Oroboros O2k-Manual

O2k-checklist: get started with an O2k-experiment

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The Oroboros checklist for high-resolution respirometry provides a short guideline through essential steps for starting an experiment.

1. State of the O2k

1. The O2k is connected to a computer with DatLab installed.
2. Oxygen sensors are mounted properly. » MiPNet19.18B POS-service
3. The volume of the O2k-chambers has been calibrated. » MiPNet22.11 O2k-FluoRespirometer manual - Section 5.4
4. 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). »https://wiki.oroboros.at/index.php/O2k-chamber
4. Insert stoppers fully (prevent 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) and copy calibration values to your calibration list for quality control (Excel file: MiPNet06.03 POS-calibration-SOP - Section 6).

7. Check oxygen solubility factor F_M before confirming calibration.

8. Prepare sample, Hamilton injection syringes, and chemicals. »MiPNet19.14 SOP Hamilton microsyringes.

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 copied into the experimental file. »MiPNet14.06 Instrumental O2 background.

10. For a better overview of your 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).

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

Acknowledgements