

O2k-Manual: POS service

Mitochondrial Physiology Network 19.18(B09):1-7 (2021)

Version B09: 2021-06-23 ©2014-2021 Oroboros

Updates: http://wiki.orooboros.at/index.php/MIPNet19.18B_POS-service



Service of the polarographic oxygen sensor OroboPOS

Erich Gnaiger

Oroboros Instruments

High-Resolution Respirometry

Schoepfstrasse 18, 6020 Innsbruck, Austria

Email: instruments@orooboros.at

www.orooboros.at



Contents

1. Summary.....	1
2. OroboPOS-Service Kit.....	2
3. Cleaning.....	2
3.1. Cathode cleaning	3
3.2. Anode cleaning.....	4
3.3. Clean the electrical connection	4
4. Membrane mounting	4
5. Cable connection	5
6. Storage of the OroboPOS.....	5
6.1. Short-term storage	5
6.2. Long-term storage	6
7. High signal at zero oxygen.....	6
8. References.....	6
9. Acknowledgements	7

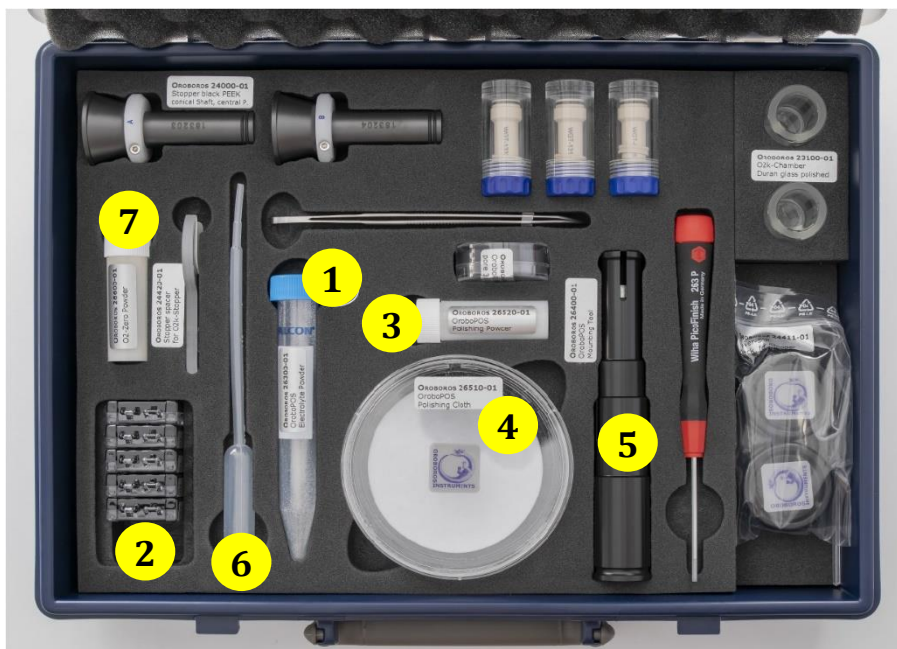
1. Summary

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 that are maintained in a well-monitored functional state. POS service may only need to be performed every few months. However, POS service is necessary if (1) a new sensor is installed, (2) the signal during air calibration is not stable over time, (3) the signal-to-noise ratio 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 % - 5 % of the signal at air saturation. For each sensor, the frequency of POS service is optimized based on long-term calibration records for **quality control** (O2-calibration.xlsx).

2. OroboPOS-Service Kit



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 O₂-Zero Powder, dithionite (Na₂S₂O₄)

Accessories for sensor service are provided in the [OroboPOS-Service Kit](#). In addition, distilled water and 25 % ammonia solution are required. To ensure premium quality of the content, the OroboPOS-Service Kit should be stored in the dark.

3. 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 observe many small bubbles in the electrolyte reservoir. This does not indicate that the bubbles caused a problem while the sensor was in use. Remove the [OroboPOS-membrane ring](#) (2), (3) and [membrane](#) (4). Wash off electrolyte with distilled water.

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 needs to be cleaned if 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-to-noise ratio or reduce the signal output by >30 %, reflected by the necessity to increase the gain. Such sensor problems can be improved by cleaning the cathode, anode and gold connections.

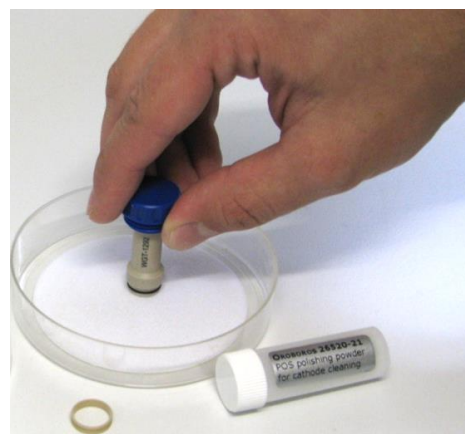


3.1. Cathode cleaning

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

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 and a spatula tip of [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. After you have finished cleaning the cathode, wash the polishing cloth with distilled water and make sure that it is completely dry before storage. Subsequently, the anode is always cleaned as well (Section 3.2). If the 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.



In extreme cases, the cathode may be cleaned by adding a drop of 50-75 % nitric acid onto the surface of the cathode for no longer than 15 seconds with extreme care. Carefully remove any traces of nitric acid by washing with distilled water and proceed as described above.

3.2. Anode cleaning

Fill the electrolyte reservoir of the POS with fresh 25 % ammonia solution. Within 10 min the silver/silver chloride should appear bright gray. Wash the sensor with distilled water and if the anode is still dark gray, repeat the anode cleaning. For older sensors it becomes necessary to prolong the exposure to ammonia up to several hours or even overnight, under the cap of the Perspex housing. Protect the POS from light, since the silver/silver chloride anode is light sensitive.



3.3. Cleaning the electrical connection

Unscrew the POS head and inspect both sides of the electrical connection (gold pin of the OroboPOS-Connector and threads of the POS head). Remove any contamination such as salt crystals, grease and moisture with a fine paper cloth. If necessary, moisten a paper cloth with 100 % ethanol and gently clean the gold pin and threads. Similarly, clean the plug of the electrical cable connecting to the O2k-Main Unit. Before screwing the POS head onto the [OroboPOS-Connector](#) for membrane application, clean the body of the OroboPOS-Connector of moisture and any other contamination (particularly any salt crystals from the electrolyte). If the O2k is used in a humid environment, the OroboPOS-Connector and OroboPOS can be dried in an oven at up to 60 °C for 1-2 h.

4. Membrane mounting

The [POS](#) 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.

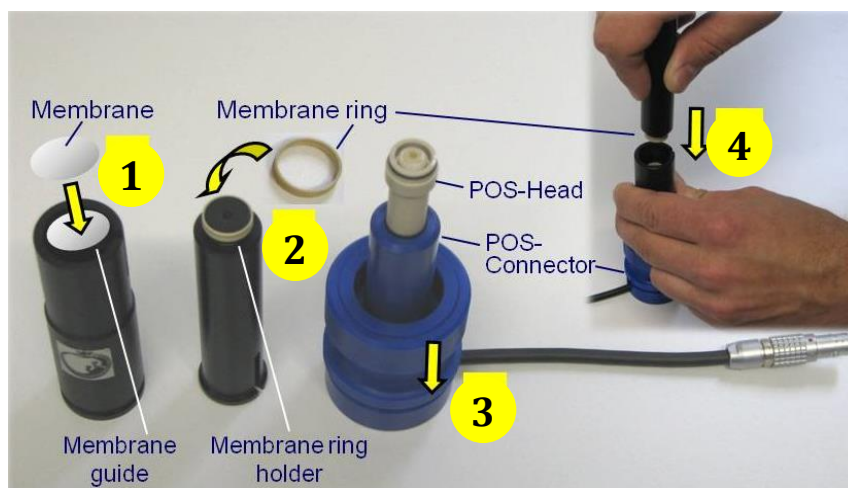


Use KCl solution as electrolyte (1 mol·dm⁻³; 74.56 g potassium chloride per liter, in distilled water) provided in the OroboPOS-Service Kit. Add distilled water to the [electrolyte powder](#) up to the 10 mL mark. Alternatively, dissolve 1.49 g KCl in distilled water with a total volume of 20 mL. Store at 4-8 °C in a closed vial. To prevent the formation of gas bubbles in the electrolyte solution, heat the solution by gently agitating the electrolyte vial in hot water (40-70 °C) before membrane mounting.

[POS membranes](#), which are transparent, are kept in small boxes in the [OroboPOS-Service Kit](#). Each membrane is separated by a non-transparent white paper sheet. Do not add the paper to the oxygen sensor. Carefully separate a membrane from the stack of paper sheets and membranes, avoiding any mechanical damage of the membrane. Do not touch the central area of the membrane.

The black [OroboPOS-Mounting Tool](#) consists of two parts, (i) the membrane guide (larger diameter) and (ii) the 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 **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) 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 bubbles should be trapped in the electrolyte reservoir after membrane application. Nor should folds be visible in the membrane in the central area. Inspect the electrolyte reservoir under a magnifying glass. Small folds in the membrane near the outer circumference have no negative effects, 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**

After POS service, the OroboPOS needs some time in operation to stabilize in pure water or MiR05, which may be a few or several hours (overnight). During this stabilization time the O2k has to be running (25 °C; illumination off). After a POS has been used and the seal tip has been removed from the POS it is normal to see many small bubbles. This does not indicate that there was a problem while the sensor was in use.

5. Cable connection

For connection of the OroboPOS to the O2k-Main Unit, refer to the following manual:
» [MiPNet22.11 O2k-FluoRespirometer manual](#)

6. Storage of the OroboPOS

6.1. Short-term storage

For short periods of time, *i.e.* days or several weeks, the OroboPOS is maintained in the O2k-chamber. The chamber is prewashed with distilled water and 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 lifetime of the sealing rings. The receptacle of the stopper is completely filled with 70 % ethanol and 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](#)).

For shelf storage (for a couple of days), unscrew the OroboPOS from the OroboPOS-Connector and unplug the OroboPOS-Connector from the O2k-Main Unit. Clean the sealing tip and membrane with distilled water. Mount the OroboPOS to the blue storage base and seal it with the Perspex cup to **prevent the electrolyte from drying out**. Store in the dark.

6.2. Long-term storage

For storage of the POS for several months, the sealing tip and membrane are removed 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). Wash the electrolyte off the POS with distilled water. The POS head is **stored dry and in the dark**. Check the electrical connections for any moisture and salt contamination and clean with a soft tissue if needed.

7. High signal at zero oxygen

A 'high signal at zero oxygen' may be observed during [zero calibration](#) and can be a sign of (1) a damaged OroboPOS, OroboPOS-Connector or O2k-Main Unit, (2) a dirty OroboPOS, or (3) contamination of the electrical connections. It is therefore recommended to perform cathode cleaning and several rounds of anode cleaning, and [cleaning of the electrical connections](#). After cleaning, the OroboPOS and OroboPOS-Connector can be dried at 50-60 °C for at least 1-2 h. Additionally, the zero current of the O2k-Main Unit, the OroboPOS-Connector and the OroboPOS (without membrane) should be tested for any [current leakage](#). If the latter test excludes any sources of leakage other than the POS and if repetition of POS service is not enough to solve the issue, the sensor head must be replaced.

8. References

Gnaiger E (2008) Polarographic oxygen sensors, the oxygraph and high-resolution respirometry to assess mitochondrial dysfunction. In: Mitochondrial dysfunction in drug-induced toxicity (Dykens JA, Will Y eds) John Wiley:327-52.

»[Bioblast link](#)

Gnaiger E, Forstner H, eds (1983) Polarographic Oxygen Sensors. Aquatic and Physiological Applications. Springer, Berlin, Heidelberg, New York:370 pp. »[Bioblast link](#)

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. »[Bioblast link](#)

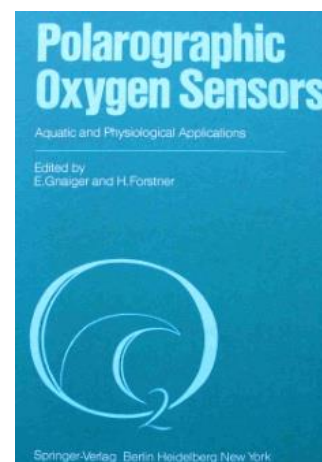
Hitchman ML, Gnaiger E (1983) A thermodynamic consideration of permeability coefficients of membranes. In: Polarographic oxygen sensors, Gnaiger E and Forstner H, eds., Springer, Berlin, Heidelberg, New York:31-6. »[Bioblast link](#)

Related MiPNets



»[MiPNet26.06 DatLab 7:Guide](#)

»[MiPNet22.11 O2k-FluoRespirometer manual](#)



9. Acknowledgements

Mario Fasching contributed to this MiPNet as a former member of Oroboros Instruments. Videos: Johannes Aitzetmüller, fancy tree films, Innsbruck.

Supported by the NextGen-O2k project.



This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 859770.

