Membrane Potential Analysis by Flow Cytometry

Preparation of the dyes:

$DiBAC_4(3)$ (Molecular Probes) dissolved in DMSO.
Working concentration 20 uM

$DiOC_6$ (Molecular Probes) dissolved in DMSO.
Working concentration 20 uM

JC-1 (Molecular Probes) dissolved in DMSO.
Working concentration 10 mM

Loading of the dyes:

Total cell count should be between 5×10^5 and 1×10^6 cells/ml. Dye concentrations and incubation time may vary depending on cell type and will need to be determined independently

$DiBAC_4(3)$: Use 7. 5 ul of a 20 uM stock per ml of cells for a final concentration of 150 nM. The cells are incubated at 37°C for 30 minutes to load the dye. Prior to acquisition in the flow cytometer, add 1 ul of propidium iodide (PI; 10 mg/ml stock) for a final concentration of 10 ug/ml.

$DiOC_6$: Use 7. 5 ul of a 20 uM stock per ml of cells for a final concentration of 150 nM. The cells are incubated at 37°C for 30 minutes to load the dye. Prior to acquisition in the flow cytometer, add 1 ul of propidium iodide (PI; 10 mg/ml stock) for a final concentration of 10 ug/ml.

JC-1: 1 ul of a 10 mM stock for a final concentration of 10 uM. The cells are incubated at 37°C for 30 minutes to load the dye. No PI is added to this sample. We have found that adding the cells to the dye already in a tube allows for better loading of this dye.

Flow Cytometric Analysis:

Cells are examined by exciting the dyes/PI with a 488 nm laser. A standard optical set-up is used on FACSort.

$DiBAC_4(3)$ and $DiOC_6$ are detected using a 530 nm filter (FL-1) and PI in FL-2 or FL-3.

JC-1 monomers and aggregates are detected in FL-1 and FL-2, respectively.