PROTOCOL 203_ CFSE LABELLING FOR T CELLS

Reagents

0.5mM CFSE in DMSO

Ice cold PBS

BSS

Waterbath set to 37°C and prewarmed

Smashing grids, tubes etc.

1. Smash spleens (or sorted $CD4^+$ T cells) to be labeled in BSS (or MEM) and clear the debris with a quick spin and then pellet the cells (1,500rpm, 8 mins).

2. Lysis step only required when labelling whole spleens. NOT required for sorted $CD4^+$ T cells.

3. Resuspend at $2x10^7$ cells/ml in PBS (about 3ml/spleen).

4. Add 1:1000 CFSE (Make up CFSE stock at 0.5mM in DMSO).

5. Transfer to a prewarmed waterbath at 37°C for exactly 10 minutes (or use 37C CO_2 incubator).

6. To stop/block CFSE incorporation into the membrane add fetal calf serum to a final concentration of 2%.

7. Spin 1,500rpm 8 minutes and wash in PBS.

8. Resuspend the labelled cells at the appropriate concentration.

Note:

This protocol was established for in vivo transfer of cells. Right after labelling these cells are usually much too bright for FACS analysis. After a few days in vitro restimulation and CFSE dilution the brightness should be OK for FACS.