oroboros INSTRUMENTS high-resolution respirometry

Oxygraph-2k Manual

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Service of the polarographic oxygen sensor OroboPOS

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Section

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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 is not stable over time, (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



- (7) 26600-01 O_2 -Zero Powder, dithionite (Na₂S₂O₄)
- 3 26550-01 Pen-Contact Oil for stable low contact resistance

Accessories for sensor service are provided in the OroboPOS-Service Kit. In addition, you need distilled water and 25% ammonia solution (fresh). 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 POS connectors or cable connections to the O2k (<u>MiPNet14.01</u>).

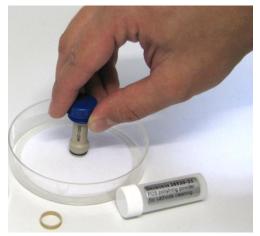
For sensor service, remove the black POS seal tip (1). It is normal to see many small bubbles in the electrolyte reservoir. This does not indicate that the bubles caused a problem while the sensor was actually in use. Remove the PEEK membrane ring (2, 3) and membrane (4). Wash off the electrolyte with distilled water. For cleaning the anode and cathode, the sensor head is best removed from the OroboPOS-Connector and mounted to the blue base of the Perspex housing of the POS (OroboPOS-Service kit).

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 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.

Wash off the 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, add some of the provided aluminum oxide powder (0.3 μ m) with the tip of a spatula and moisten it with a few drops of distilled water to obtain a thin paste. Hold the sensor in a vertical position and polish the



Cathode cleaning on the polishing cloth.

cathode for one minute in a figure-eight motion. Wash the aluminum oxide powder carefully off the sensor with distilled water, and wash the polishing cloth.

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.

If the response time of the sensor signal remains 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 salt crystals, 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.

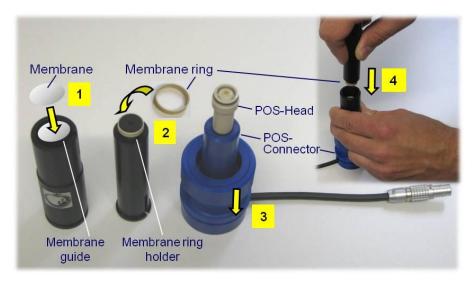
3. Membrane mounting

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. Rinse the OroboPOS-Mounting Tool (OroboPOS-Service Kit) with distilled water to wash off any electrolyte crystals.

As electrolyte, use KCl solution (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 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 two 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 PEEK membrane ring (which seales the membrane against the sensor body) to the membrane ring holder. Fill the POS head with electrolyte. [3] Push the lower ring on the POS connector stongly down, 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 PEEK 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.

No gas bubbles should be trapped in the electrolyte after reservoir 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 POS and POS connector. Apply a POS seal tip and attach the POS connector to the POS holder at the O2k-Main Unit (Section A).

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 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 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

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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).

Short-term shelf storage 5.2.

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.

Replacement of the OroboPOS head 6.

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

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Updates » www.bioblast.at/index.php/MiPNet19.18B POS-Service » www.bioblast.at/index.php/MiPNet19.18A O2k-Start 02k-Manual Next step - O2k-Core Manual D » MiPNet19.18D O2k-Calibration