CELL-BASED ASSAYS

Mitochondrial membrane potential

Mitochondrial membrane potential (ΔΨm) is critical for maintaining the physiological function of the respiratory chain to generate ATP. JC-1 is a dye that accumulates in mitochondria. The dye exists as a monomer at low concentrations and yields green fluorescence. At higher concentrations, the dye forms J-aggregates that exhibit a red fluorescence. These characteristics make JC-1 a sensitive marker for mitochondrial membrane potential[1,2].

Test system: A-375 (human melanoma) - ATCC®.
Experimental number: Three per group in triplicate.
Reference Item: Valinomicyn 1 µM.

Main read-outs:
Fluorescence in 490 nm (excitation) and 530 nm (emission) (green);
Fluorescence in 525 nm (excitation) and 590 nm (emission) (red).

Validation Data

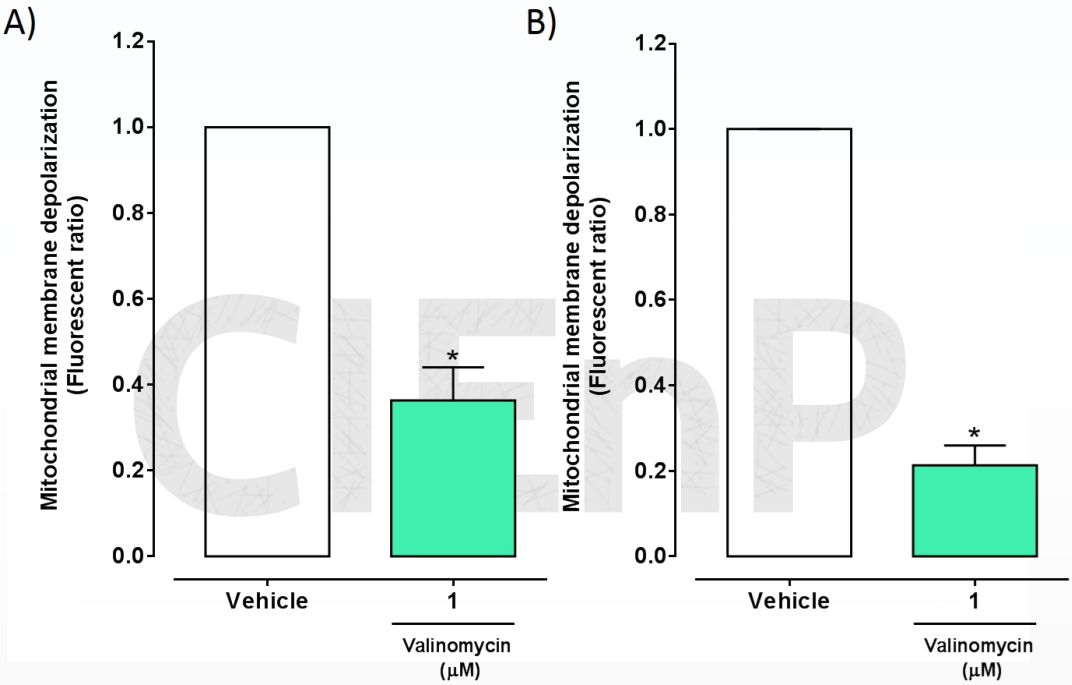

Figure: Mitochondrial membrane potential activity of valinomycin. The figure represents the reduction of mitochondrial membrane potential by valinomycin (1 µM) treatment for 24 hours **(A)** or 48 hours **(B)**, when compared with control group (vehicle). Each column represents the mean ± SEM of 3 experiments per group in triplicate. Statistical analyses used was t-test. *P < 0.05, versus vehicle group.

To avoid bias and to allow reproducibility and reliability of all in vitro experiments we follow the guidance on good cell culture practice[3]. All in vitro experiments are performed in triplicate wells for each condition and repeated at least three times.

References:

[1]JC-1: alternative excitation wavelengths facilitate mitochondrial membrane potential cytometry. Perelman A, C Wachtel, M Cohen, S Haupt, H Shapiro and A Tzur.. Cell Death and Disease (2012) 3..

[2]Determination of Mitochondrial Membrane Potential and Reactive Oxygen Species in Live Rat Cortical Neurons. Dinesh C. Joshi and Joanna C. Bakowska J Vis Exp. 2011; (51): 2704.

[3]Coecke S; Balls M; Bowe G; Davis J; Gstraunthaler G, Hartung T, Hay R, Merten OW, Price A, Schechtman L, Stacey G, Stokes W. Guidance on good cell culture practice: a report of the second ECVAM task force on good cell culture practice. Altern Lab Anim. 2005, 33(3):261-87.

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