PROTOCOL VIABILITY DYES & GATING

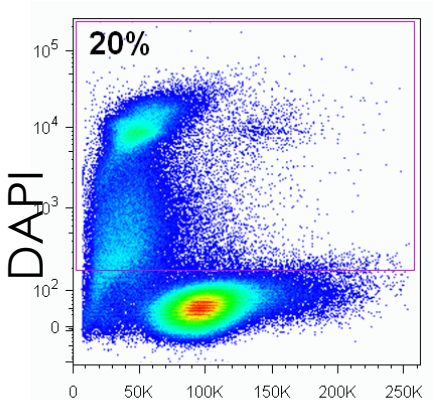
Aim: Accurate gating of live cells using viability dyes.

Dead or dying cells can cause false positive data if not excluded. Gating using an FSC v SSC plot is NOT an accurate way of doing this.

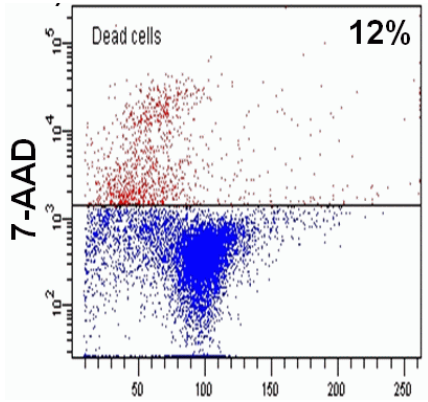
Resuspend cells in FACS buffer.
Add a viability dye to make a final concentration in the sample of:
A) 0.1 ug/ml **DAPI** or
B) 2.5 mg/ml **7- AAD** or
C) 3uM by adding 1ul of 0.3mM **DRAQ7** stock solution per 100ul cells or
D) 5 ug/ml **PI**
Collect events as soon as possible, using the required fluorescence channel, see below.
A). DAPI 440 nm channel fromViolet laser (405nm)
B). 7-AAD 670 nm channel from yellow/green laser (561nm)
C). DRAQ7 695 nm or 760 nm channel from red laser (640 nm).
D). PI 575 nm, 610 nm or 670 nm channels from yellow/green laser (561nm)

Instrument Set Up
Select viability dye for the Y axis and use FSc for the X axis. Populations of cells seen above the gate are dead or dying cells – select the negative population as viable. Use this as the first plot in your gating strategy.

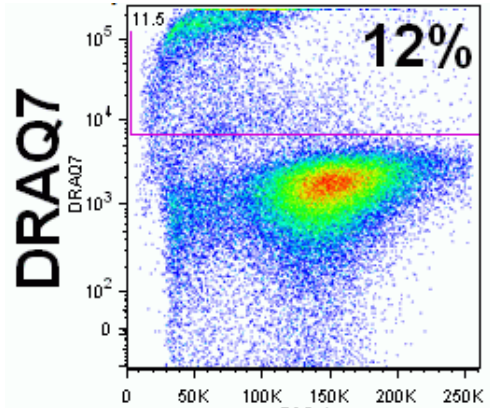
A) DAPI



B) 7 AAD



C). DRAQ7



D). Propidium Iodide (PI)