



Checklist: Getting started with an experiment

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The OROBOROS checklist for high-resolution respirometry provides a short guideline through essential steps before starting an experiment. For more details please read the respective manual provided with the OROBOROS USB-flash drive or download the manuals from <http://wiki.orooboros.at/index.php/O2k-Manual>.

Recommended background reading:

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. » 

1. Switch on instrument, start DatLab programme and set temperature to desired value. Choose Layout "01 Calibration Exp. Gr3-Temp".
2. Aspire 70% ethanol from chambers (chambers should have been stored with 70% ethanol after last use) and wash chamber three times with distilled water; also rinse stoppers.
3. Add required medium to the chambers (approx. 2.2 ml for standard chamber volume).
4. Fully insert stoppers (prevent trapping of bubbles!), aspire surplus medium, lift stopper again to position "Air calibration" (use stopper spacer tool).
5. Let medium equilibrate and perform a Stirrer test after stabilization of sensor signal.
6. Prepare sample, Hamilton injection syringes and required chemicals.
7. After stabilization of flux (approx. 35 min) perform air calibration - check solubility factor SF before calibrating. Flux should not exceed $\pm 1 \text{ pmol}/(\text{s}\cdot\text{ml})$.
8. Copy calibration values to your calibration list for quality control (excel file: [Calibration list.xls](#)).
9. Check if correct Background values are used - normoxygen/highoxygen (this can be done also after the experiment and copied into the file).
10. Fully insert stoppers again and observe flux to check for biological contamination; flux should not exceed $4 \text{ pmol}/(\text{s}\cdot\text{ml})$.
11. For better overview of your experiment start a new DatLab file shortly before adding sample (calibration values will be carried over).
12. Start your experiment.