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Updates: http://wiki.oroboros.at/index.php/MiPNet17.09_IOC_Questions

Questions for the O2k-Workshop

The **O2k-Guide (O2k-Manual)** and **O2k-Protocols** provide answers to many of the following questions. [MiPNet] numbers on the Oroboros.USB and website. More information: » www.oroboros.at / www.bioblast.at.

1. Oroboros O2k assembly [MiPNet22.11]

1.1. What is the most important consideration for positioning the glass chamber during assembly of the O2k?
1.2. How do you detect an oxygen leak in the chamber?

2. Polarographic oxygen sensor (POS)

2.1. Why is it important to check the non-calibrated raw signal (voltage, after current-to-voltage conversion) of the OroboPOS, and how can you quickly see the raw signal real-time?
2.2. The sensor voltage is 9.9 V. What should you do?
2.3. Why is it important to maintain an extremely constant temperature in and around the O2k-chamber?
2.4. Does the POS respond to oxygen concentration, c_{O2} [µmol·dm^{-3} = µM], or partial oxygen pressure p_{O2} [kPa]?

3. OroboPOS calibration [MiPNet19.18D]

3.1. How many calibration points are required for proper calibration of the polarographic oxygen sensor (POS)?
3.2. Should the chamber be open or closed during air calibration?
3.3. What is an acceptable voltage (raw signal) of the POS at (i) air calibration, and (ii) zero oxygen calibration, and how are these raw signals affected by the gain setting?
3.4. Why should you check the raw voltage during calibration?
3.5. How do you perform a zero oxygen calibration?
3.6. The oxygen solubility, S_{O2} [µM·kPa^{-1}], relates oxygen concentration to partial pressure. How is S_{O2} related to the solubility factor, F_M? Which variables need to be considered for estimation of the oxygen solubility of an aqueous solution, for example of mitochondrial respiration medium MiR06? [MiPNet06.03]
3.7. When is the oxygen calibration of a POS preferentially performed?
3.8. How long does it take approximately (5, 15, 30 or 45 min) to perform an oxygen calibration at air saturation, after the O2k is switched on (at experimental temperature in the range of 20 to 37 °C)?
3.9. Do you have to consider the instrumental background when performing an oxygen calibration of the POS at zero oxygen concentration?
3.10. Do you need to consider the instrumental background when performing an oxygen calibration of the POS at air saturation?

3.11. Does the oxygen signal have to be stable for an oxygen calibration of the POS?
3.12. How do you define POS signal stability? [MiPNet06.05]
3.13. Do you have to perform a zero oxygen calibration of the POS before air calibration?
3.14. Can you calibrate the POS with biological sample and respiratory activity in the aqueous solution, when equilibration is performed with a gas phase in the chamber and stability of the signal is observed?
3.15. What is the difference between static calibration [MiPNet19.18D] and dynamic sensor calibration (time constant – for advanced users)? How can you use a dynamic calibration (stirrer test) as a quick sensor test? [MiPNet02.04]

4. OroboPOS Service [MiPNet19.18B]

4.1. What should be done if the sensor connector threads appear dark and dirty?
4.2. The POS membrane box appears to have two types of membranes, which one should be applied to the sensor?
4.3. How can you avoid creating bubbles when filling the electrolyte reservoir of the POS?
4.4. Can the ammonia treatment be applied repeatedly?
4.5. How can you check sensor performance?
4.6. What precautions should be taken when handling the sensor connector?

5. Cleaning of the Chamber [MiPNet19.03]

5.1. Which solution should be placed in the chamber when the O2k is not in use (i.e. overnight, for a few days)?
5.2. Can detergents be used to clean the chamber and the PVDF stoppers?
5.3. What is the recommended cleaning procedure between experimental runs?
5.4. The glass chambers appear to have surface residue. Can this be removed, what is the procedure?
5.5. The stirrer bar gets stuck. What can be done?

6. Instrumental background test [MiPNet19.18E; MiPNet14.06]

6.1. Does the oxygen signal have to be stable (constant) for setting a mark in an instrumental background test?
6.2. Does the oxygen flux have to be constant for setting a mark in an instrumental background test?
6.3. How do you define flux stability? Is a flat horizontal red line always an indication of a stable flux?
6.4. Do you need to determine instrumental background flux at air saturation and zero oxygen concentration?
6.5. Do you need to calibrate the POS before performing an instrumental background calibration?
6.6. We use the symbol $a°$ for the intercept at zero oxygen concentration, and the symbol $b°$ for the slope of background oxygen flux as a function of oxygen concentration. In the analysis of instrumental background, we have obtained 0.022 and -1.7. Which value is $a°$ and $b°$, respectively?
6.7. Does the background-corrected flux have to be zero when the oxygen signal is stable?
6.8. How often do you have to check the instrumental background?