoid bending and torsions of the cable.

See MiPNet19.01 for O2k Series B to C.

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 switched on and run overnight.

5. Storage of the OroboPOS

5.1. Short-term storage in the O2k

For short periods of days or several weeks, the POS is maintained in the O2k-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





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 OroboPOS-Mounting Tool (see above). 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.

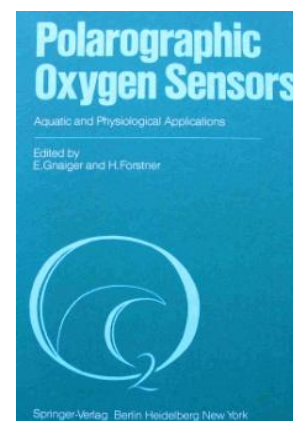
6. Replacement of the OroboPOS head

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 (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:327-52. » 
- Gnaiger E, Forstner H, eds (1983) Polarographic Oxygen Sensors. Aquatic and Physiological Applications. Springer, Berlin, Heidelberg, New York:370 pp. » 
- 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:31-6. » 



Updates » http://wiki.orooboros.at/index.php/MiPNet19.18B_POS-Service

» http://wiki.orooboros.at/index.php/MiPNet19.18A_O2k-Start

Next step – O2k-Core Manual D » [MiPNet19.18D O2k-Calibration](#)

Supplement A. Videosupport*

*Links are functional on the OROBOROS USB flash drive.

➤ [B1 OroboPOS from Perspex housing](#)

➤ [B2 Disassembly of OroboPOS](#)

➤ [B3 Cathode cleaning](#)

[OroboPOS-Polishing Cloth](#), [OroboPOS-Polishing Powder](#).

➤ [B4 Anode cleaning](#)

➤ [B5 Membrane mounting](#)

Supplement B. Links to O2k-Catalogue

- » <http://wiki.oroberos.at/index.php/OroboPOS>
- » <http://wiki.oroberos.at/index.php/O2k-Catalogue: OroboPOS components>
- » <http://wiki.oroberos.at/index.php/OroboPOS-Service Kit>

