



### DL-Protocols

**DLP:** DL-Protocols are provided in DatLab as files with the extension \*DLP. A DL-Protocol defines the sequence of [Events](#) and [Marks](#). Templates are linked to DL-Protocols for storing exported data in a database and for data analysis. A DL-Protocol can be assigned to O2k-Chamber A or B, or both.

**Instrumental:** Instrumental DL-Protocols are used for calibrations and instrumental quality control, without experimental sample in the incubation medium.

**SUIT:** DL-Protocols for [substrate-uncoupler-inhibitor titrations](#) (SUIT) provide a guide through a sequence of [coupling control states](#) and [electron transfer-pathway states](#).

**Lower O2 limit [ $\mu\text{M}$ ]:** This can be set for each chamber, to trigger an automatic warning when the experimental O<sub>2</sub> concentration declines below this limit as a WARNING to remind the user that re-oxygenation of the medium may be required. In many cases the lower limit is set at 30  $\mu\text{M}$ .

**Titration volumes and concentrations:** Users can edit titration volumes and concentrations. In [Protocols] select [Enable DL-Protocol editing] and edit the DL-Protocol in the Overview window, save the overview, and export the file as a user-specific DL-Protocol [File \ Export \ DL-Protocol User (\*.DLPU)].

**Events and marks:** Users can modify steps (instructions, events, E and marks, M) in a DL-Protocol. In [Protocols] select [Enable DL-Protocol editing] and edit the DL-Protocol in the Overview window, save the overview, and export the file as a user-specific DL-Protocol [File \ Export \ DL-Protocol User (\*.DLPU)].

**DLPU:** DL-Protocol User, with modified steps, titration volumes and final concentrations.

- E:** Event in DatLab, an action at a time point in the SUIT protocol.
- M:** Mark in DatLab, a selected section over a period of time for numerical data analysis (Mark statistics).

## SUIT

- O2** O2 channel only.
- AmR** O2 channel and Amperometric channel (Amp) for Amplex UltraRed assay (AmR) for measurement of H<sub>2</sub>O<sub>2</sub> production.
- TPP** O2 channel and Potentiometric channel (pX) for TPP<sup>+</sup> or TPMP<sup>+</sup> assay for measurement of mt-membrane potential difference.
- Fluo** O2 channel and Amperometric channel (Amp) for fluorescence dye (e.g. safranin, TMRM) for measurement of mt-membrane potential difference.
- MgG** O2 channel and Amperometric channel (Amp) for Magnesium green assay (MgG) for measurement of mitochondrial ATP production.

## Abbreviations [1]

ce	living cells; $N_{ce} = N_{vce} + N_{dce}$
dce	dead cells
imt	isolated mitochondria
MiR	mitochondrial respiration medium
mt	mitochondria
mtprep	mitochondrial preparations
pce	permeabilized cells
pfi	permeabilized muscle fibers
pti	permeabilized tissue
SUIT	substrate-uncoupler-inhibitor protocol
thom	tissue homogenate
vce	viable cells

**Units** Report flow per cell in units [ $\text{amol}\cdot\text{s}^{-1}\cdot\text{cell}^{-1}$ ] equivalent to [ $\text{pmol}\cdot\text{s}^{-1}\cdot 10^{-6}$  cells].

- [1] MitoEAGLE preprint 2019-08-30 Mitochondrial respiratory states and rates.  
[http://www.mitofit.org/index.php/Gnaiger\\_2019\\_MitoFit\\_Preprint\\_Arch](http://www.mitofit.org/index.php/Gnaiger_2019_MitoFit_Preprint_Arch)



» [NextGen-O2k](#): Supported by the NextGen-O2k project, which has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 859770.