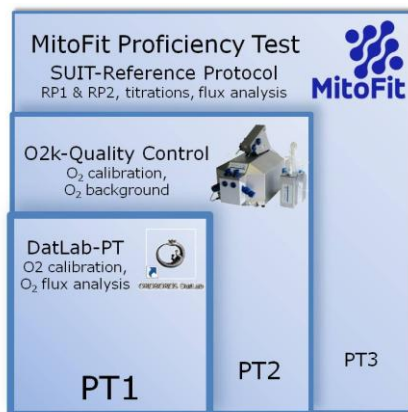# OROBOROS O2k-Protocols

MitoFit PT1: DatLab proficiency test

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Summary: The MitoFit Proficiency Test Part 1 (MitoFit-PT1) is the DatLab-PT for flux analysis. It can be considered as a first-level 'dry' MitoFit-PT for evaluation of inter-individual and inter-laboratory reproducibility and accuracy of DatLab flux analysis of a data file. Proficiency will be tested for (1) O_2 calibration at air saturation and zero O_2 concentration; (2) analysis of instrumental O_2 background; and (3) DatLab flux analysis of a data file obtained in an experiment using the O2k-Core with human cells and the SUIT reference protocol.

1. Experimental basis

The Power-O2k P7 was used for the instrumental tests and the cell respirometric experiment to generate the DatLab files for the MitoFit PT1.

> 2016-04-15 P7-01.DLD (O_2 background)
> 2016-05-24 P7-01.DLD (O_2 calibration)
> 2016-05-24 P7-02.DLD (RP1-RP2 with Pc)

2.1 ml medium; O2k-chamber with stopper in 'open' position for air calibration.

Closed: Stoppers fully inserted into O2k-chamber, extruding all gas space and excess aqueous medium, for final volume of 2.0 ml.

RP1-RP2: During the experiment, the smoothing factor for flux was set at 40 data points in DatLab. For analysis, the smoothing factor should be set to 20 data points.

2. Data analysis

Please read the instructions carefully and make sure that DatLab is installed on your PC before you proceed.

Step 1:

Open the file in DatLab:

2016-05-24 P7-01.DLD (O$_2$ calibration)

This is an example of an O$_2$ calibration including a stirrer test for quality control. You should now set the according mark and calibrate the data.
» http://wiki.oroboros.at/index.php/MiPNet06.03_POS-Calibration-SOP

Save the file with your name abbreviation as extension.

Step 2

Open the file in DatLab:

2016-04-15 P7-01.DLD (O$_2$ background)

This is an example of an evaluation of instrumental oxygen background flux required for automatic correction of oxygen diffusion into or out of the O2k-Chamber. Mitochondrial respiration medium MiR05 (http://wiki.oroboros.at/index.php/MiR05) without biological sample was added to the O2k-Chamber and oxygen fluxes were measured at different levels of oxygen concentration obtained by TiP2k mediated titration of dithionite. **Note that this file has not been calibrated.**

You should now calibrate the data and determine the parameters required for background correction. Set the appropriate marks and calculate the background correction values. Then click the button "Save and Recalculate".
» http://bioblast.at/index.php/MiPNet14.06_InstrumentalO2Background

Save the file with your name abbreviation as extension.

Step 3

Open the following file in DatLab:

2016-05-24 P7-02.DLD (RP1-RP2 with Pc)

This is an experimental file containing the measurement of respiration of permeabilized blood cells (PBMCs) according to the SUIT reference protocol:

» http://www.bioblast.at/index.php/MiPNet21.06_SUIT-RP

This file has been calibrated (air saturation and zero oxygen). You should analyze all respiratory states in accordance with this example by marking sections of the O2 flux in the experiment as you feel is appropriate.

The O2 calibration for the experimental file is taken from the calibrated 2016-05-24 P7-01.DLD file. The instrumental O2-background is at the system default setting.

When you have set all necessary marks, save and copy the information to the DatLab-Excel template:

» http://www.mitofit.org/index.php/MiPNet21.06_SUIT-RP#DatLab-Excel_templates_for_RP_analysis

Save both files with your name abbreviation as an extension.

Step 4

Send all files with your name abbreviation to:
» christina.plattner@oroboros.at

Help:

RP1 and RP2:
» http://www.mitofit.org/index.php/MiPNet21.06_SUIT-RP

DatLab:
» http://wiki.oroboros.at/images/e/ee/MiPNet19.18C_DatLab-Guide.pdf
» http://wiki.oroboros.at/images/1/18/MiPNet19.18E_O2FluxAnalysis.pdf

3. Author contributions, publication versions, references

This communication is a pre-publication prepared by PC, KG, DC, GE. SZ performed the test experiments.

Full version with references
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http://www.mitofit.org/index.php/MiPNet21.07_MitoFit_DatLab_PT