Labelling cells with CFSE (carboxyfluorescein diacetate succinimidyl ester)

Please note – this protocol has been optimised for certain conditions and is meant as a guide only, you may need to alter some parameters to suit your own experiment

NOTE: to succeed this protocol MUST be followed EXACTLY!

- Transfer the required number of cells to a sterile round-bottomed FACS tube (these tubes seem to result in less clumping than the conical 15ml tubes)
- Centrifuge and resuspend to 2 x 10^7 cells/ml in the appropriate staining buffer (see below).
  Note: if cells were originally in complete medium, the cells must be washed once in staining buffer to remove the large amounts of protein contained in complete medium.
- Dilute CFSE stocks (5 mM in DMSO) 1:20 in staining buffer
- Add 4 μl of diluted CFSE per 100 μl of cells then mix IMMEDIATELY and THOROUGHLY to ensure even staining (final concentration of CFSE is 10 μM).

1. Incubate at 37°C for 10 minutes *exactly*
2. Add 3 ml of complete medium, ie containing 10% FCS/huAB serum and incubate at room temperature for 5 minutes, to quench the CFSE
3. Centrifuge and repeat wash once more

Staining buffer:

Mouse cells: PBS + 0.1% FCS

Human cells: HBSS + 0.1% human AB serum

NOTES: It is important that cells have a high % viability, and are in suspension with no clumps, to ensure uniform labelling

CFSE is provided by Molecular Probes in powder form - it should be dissolved in high quality anhydrous DMSO to 5mM (2.79 mg/ml) and stored desiccated at -20°C in small aliquots. Each aliquot can be thawed and refrozen 3 times, then should be discarded. Correctly stored CFSE is stable for at least 6 months - some deterioration may occur after this time

A small amount of protein is included in the staining buffer to increase viability of the cells, but be careful not to include too much, as this will quench the CFSE, resulting in poor labelling

This protocol uses a final concentration of 10 μM CFSE, which seems to work well for human PBMC and the mouse primary cells. However, other concentrations (in the range 0.5 - 20 μM) may be more appropriate for some cells. High concentrations of CFSE can affect the function of the cells, although not necessarily kill them (so this cannot be detected with Trypan Blue); therefore, a titration of CFSE concentration in a functional assay (eg proliferation assay) is a good idea when trying this protocol on new cells