

# Hydrogen peroxide flux analysis using Amplex UltraRed assay in MiR05-Kit with DatLab 7.4

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## Contents

1.	General information .....	1
2.	Instructions .....	2
2.1	Starting a new data analysis .....	2
3.	Data analysis .....	2
3.1.	Oxygen flux analysis.....	3
3.2.	Hydrogen peroxide flux analysis .....	4
4.	References .....	5

## 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 are provided for data analysis of O<sub>2</sub> flux and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) flux using Amplex UltraRed assay in [MiR05-Kit](#) for isolated mitochondria, tissue homogenate (except of liver homogenate), permeabilized cells and living 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](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:

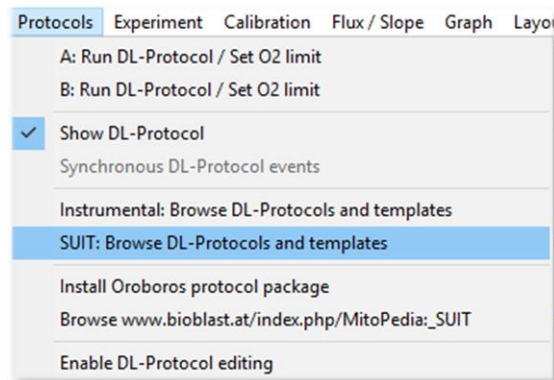
- <https://suitbrowser.orooboros.at/>

## 2. Instructions

### 2.1 Starting a new data analysis

Upon completion of real time respirometry measurement in DatLab 7.4 or anytime while performing 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, open SUIT-###\_AmR folder. Inside this folder, you will find another folder for the specific DL-Protocol (named SUIT-###\_AmR\_mt/ce/ce-pce\_D###). In each folder, two Excel files can be found:
  - a. A blank template (named SUIT-###\_AmR\_mt/ce/ce-pce\_D###.xlsx)
  - b. A demo version of the template (named SUIT-###\_AmR\_mt/ce/ce-pce\_D###\_demo.xlsx), which provides an example of the file already with data.
3. Create a copy of the SUIT-###\_AmR\_mt/ce/ce-pce\_D### 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. Data analysis

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 \(See section 2\)](#)

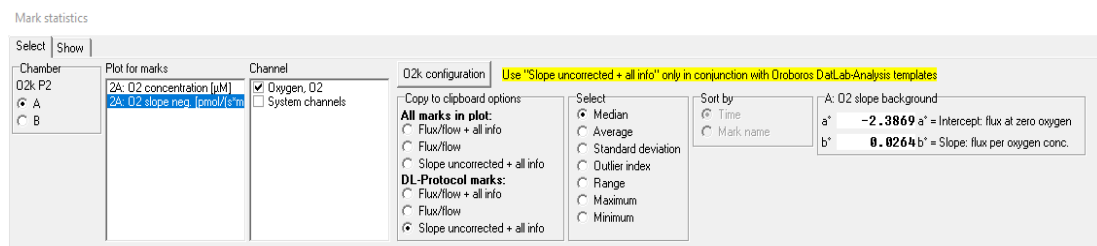
### 3.1. Oxygen flux analysis

The calculations of the O<sub>2</sub> fluxes are provided under the following link complying with Oroboros transparency policy:

➤ <https://wiki.oroboros.at/index.php/Flux / Slope#O2>

3.1.1 In DatLab 7.4, after setting the marks separately to the O<sub>2</sub> flux and H<sub>2</sub>O<sub>2</sub> flux, go to 'Marks'(F2), and select 'Slope uncorrected+all info'. In the new window select:

- Your chamber of interest.
- Plot for Marks: 'O<sub>2</sub> slope neg. [pmol/s\*mL]'.
- Channel: 'Oxygen, O<sub>2</sub>'. Leave only this channel selected.
- Select: 'Median'.
- Sort by: 'Time' (default).
- Then, click on [Copy to clipboard] to copy the selected values.



3.1.2 In the Excel template: Click on the yellow cell L4 and paste these selected values (only O<sub>2</sub>) from DatLab [Ctrl+V].

3.1.3 The calculated values for the *FCR*, *FCR* (bc), specific O<sub>2</sub> flux and specific O<sub>2</sub> flux (bc) on each step of the protocol can be found in the rows 19 to 22, starting at column V.

3.1.4 Paste the DatLab graphs showing the traces for the chamber:

- In DatLab: Select the graph (left mouse click into the respirometry graph of interest) → select 'Graph\Copy to Clipboard\WMF'.
- In the Excel template: Click on the yellow cell A6: 'Paste DatLab graph here, reduce to width 22 cm (8 inches)' → press [Ctrl+V] to paste.
- Select the graph (right click on the graph) → select 'Size and properties' and set the width of the graph to 22 cm (8 inches).

## 3.2. Hydrogen peroxide flux analysis

The calculations of the H<sub>2</sub>O<sub>2</sub> fluxes are provided under the following link complying with Oroboros transparency policy:

- <https://bioblast.at/index.php/Flux / Slope#How to Analyse with DatLab 7.4 2>

3.2.1 In DatLab 7.4, set the marks separately to the O<sub>2</sub> flux and H<sub>2</sub>O<sub>2</sub> flux:

- Go to [Layout] menu and click into 'O<sub>2</sub>&Amp' and select 'Standard Layouts/01 Amp Amperometric\_Raw signal'.
- Go to 'Marks' and select 'Slope uncorrected + all info'. In the new window select 'H<sub>2</sub>O<sub>2</sub> slope [pmol/s\*mL]' in 'Plot for Marks'.
- Channel: 'Amperometric,Amp'. Leave only this channel selected.
- Select: 'Median'.
- Sort by: 'Time'(default).
- Then, click on [Copy to clipboard] to copy the selected values.

3.2.2 In the Excel template: Click on the yellow cell L41 and paste [Ctrl+V] Amp slope from the DatLab.

3.2.3 Copy the sensitivity values (Amp calib.) from the DatLab: go to 'Calibration\Amperometric, Amp' to open Amp calibration window (be sure that you select the one for your trace of interest, i.e., A or B).

a. Select marks (1)0.0 and (1)0.1 and copy the sensitivity value [Ctrl+C] from the box and paste [Ctrl+V] in the excel template in cell V36.

b. Continue for each pair of H<sub>2</sub>O<sub>2</sub> calibration and paste in the yellow boxes (at the right of cell V36) to the corresponding titration steps. In the empty yellow boxes use the previous sensitivity values.

c. The value for sensitivity "before sample" is taken from the previous calibration file recorded in the absence of any sample. Open this calibration file and in the Amp calibration window select (0)0.0, (0)0.1 and (0)0.2 to calculate the sensitivity value. Then, copy the value in the excel template into cell U36. This box cannot be left empty, otherwise the H<sub>2</sub>O<sub>2</sub> values cannot be calculated. An example of a calibration file can be found here: in DatLab, open in the 'Protocols' menu: 'Instrumental: Browse DL-Protocols and templates' and open the provided demo file in the 'AmR calibration' folder.

3.2.4 Copy the Amp graph as it is explained in the 3.1.4 and paste on the yellow cell A30.

3.2.5 Specific H<sub>2</sub>O<sub>2</sub> flux can be found in row 30 and specific flux H<sub>2</sub>O<sub>2</sub> (bc) in row 31, both starting at column V.

## 4. 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, Fontana-Ayoub M, Fasching M, Gnaiger E (2019) O2k-FluoRespirometry: HRR and simultaneous determination of H<sub>2</sub>O<sub>2</sub> production with Amplex UltraRed. Mitochondr Physiol Network 20.14(04):1-6. – [»Bioblast link«](#)
3. Eigentler A, Fontana-Ayoub M, Gnaiger E (2019) O2k-FluoRespirometry: HRR and H<sub>2</sub>O<sub>2</sub> production in mouse cardiac tissue homogenate. Mitochondr Physiol Network 18.05(03):1-6. – [»Bioblast link«](#)



» [MitoPedia: Measurement of hydrogen peroxide](#)

» [Flux / Slope\\_O2](#)

» [Flux / Slope\\_H2O2](#)

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