O2k-Protocol

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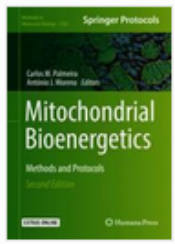
High-resolution respirometry and OXPHOS protocols



High-Resolution FluoRespirometry and OXPHOS Protocols for Human Cells, Permeabilized Fibers from Small Biopsies of Muscle, and Isolated Mitochondria

Carolina Doerrier, Luiz F. Garcia-Souza, Gerhard Krumschnabel, Yvonne Wohlfarter, András T. Mészáros, Erich Gnaiger

High-resolution respirometry combining a linear coupling control protocol analysis in living cells and the respirometric cell viability test

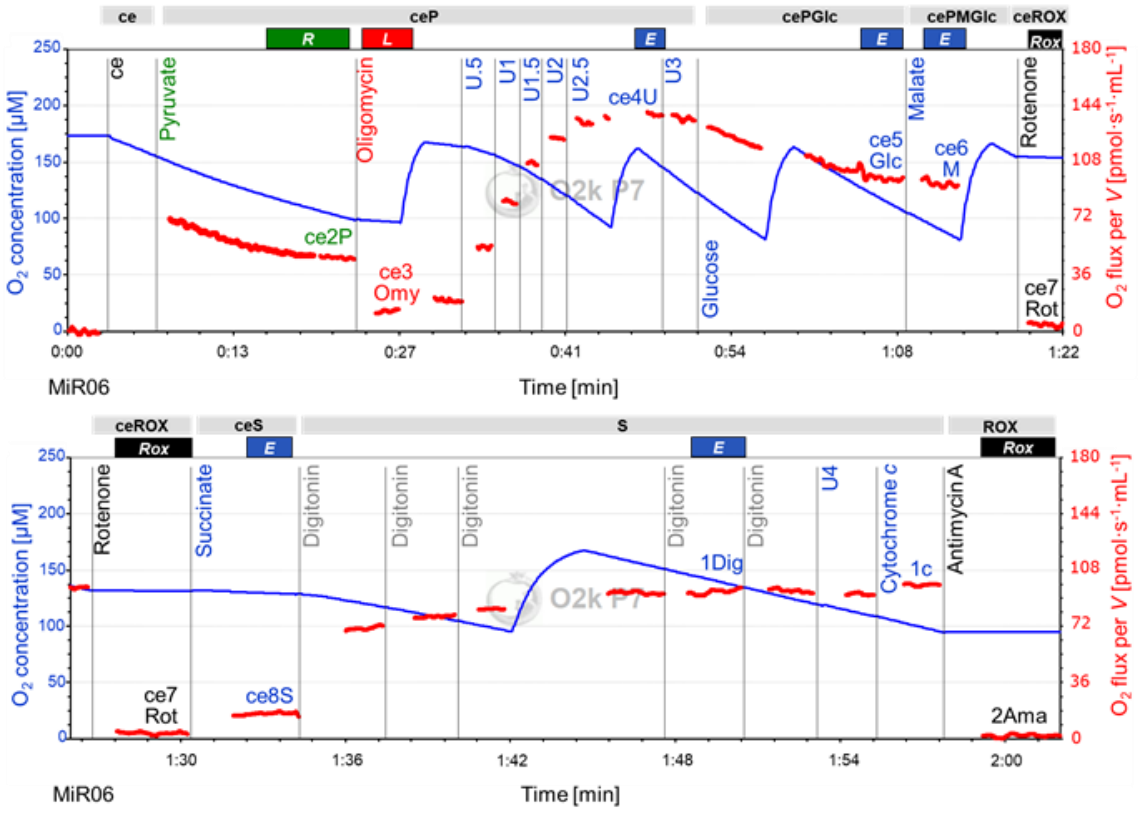


Figure 1. Respiration of cryopreserved **(A)** living and **(B)** permeabilized HEK293 cells in the mitochondrial respiratory medium MiR06. **ce2P: ROUTINE respiration (*R*)** supplemented with pyruvate. **ce3Omy: LEAK state (*L*)** induced by oligomycin addition. **ce4U: ET capacity** after multiple uncoupler titrations. **ce5Glc:** glucose addition for the evaluation of the Crabtree effect. **ce6M:** malate. **ce7Rot:** inhibition by rotenone (***Rox***: residual oxygen consumption). **ce8S:** succinate to stimulate death cells with functional mitochondria. **1Dig:** digitonin for selective plasma membrane permeabilization. **1c:** cytochrome *c* for testing the integrity of the outer mitochondrial membrane. **2Ama:** antimycin A to induce **ROX** state.

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SUIT reference protocol (RP1&2) for mitochondrial pathways mapping

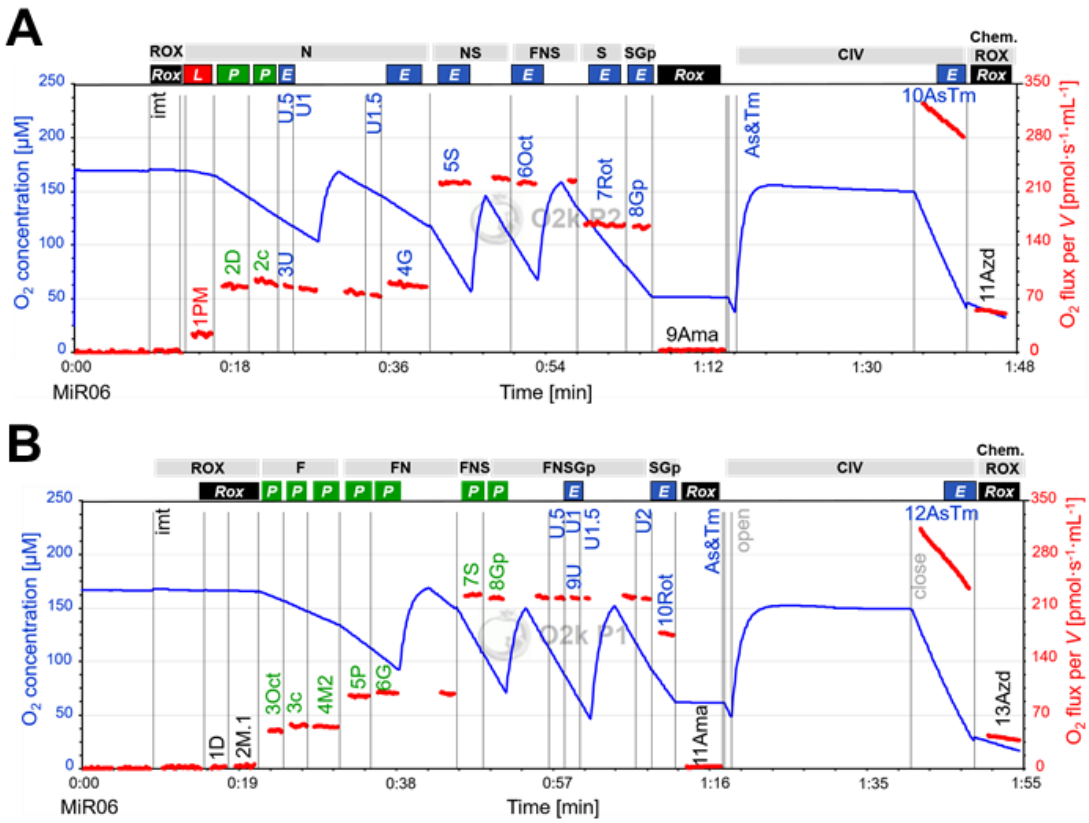

Figure 2. SUIT RP1&RP2. **N:** Linear coupling control (LEAK, ***L***; OXPHOS, ***P*** and ET, ***E***) with NADH-linked substrates. **F:** OXPHOS capacity in β-oxidation of fatty acids in presence of low malate to prevent the overestimation of F-pathway capacity if anaplerotic pathways are present in our sample. **FN:** OXPHOS capacity with convergent flow in the fatty acid oxidation&NADH-pathway. **NS:** Combined NADH and succinate-linked ET capacity. **FNS:** OXPHOS and ET capacities in the presence of fatty acid oxidation&NADH&succinate-linked substrates. **FNSGp:** OXPHOS and ET capacities at covergent electron flow into the Q-cycle through fatty acid oxidation&NADH&succinate&glycerophosphate-pathways. **S:** Succinate-pathway supporting ET-capacity. **SGp:** ET-capacity in the combined succinate&glycerophosphate-pathway. **CIV:** Complex IV activity.

SUIT RP1&2 provide a common reference in different mitochondrial coupling control and electron transfer (ET) pathways states

Diagnostic: specific mitochondrial defects can be identified by high-resolution respirometry

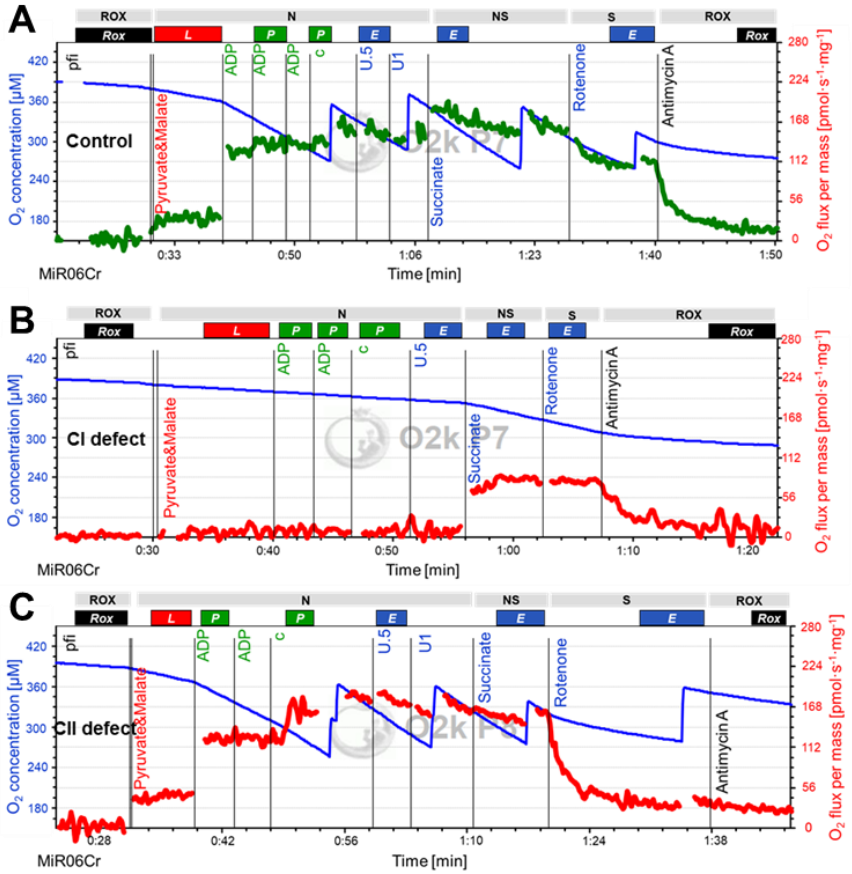

Figure 3. Shortened SUIT protocol linked to RP1 with diagnostic examples. N-linked **LEAK**, **OXPHOS**, and **ET** states with pyruvate (5 mM) and malate (2 mM). Sequential addition of succinate (10 mM) and rotenone (0.5 µM) yields NS-ET and S-ET capacity. ROX after inhibition of CIII with antimycin A (2.5 µM). Oxygen concentration (µM) and tissue mass-specific oxygen flux [pmol·s^{-1}·mg^{-1}] of permeabilized mouse cardiac fibers as a function of time. Reoxygenations in MiR06 were performed using H_2O_2 titrations. **(A) Control sample**. **(B) CI defect** induced by 0.5 µM of rotenone. **(C) CII defect** induced by 5 mM malonic acid.

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Evaluation of oxygen kinetic parameters by high-resolution respirometry provides relevant information for pathophysiological conditions

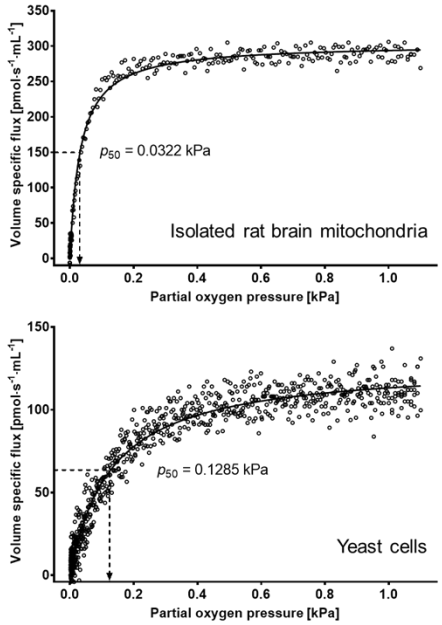

Figure 4. Oxygen kinetics is assessed in a closed-chamber of the O2k-FluoRespirometer during aerobic-anaerobic transitions when O_2 is consumed by mitochondria until the O_2 concentration declines practically to zero. Kinetic parameters are calculated from a hyperbolic fit of volume-specific O_2 flux, $J_{V'O2}$, plotted as a function of pO_2. Circles represent individual data points. Solid lines are hyperbolic fits over the low oxygen range (<1.1 kPa, ca. 10 µM), yielding the pO_2 at half-maximum $J_{V'O2}$ (p_{50}) as a parameter. **(A) Rat brain mitochondria** in MiR06 (oxygen solubility 9.72 µM·kPa^{-1}) at 37 °C in the presence of glutamate (10 mM), malate (2 mM), succinate (50 mM), and ADP (2.5 mM). **(B)** Baker's **yeast** (freeze-dried) in Na-phosphate buffer (50 mM, pH 7.1, oxygen solubility 10.05 µM·kPa^{-1}) at 37 °C in the ROUTINE state of respiration with endogenous substrates.

Protocols for high-resolution respirometry of living cells, permeabilized cells, permeabilized muscle fibers, isolated mitochondria, and tissue homogenates offer sensitive diagnostic tests of integrated mitochondrial function using standard cell culture techniques, small needle biopsies of muscle, and mitochondrial preparation methods

Reference: Doerrier C, Garcia-Souza LF, Krumschnabel G, Wohlfarter Y, Mészáros AT, Gnaiger E (2018) High-Resolution FluoRespirometry and OXPHOS protocols for human cells, permeabilized fibers from small biopsies of muscle, and isolated mitochondria. Methods Mol Biol 1782:31-70.

Figures and texts slightly modified based on the recommendations of the COST Action MitoEAGLE CA15203. Doi:10.26124/mitofit:190001.v4

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