Mitochondrial Physiology Network 24.09(01):1-5 (2019)

Updates:https://bioblast.at/index.php/MiPNet24.09_Mt-membrane_Potential_Analysis

Data analysis of mitochondrial membrane potential estimation using various fluorescence dyes

Komlodi T[1], Antunes D[1], Iglesias-Gonzalez J[1], Sobotka O[2,3], Gnaiger E[1]

[1] **Oroboros Instruments**
Schoepfstrasse 18, A-6020 Innsbruck, Austria
Email: instruments@oroboros.at
www.oroboros.at

[2] 3rd Department of Internal Medicine, Gerontology and Metabolism
University hospital and Faculty of Medicine in Hradec Kralove
Charles University, CZ

[3] Department of Physiology, Faculty of Medicine in Hradec Kralove
Charles University, CZ

Contents

1. General information

Substrate-uncoupler-inhibitor-titration (SUIT) protocols are designed to study respiratory control in a sequence of coupling and pathway control states induced by multiple titrations within a single experimental assay. DatLab 7.4 has been specifically designed to guide the user through SUIT protocols (DL-Protocols in DatLab). Excel templates (SUIT-###_Fluo_mt_D###_general.xlxs) are provided for data analysis of O_2 flux and mitochondrial membrane potential (mtMP) using different fluorescence dyes (*e.g.*, safranin, TMRM, Rhodamine123) for isolated mitochondria, tissue homogenate and permeabilized cells. Each DL-Protocol is defined with a unique D-number (D###), for a detailed list see:

- https://www.bioblast.at/index.php/SUIT_protocol_library#List_of_SUIT_protocols_with_D-numbers

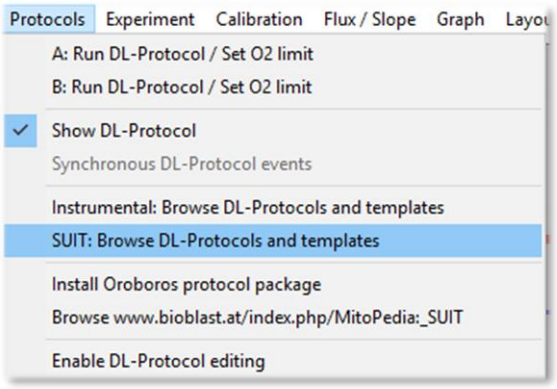
Use the SUITbrowser to find the best SUIT protocol for your research questions:

- https://suitbrowser.oroboros.at/

2. Starting data analysis

Upon competition of real time respirometry measurement in DatLab 7.4 or anytime while doing the DatLab analysis, open our Excel template to analyze your data in a time efficient way.

1. In DatLab 7.4, select the menu [Protocols] and click on [SUIT:Browse DL-Protocols and templates].
2. Select your SUIT protocol and open the SUIT-###_Fluo folder. Inside this folder, you will find another folder for the specific DL-Protocol (named SUIT-###_Fluo_mt _D###). In each folder, four Excel files can be found:



   a. A blank template (named SUIT-###_Fluo_mt_D###_general.xlxs) to calculate the relative mtMP values.
   b. A demo version of the general template (named SUIT-###_Fluo_mt_D###_general_demo.xlsx), which provides an example of the file already with data showing the relative mtMP values.
   c. A blank template (named SUIT-###_Fluo_mt_D###_safranin.xlxs) to convert the fluorescence values measured by safranin into absolute mtMP values expressed as mV.
   d. A demo version of the specific template (named SUIT-###_Fluo_mt_D###_general_demo.xlxs) which provides an example of the file already with data showing the mtMP values expressed in mV.

3. Create a copy of SUIT-###_Fluo_mt_D###_general.xlxs analysis template for your data analysis and rename it. You can

rename the template by opening it and choosing the option ‘Save as´ in the archive top menu.

3. Calibration

3.1. Open the DatLab file containing the data from the calibration.

3.2. Open the menu [Calibration] and select 'Amperometric, Amp' to calibrate and convert the amperometric signal into the concentration of the fluorescence dye.

3.3. Select the first four marks (*e.g.*, Saf0, Saf0.5, Saf1, Saf1.5, Saf2) for calibration. Check the r^2 of the linear regression and press the 'Show graph' button to check the linearity of regression. The sensitivity [V/µM] can be also found in the same window.

3.4. Press 'Calibrate' and the [Y1: Amp raw] will turn into calibrated [Y1: Amp]. Adjust the scaling [F6] of [Y1: Amp].

4. Biological Experiment

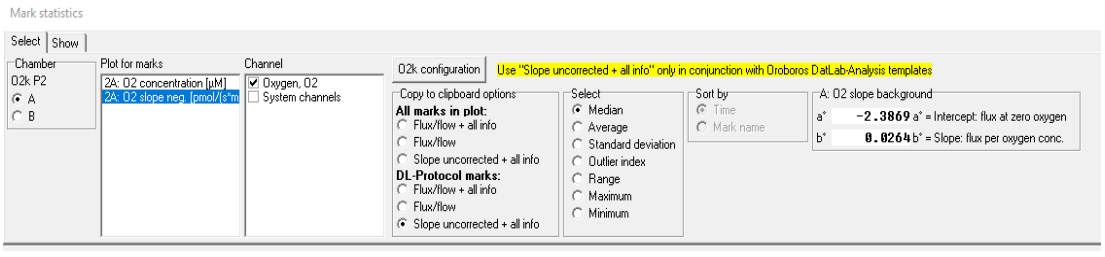
In the excel template you can select the setting by ticking the boxes `Titration volume correction´ and `Known sample concentration´. More information can be found here: [MiPNet24.06 Oxygen flux analysis with DatLab7.4]

4.1. Oxygen flux analysis

The calculations of the O_2 fluxes are provided under the following link complying with Oroboros transparency policy:
https://wiki.oroboros.at/index.php/Flux_/_Slope#O2

4.1.1. In DatLab 7.4, after setting the marks separately to the O_2 flux, go to [Marks], and select ‘Slope uncorrected + all info´ in `DL-Protocol marks´. In the new window select:

a. Your chamber of interest.
b. Plot for Marks: `O2 slope neg. [pmol/s*mL]´.
c. Channel: `Oxygen, O2´. Leave only this channel selected.
d. Select: ‘Median´.
e. Sort by: ‘Time´(default).
f. Then, click on [Copy to clipboard] to copy the selected values.



4.1.2. In the Excel template: Click on the yellow cell A7 and paste these selected values (only O_2) from DatLab [Ctrl+V].

4.1.3. The calculated values for the specific O_2 flux, specific O_2 flux (bc), *FCR* and *FCR* (bc), on each step of the protocol can be found from column K in the rows 24, 25, 27 and 28, respectively.

4.1.4. Paste the DatLab graphs showing the traces for the chamber:

a. In DatLab: Select the graph (left mouse click into the respirometry graph of interest) → select `Graph\Copy to Clipboard\WMF´.

b. In the Excel template: Click on the yellow cell A27: `Paste DatLab graph here, reduce to width 22 cm (8 inches)´ → press [Ctrl+V] to paste.

c. Select the graph (right click on the graph) → select `Size and properties´ and set the width of the graph to 22 cm (8 inches).

4.2. Membrane potential analysis

4.2.1. In DatLab 7.4, copy the calibration values of the fluorescence signal from the previous calibration file: Open the menu [Calibration] and select 'Amperometric, Amp', press 'Copy from file' to open the calibration file and copy the sensitivity value. Then press the 'Calibrate' button in the 'Amp calibration' window.

4.2.2. Select [Y1: Amp] as the active plot for setting marks and place marks according to your protocol as done for the O_2 flux.

4.2.3. Use the macro in DatLab 7.4 as explained in MiPNet20.13 to obtain normalized (calculated) fluorescence plots.

4.2.4. Copy marks from the original amperometric trace to the calculated one: select the calculated trace and open the menu [Marks] and select 'Copy marks from\5A: Amp [µM] or 5A: Amp raw'.

4.2.5. Open [Marks] window and select 'Slope uncorrected + all info´; in the new window select channel 'Calculated' and use DL-Protocol marks: 'Slope uncorrected + all info' to display data for the marked regions.

4.2.6. Export data with 'Copy to Clipboard'.

4.2.7. Paste the calculated fluorescence values in the Excel template into the yellow cell A42.

4.2.8. The calculated mtMP values can be found in row 50 starting with column J.

4.2.9. Copy the Amp graph as it is explained in the 4.1.4 and paste on the yellow cell A63.

5. References

1. Cardoso LHD, Antunes D, Iglesias-Gonzalez J, Komlodi T, Doerrier C, Garcia-Souza LF, Gnaiger E, Sobotka O (2019) Oxygen flux analysis with DatLab 7.4. Mitochondr Physiol Network 24.06(01):1-5. – »Bioblast link«
2. Krumschnabel G, Fasching M, Gnaiger E (2019) O2k-FluoRespirometry: HRR and simultaneous determination of mt-membrane potential with safranin or TMRM. Mitochondr Physiol Network 20.13(03):1-5. - »Bioblast link«

» MitoPedia:_Mitochondrial_membrane_potential

» Flux / Slope

» Safranin

» TMRM

» Rhodamine123

Acknowledgements

The project NextGen-O2k has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 859770.