Mitochondrial Physiology Network 20.04(03):1-2 (2016)
Version 04: 2016-08-05
Updates: http://wiki.oroboros.at/index.php/MiPNet20.04_O2k-checklist

O2k-checklist: get started with an O2k-experiment

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The Oroboros checklist for High-Resolution FluoRespirometry provides a short guideline through essential steps for starting an experiment.

More details: O2k-Manual

- Oroboros USB-flash drive
- http://wiki.oroboros.at/index.php/O2k-Manual.

1. Recommended background reading

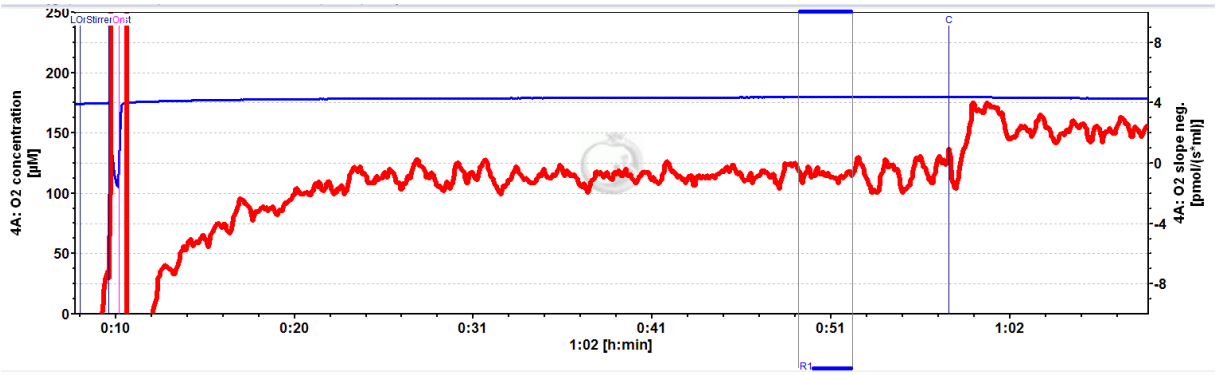
- Gnaiger E (2014) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 4th ed. Mitochondr Physiol Network 19.12. Oroboros MiPNet Publications, Innsbruck:80 pp. »
- Gnaiger E (2014) O2k-calibration by DatLab »
- Pesta D, Gnaiger E (2012) High-resolution respirometry. OXPHOS protocols for human cells and permeabilized fibres from small biopsies of human muscle. Methods Mol Biol 810: 25-58. »

2. State of the O2k

1. The O2k is connected to a computer with DatLab installed.
2. The volume of the O2k-Chambers (2 ml) has been calibrated.
3. O2k-Chambers should have been stored with 70% ethanol after last use.

3. Steps to start the O2k

4. Switch on the O2k, start DatLab program and set temperature to selected value. Standard graph layout "01 Calibration show Temp".
5. Aspire 70% ethanol from chambers and wash chamber three times with distilled water; also rinse stoppers.
6. Add experimental medium to the chambers (approx. 2.2 ml for standard chamber volume).
7. Fully insert stoppers (prevent trapping of bubbles), aspire surplus medium, lift stopper again to position "Air calibration" (use stopper spacer tool).
8. Let medium equilibrate and perform a Stirrer test during stabilization of sensor signal.
9. Prepare sample, Hamilton injection syringes and chemicals.
10. After stabilization of blue and red plot (approx. 35 min) perform air calibration - check solubility factor SF before calibrating. Uncalibrated slope should not exceed ± 1 pmol·s^{-1}·ml^{-1}. Standard graph layout "02 Calibration - Background".



11. Calibrate at air saturation (R1) and copy calibration values to your calibration list for quality control (Excel file: O2-calibration.xlsx).
12. Check if correct background values are used – normoxia versus high oxygen. A background test can be performed before (automatically copied into the file) or after the experiment and copied into the experimental file.
13. Fully insert stoppers and observe flux to check for stability and biological contamination; flux should not exceed +4 pmol·s^{-1}·ml^{-1} after 5-10 min. Standard graph layout "02 Calibration - Background".
14. For better overview of your experiment, start a new DatLab file shortly before adding sample (calibration values will be transferred automatically).
15. Start your experiment.