MEDICAL UNIVERSITY OF VIENNA

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JC-1 stain of apoptotic cells

JC-1 (5, 5´, 6, 6´-tetrachloro-1, 1´, 3, 3´-tetraethylbenzimidazol-carbocyanine iodide) is a lipophilic fluorescent cation that incorporates into the mitochondrial membrane, where it can form aggregates due to the physiological membrane potential of mitochondria. This aggregation changes the fluorescence properties of JC-1 leading to a shift from green to orange fluorescence. Intact living cells stained with JC-1 therefore exhibit a pronounced orange fluorescence of mitochondria, which is detectable by flow analysis (in the FL-2 channel). Apoptosis results in a break-down of the mitochondrial membrane potential and a subsequent decrease of the orange fluorescence (and a slight increase of the green fluorescence). By that means, apoptotic cells can be easily distinguished from non-apoptotic cells.
For a review see this website

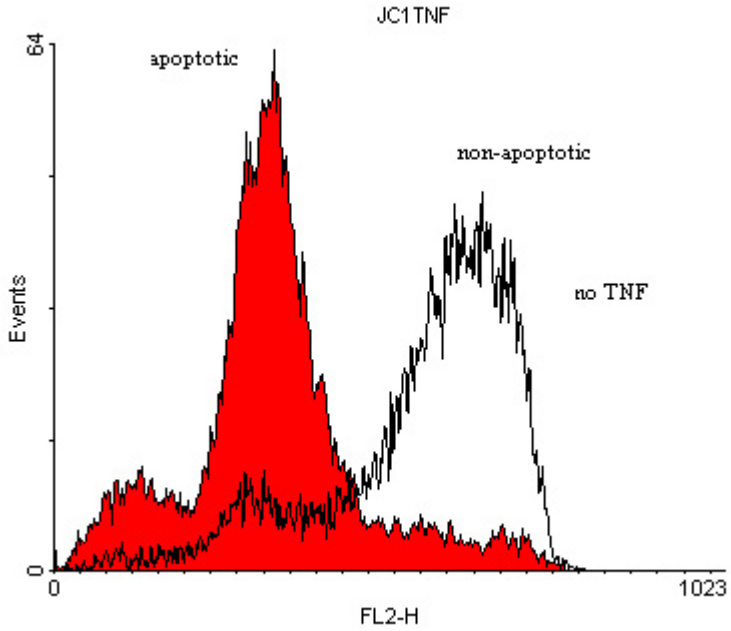
JC-1 is prepared as a 1000x stock solution in DMSO (5 mg/ml).
For the staining of adherent cells it is diluted in medium to 5 µg/ml (with vortexing during the dilution to prevent the formation of precipitates); the JC-1 containing medium is added to the cells, followed by incubation for 10 min at 37°C (or RT for 15 min).
Subsequently the cells are washed twice with PBS, trypsinized, suspended in 500 µl PBS and analyzed by flow analysis.
Suspension cells (lymphocytes): suspend 1:1 with 10 µg/ml JC-1 in medium (final conc.: 5 µg/ml)

Approximate detection settings on FACSort:

FL1: 360 V (log)
FL2: 310 V (log)
Compensation : FL1-7% FL2 und FL2-74% FL1

other settings: cell type specific, e.g. for TF-1:
SSC: 336 V lin
FSC: E00 lin 1.0
Threshold: FSC: 52 (or FL2)

Example of an analysis:


JC1TNF
apoptotic
non-apoptotic
no TNF
Events
64
0
0
1023
FL2-H