Training Guide

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Introduction to the Agilent Seahorse XF Cell Energy Phenotype Test

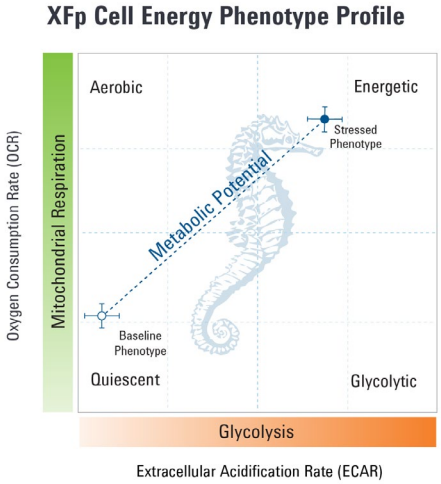
The ability of XF technology to simultaneously measure the two major energy producing pathways in live cells- mitochondrial respiration and glycolysis - has accelerated understanding of cellular function, including activation, proliferation, differentiation, and disease etiology. The XF Cell Energy Phenotype Test rapidly measures mitochondrial respiration and glycolysis under baseline and stressed conditions to reveal key parameters of cell energy metabolism, including Baseline and Stressed Energy Phenotypes, as shown in the figure below. There are four relative bioenergetic phenotypes that a cell can display:

Quiescent: The cell is not very energetic via either metabolic pathway.

Energetic: The cell utilizes both metabolic pathways.

Aerobic: The cell utilizes predominantly mitochondrial respiration.

Glycolytic: The cell utilizes predominantly glycolysis.



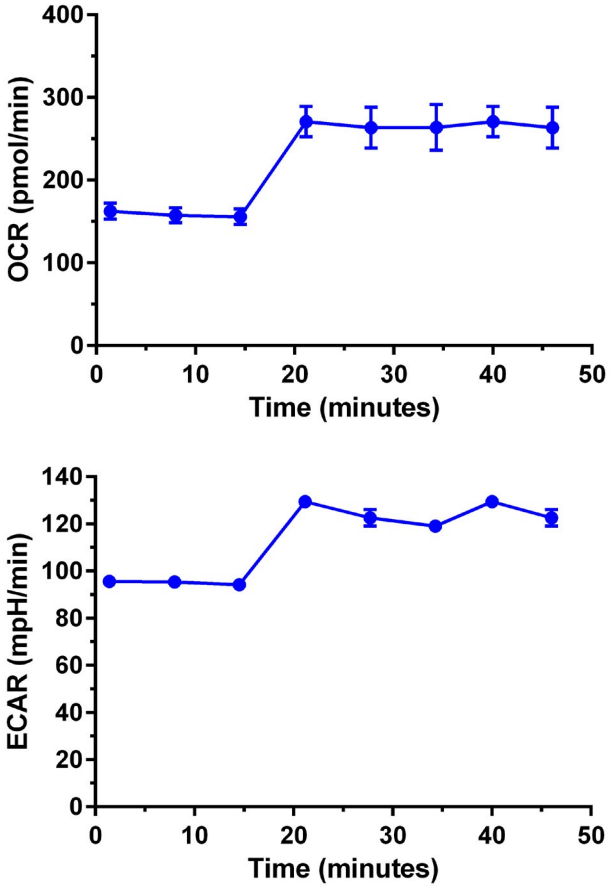
The XF Cell Energy Phenotype Test employs a simultaneous injection of two compounds that create stressed conditions with respect to cellular energy production. Oligomycin inhibits mitochondrial ATP production, often resulting in a compensatory increase in the rate of glycolysis (ECAR) as the cells attempt to meet their energy demands via the glycolytic pathway; FCCP drives mitochondrial respiration (OCR) to maximal rates by uncoupling electron transport and oxidative phosphorylation processes.

The assay is simple, consisting of three basal and five stressed measurements. The kinetic OCR and ECAR data (see figure to right) is transformed by the XF Cell Energy Phenotype Test Report Generator, which plots basal and stressed OCR and ECAR values on an XF Energy Map showing OCR v. ECAR (see figure on first page).

In addition to providing energy phenotype information, the Cell Energy Phenotype Test is also an ideal assay for initial cell characterization and optimization with respect to cell seeding density and the FCCP concentration required to elicit maximal respiration (i.e. creating a stressed condition).

Optimizing cell density and FCCP concentration for each cell type or cell line used will provide the best chances for a successful XF assay in research, including robust and consistent data that may be analyzed and interpreted with confidence.

Methods and assay designs for using the XF Cell Energy Phenotype Test for cell characterization are presented in Agilent Seahorse XF Training Module 3: Performing the XF Assay.



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