PRINCIPE FELIPE
CENTRO DE INVESTIGACION

CIPF Seminar

Measuring mitochondrial respiration and other bioenergetic parameters using the O2k-FluoRespirometer from Oroboros Instruments

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CIPF Immunobiology Lab

Abstract: The O2k-FluoRespirometer is a modular system designed by Oroboros Instruments for high-resolution FluoRespirometry (HRFR), a method that enables the combined measurement of respiration and fluorometric signals for assessing not only mitochondrial respiration but also ROS production, mitochondrial membrane potential, ATP production or Ca2+ levels. With this system, the experimenter follows the respiration (oxygen consumption, oxygen flux) or the other bioenergetic parameters of biological and biochemical samples, transferred into the experimental chambers, while the experiment is still running. This methodology can be routinely applied for primary and cultured cells, tissue biopsies, permeabilized cells and tissue biopsies, microbial cultures, oxygen-consuming enzymes, antioxidant systems, and isolated mitochondria. Specific substrate-uncoupler-inhibitor titration (SUIT) protocols are applied in studies of cell respiration or mitochondrial activity. In experiments with intact cells, different coupling control states can be studied to evaluate ROUTINE respiratory activity, respiration coupled to ATP production, respiratory excess capacity, residual oxygen consumption, and intactness of cell membranes. In mitochondrial preparations such as isolated mitochondria, permeabilized cells and tissue samples, or homogenized tissue, quantitative information is obtained on selected mitochondrial pathways, particularly of the process of oxidative phosphorylation (OXPHOS) in mitochondria. The O2k is specially used in basic and applied research to study respiration, to assess either basal mitochondrial characteristics or to obtain diagnostic insight into respiratory pathways.

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