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Characterization of mitochondrial membrane potential

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes the procedure used by the Seifert Lab to characterize the membrane potential of mitochondria from fibroblasts using JC-1 dye.

Protocol materials

⊗ HBSS Merck MilliporeSigma (Sigma-Aldrich) Catalog #H4891

⊗ JC-1 Dye (Mitochondrial Membrane Potential Probe) Thermo Fisher Catalog #T3168

⊗ 250 mg FCCP biorbyt Catalog #orb385553

⊗ Oligomycin Merck MilliporeSigma (Sigma-Aldrich) Catalog #495455



- 1 Seed cells on a 96-well black clear glass bottom plate at a density of ~30,000 cells per well
- 2 Culture for 24:00:00 to 48:00:00 until cells reach 80% confluency
- 3 Wash cells in 1X HBSS Merck MilliporeSigma (Sigma-Aldrich) Catalog #H4891
- 4 Add 100 μ L of JC-1 Dye (Mitochondrial Membrane Potential Probe) Thermo Fisher Catalog #T3168 staining mix
- 5 Incubate for 00:30:00
- 5.1 Optional: to measure changes in mitochondrial membrane potential in response to inhibitors
- 5.2 Halfway through incubation, add 1.5 micromolar (μ M) Oligomycin Merck MilliporeSigma (Sigma-Aldrich) Catalog #495455 and 10 millimolar (mM) 250 mg FCCP biorbyt Catalog #orb385553
- 5.3 Incubate for remaining 00:15:00
- 6 Wash cells with warm 1X HBSS Merck MilliporeSigma (Sigma-Aldrich) Catalog #H4891
- 7 Scan plates with 485 nm excitation and 529 nm detection to quantify green fluorescence (monomeric)
- 8 Scan plates with 590 nm detection to quantify red fluorescence (aggregate)



- 9 Calculate mitochondrial membrane potential by the ratio of green to red fluorescence intensities, normalized to sample protein level (μg)