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B: http://wiki.oroboros.at/index.php/MiPNet19.18B_POS-service

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 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** (O2-calibration.xlsx).

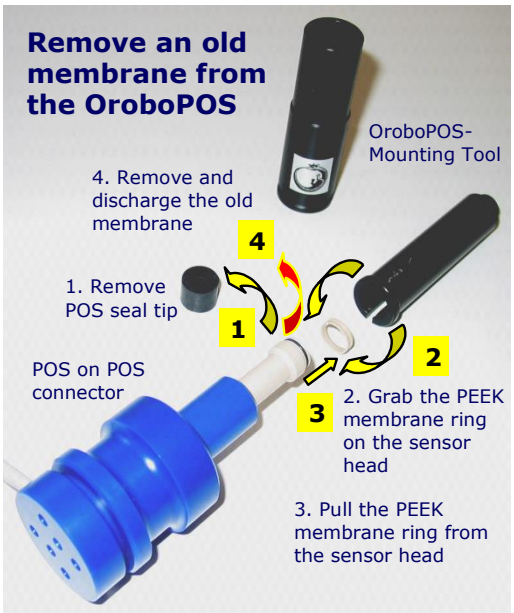
1. OroboPOS-Service Kit

Clip B1a

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 ($Na_2S_2O_4$)

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 OroboPOS-membrane ring 2,3 and membrane 4. Wash off electrolyte with distilled water.

Clip B1b

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

Cathode cleaning on the polishing cloth.

The cathode must be treated with extreme care. Do not touch with fingers, nor expose to detergents or greasy liquids.

Clip B1b Mount the OroboPOS onto the blue storage base. Wash off electrolyte from the POS with distilled water.

Place the Petri dish with the Polishing Cloth (OroboPOS-service kit) on a flat surface. Add a few drops of distilled water. Add on the tip of a spatula OroboPOS-Polishing Powder (aluminum oxide, 0.3 µm). Hold the sensor in a vertical position and polish the cathode in the thin paste for one minute in a figure-eight motion. Wash the polishing powder carefully off the sensor with distilled water. Subsequently, the anode is always cleaned as well (Section 2.2).

Clip B2

If noise remains high or the response time of the sensor signal is biphasic (exponential phase followed by a slow drift) after polishing the cathode and cleaning the anode 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

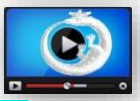
Clip B3

Fill the electrolyte reservoir of the POS with fresh concentrated (25%) ammonia solution. Within 10 min 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. Clean 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 Pen-Contact Oil (available from Oroboros Instruments) to the connections only if necessary. Before screwing the POS head onto the OroboPOS-Connector for membrane application, clean the POS connector of moisture and any other contamination (particularly any salt crystals from the electrolyte). Similarly, clean the plug of the electrical cable connecting to the O2k-Main Unit.

3. Membrane mounting


Clip B4

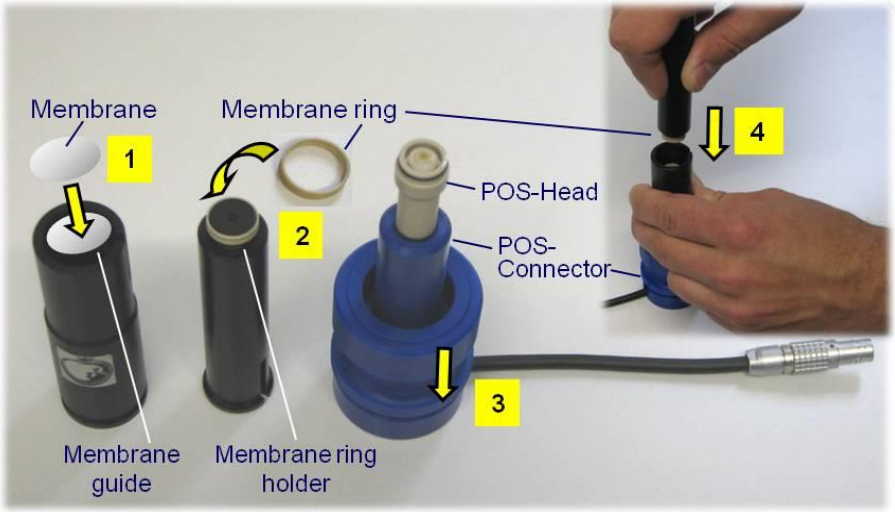
The POS head is screwed onto the blue OroboPOS-Connector. Check the O-ring on the POS head to ensure that its surface is smooth and intact. In exceptional cases, apply a tiny amount of grease to the O-ring of the sensor head.

Use KCl solution as electrolyte (1 mol·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 °C in a closed vial. To prevent the formation of gas bubbles in the electrolyte, heat the electrolyte solution by shaking the electrolyte vial in hot water (40-70 °C) before membrane mounting.

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 a membrane from the stack of paper sheets and membranes, carefully avoiding any mechanical damage of the transparent membrane. Do not touch the central area of the membrane with your fingers.

The black OroboPOS-Mounting Tool consists of two parts, (i) the membrane guide (larger diameter) and (ii) membrane ring holder with O-ring. Wash off any electrolyte and salt crystals with distilled water.

1 Position a new membrane into the **membrane guide** using the pair of forceps. 2 Fix the OroboPOS-Membrane Ring (which seales the membrane against the sensor body) to the **membrane ring holder**. Fill the POS head with electrolyte. 3 Slide the membrane guide downwards across the POS head while pushing the lower ring (arrow) of the blue OroboPOS-Connector strongly downwards. By releasing this ring, the membrane guide is fixed to the POS connector. 4 To slide the OroboPOS-Membrane Ring over the POS head, slide the membrane ring holder into the membrane guide, and press firmly down in a single movement to the final position.



Mounting a membrane onto the OroboPOS. The OroboPOS-Mounting Tool 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. 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 effect, but large folds should be avoided. Wash excess electrolyte off the POS and POS connector. Apply a wet OroboPOS-Seal Tip and attach the OroboPOS-Connector to the OroboPOS-Holder (O2k-Main Unit, Chapter A).

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4. Cable connection

For connection of the OroboPOS to the O2k Main unit, refer to the following manuals:
MiPNet19.18 for O2k-Series G, MiPNet19.01 for O2k-Series up to F.

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. Mount the POS to the blue storage base and seal it with the Perspex cup 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 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. »
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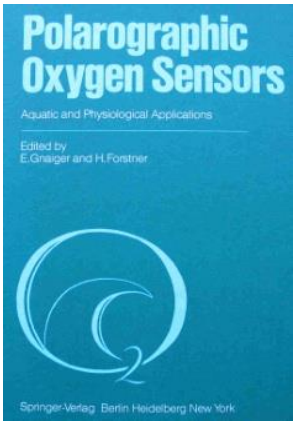

Updates »http://wiki.oroboros.at/index.php/MiPNet19.18B_POS-service

»http://wiki.oroboros.at/index.php/MiPNet19.18A_O2k-start

***Next step* – O2k-FluoRespirometer protocol »**MiPNet19.18D_O2k-calibration

Supplement A. Videosupport

Videos Johannes Aitzetmüller: fancy tree films, Innsbruck.

A.1. Videosupport weblinks

» **O2k-Videosupport**
http://wiki.oroboros.at/index.php/O2k-Videosupport

A.2. Videosupport directly on OROBOROS USB flash drive

- **B1a OroboPOS from housing**
  Start:Remove OroboPOS from Perspex housing
- **B1b Disassembly of OroboPOS**
  Service of a used OroboPOS: Disassembly of OroboPOS
- **B2 Cathode cleaning**
  OroboPOS-Polishing Cloth, OroboPOS-Polishing Powder
- **B3 Anode cleaning**
- **B4 Membrane mounting**
- **B5 Insert OroborPOS**
  OroboPOS-Connector

Supplement B. Links to O2k-Catalogue

» http://wiki.oroboros.at/index.php/OroboPOS
» http://wiki.oroboros.at/index.php/O2k-Catalogue:_OroboPOS_components
» http://wiki.oroboros.at/index.php/OroboPOS-Service_Kit