



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 [μM]: This can be set for each chamber, to trigger an automatic warning when the experimental O₂ 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 μM .

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 (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 H₂O₂ production.
- TPP** O2 channel and Potentiometric channel (pX) for TPP⁺ or TPMP⁺ 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 2019-05-20 Mitochondrial respiratory states and rates.
http://www.mitofit.org/index.php/Gnaiger_2019_MitoFit_Preprint_Arch



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