

DatLab 7.1

Innovations: DL-Protocols

Mitochondrial Physiology Network 22.16(01):1-2 (2017)

Version 01: 2017-11-13 ©2017 Oroboros

Updates: http://wiki.orooboros.at/index.php/MiPNet22.16_DL-Protocols



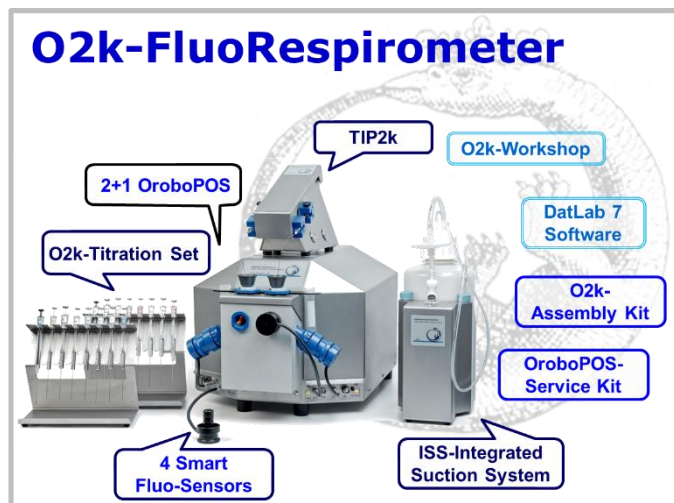
For all O2k users

Oroboros developed the advanced DatLab 7 software, featuring new dimensions in:

- DL-Protocols: support in line with MitoPedia
» <http://www.bioblast.at/index.php/MitoPedia: SUIT>
- Data acquisition & analysis for HRFR: context-sensitive help
» <http://wiki.orooboros.at/index.php/MitoPedia: DatLab>
- Quality control, traceability

Two-in-one

- DatLab 7 is optimized for High-Resolution FluoRespirometry (HRFR) – two techniques combined in each of the two O2k-chambers
- Matching O2k hardware and software
- DatLab 7 supports all



O2k configurations and files generated in previous DatLab versions.

DL-Protocols

A selected DL-Protocol defines the sequence of Events and Marks, and provides instructions on titrations in substrate-uncoupler-inhibitor titration (SUIT) protocols or calibrations and instrumental background tests.

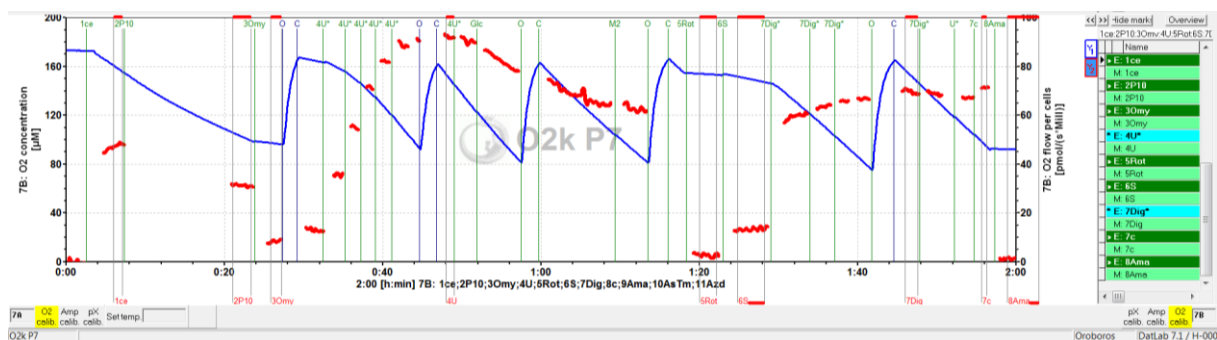
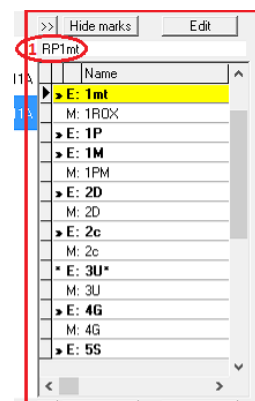
Protocol	Experiment	Calibration	Flux / Slop
<input checked="" type="checkbox"/>	Show DL-Protocol		
<input checked="" type="checkbox"/>	Synchronous DL-Protocol events		
	Set DL-Protocol / O2 limit		
	A: Export DL-Protocol		
	B: Export DL-Protocol		

Standardized DL-Protocols: An expanding collection of standardized DL-Protocols is available. While the titration steps of these SUIT protocols are fixed, the titration volumes and final concentrations can be adjusted and exported as a customized DL-Protocol by the user.

Why standardized DL-Protocols? For generating a database on mitochondrial respiratory physiology, however, standardized protocols are a requirement. In view of the variety of mitochondrial respiratory control patterns between species, tissues and cell types, and the diversity of specific questions addressed, a large library of standardized SUIT protocols will be required. Yet,

user-specific variations of SUIT-protocols do not always follow the strict rationale required for optimum protocol design. Even a minor variation of titration steps may obscure the meaning of a respiratory state, and the nomenclature requires better standardization. For instance, titration of oligomycin in the presence of ADP and a substrate combination supporting the NADH- and succinate-pathway makes sense after titration of rotenone, inducing a well-defined LEAK state with two coupling sites (CIII and CIV). In contrast, titration of oligomycin without rotenone yields LEAK respiration which cannot be interpreted with respect to coupling, if the relative contributions of NADH- and succinate pathway fluxes are not known.

Using a DL-Protocol: The user is guided stepwise through the titration sequence of a DL-Protocol (e.g. RP1mt), with standard names of Events for titrations of biochemicals and Marks for respiratory states and statistical analysis. This minimizes handling errors and greatly improves performance during O2k measurements: The **yellow** line indicates the next titration step during the experiment. Titration steps that are completed correctly are shown in **green**. **Cyan** indicates multiple titrations. Any titration event that is not properly set in sequence is indicated in **red**. An automatic warning at low oxygen levels can be set to remind the user to re-oxygenate the chamber in due time.



User-specific DL-Protocols: The full flexibility to perform user-defined experiments is maintained. New DL-Protocols, however, require programming by the Oroboros scientific support team – a contribution to Cost Action MitoEAGLE.

- In the spirit of Open Innovation and COST Action CA15203 MitoEAGLE: To obtain your user-specific DL-Protocol, the best choice is to send us an experimental DatLab file (*.DLD) with detailed description. A discussion forum on the MitoEAGLE website (or MitoPedia: SUIT) may be involved in the evaluation of the suitability of the SUIT protocol, if there are any doubts. You would be the author of the SUIT protocol which you make available for the scientific community on the website. The Oroboros team will edit the protocol according to a standardized nomenclature [1] and post the DL-Protocol on the website for Open Access.
- If you do not agree on Open Access, Oroboros will ask for a service charge to generate a DL-Protocol for your exclusive use, without publishing it on the website. Oroboros would consider such a protocol as your intellectual property, without warranty of priority rights. Any other O2k-laboratory may submit an identical protocol for Open Access, which then would become available for the scientific community.

[1] MitoEAGLE preprint 2017-11-11(16) The protonmotive force and respiratory control: Building blocks of mitochondrial physiology Part 1. http://www.mitoeagle.org/index.php/MitoEAGLE_preprint_2017-09-21.

