Fundación MEDINA

Discovering the Future

MITOCONDRIAL ASSAYS

Mitochondrial Membrane Potential

TMRE/TMRM fluorescence responds to changes in ψm .TMRE fluorescence [excitation λ (Ex λ) of 568 nm and emission λ (Em λ) > 590 nm] in mitochondria will be visualized on a HCS (BD).

Inside a healthy, non-apoptotic cell, the lipophilic TMRE/TMRMdye, bearing a delocalized positive charge, enters the negatively charged mitochondria where it accumulates in an inner-membrane potentialdependent manner. When the mitochondrial ΔΨm collapses in apoptotic cells, the TMRE /TMRM potentiometric dyes no longer accumulate inside the mitochondria and become more evenly distributed throughout the cytosol.

ROS

ROS generation will be detected with the fluorescence dye C-DCDHF-DA. The esterified fluorescence probe passes through cell membranes easily. Once inside the cell its lipophilic blocking groups are cleaved by non-specific esterases, resulting in a charged form that leaks out of the cells only slowly. The dye is useful for the detection of ROS. The plates were read in a FLIPRTETRA using excitation/ emition (495 nm /520 nm)

Superoxides

Production of superoxide was evaluated intracellularly using the superoxide-sensitive dye DHE. DHE is oxidized by superoxide to a novel product which binds to DNA enhancing intracellular fluorescence. Cells were incubated with DHE (final concentration 10 μM) in ensayo (NaCl 165 mM, KCl 4.5 mM, CaCl2 2 mM, MgCl2 1 mM, Hepes 10 mM y glucosa 10 mM, pH 7.4) for 30 min . The plates were read in a FLIPRTETRA using excitation/ emition (515 nm /595 nm)

Mitochondrial Calcium

To determinate mitochondrial calcium Rhod-2-AM will be used (Exc 549 / Em 578). Plates will be read on HCS (BD)

ATP/AMP/ADP levels

ATP, AMP and ADP concentrations will be determinated by means of anion-exchange HPLC-UV using a binary gradient of 0.3 M ammonium carbonate and waten on a ProPac PA1 column. ATP, AMP and ADP peaks will be detected compared their retention times.

NADH concentration

NADH oxidase activity will be measured as the aerobic conditions of 75 uM NADH in the absence of external quinine substrates and other respiratory chain inhibitors.

Reactions rates will be calculated from the linear decrease of NADH concentration (λ=340 nm) in a spectrophotometer using submitocondrial particules.

Oxygen consumption

The assay is based on the ability of O2 to quench the excited state of the MitoXpress probe. As the test material respires (e.g., isolated mitochondria, cell populations, small organisms, tissues and enzymes), O2 is depleted in the surrounding solution/environment, which is seen as an increase in probe phosphorescence signal. Changes in oxygen consumption reflecting changes in mitochondrial activity are seen as changes in MitoXpressTM probe signal over time.

The assay is non-chemical and reversible, a decrease in oxygen consumption (an increase in O2 levels) is seen as a decrease in probe signal. The assay will be read in a FLIPRTETRA