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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 respirometry provides a short overview of the essential steps for starting an experiment. For detailed information and step-by-step instructions refer to the corresponding MiPNets and O2k-Instructions.

1. State of the O2k

1. The O2k is connected to a computer with DatLab installed.
2. Polarographic oxygen sensors (POS) are serviced. »MiPNet19.18B POS-service
3. The O2k has been assembled. »MiPNet22.11 O2k-FluoRespirometer manual - Section 5
4. The volume of the O2k-chambers has been calibrated. »MiPNet22.11 O2k-FluoRespirometer manual - Section 5.4
5. O2k-chambers should have been stored with 70 % ethanol after last use. »MiPNet19.03 O2k-cleaning and ISS - Section 2.5

2. Steps to start the O2k

1. Switch on the O2k, start DatLab software and set temperature to selected value. Standard graph layout “01 Calibration show Temp”.
2. Wash the chambers. DL protocol “O2k-cleaning_BeforeUse” and »MiPNet19.03 O2k-cleaning and ISS - Section 2.2
3. Add experimental medium to the chambers (approx. 2.3 mL for 2.0 mL O2k-chamber and 0.54 mL for 0.5 mL O2k-chamber). »O2k-chamber

4. Insert stoppers fully (avoid trapping any bubbles), siphon off excess medium, lift stoppers to position "Air calibration" (use stopper spacer tool).
5. Perform an air calibration. DL-Protocol "O2 calibration_air" and »MiPNet06.03 POS-calibration-SOP - Section 4
6. Calibrate at air saturation (R1). Check oxygen solubility factor F_M before confirming calibration.
7. Copy calibration values to your calibration list for quality control. »MiPNet06.03 POS-calibration-SOP - Section 6
8. Prepare sample, Hamilton microsyringes, and chemicals. »MiPNet19.14 SOP Hamilton microsyringes and »MiPNet09.12 O2k-Titrations
9. Check if correct background values are used – normoxia versus high oxygen, 2.0 mL or 0.5 mL chamber. A background test can be performed before (automatically copied into the file) or after the experiment and manually copied into the experimental file. »MiPNet14.06 Instrumental O2 background
10. For a better overview of the experiment, save the calibration file and start a new DatLab file as the experimental file shortly before adding sample (calibration values will be transferred automatically).
11. Start the experiment. Set appropriate temperature. For biological experiments change slope smoothing to 20. »Smoothing

3. Recommended background reading

- Gnaiger E (2020) Mitochondrial pathways and respiratory control. An introduction to OXPHOS analysis. 5th ed. Bioenerg Commun 2020.2:112 pp. doi:10.26124/bec:2020-0002
- Gnaiger Erich et al — MitoEAGLE Task Group (2020) Mitochondrial physiology. Bioenerg Commun 2020.1. doi:10.26124/bec:2020-0001.v1
- Doerrier C, Garcia-Souza LF, Krumschnabel G, Wohlfarter Y, Mészáros AT, Gnaiger E (2018) High-Resolution FluoRespirometry and OXPHOS protocols for human cells, permeabilized fibers from small biopsies of muscle, and isolated mitochondria. Methods Mol Biol 1782:31-70. -»Bioblast link«

***Further details*: O2k-Manual**

- Oroboros USB-flash drive
- O2k Manual
- O2k-Videosupport

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