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CFSE Staining Protocol for T-cells

Reagents

1. PBS (Sterile)
2. RPMI-1640 + 10% FCS (Complete with L-Glutamine, Pen-Strep and Na Pyruvate)
3. 1 mM stock of CFSE (Molecular Probes) in DMSO (Hybriboma Grade - Sigma)
   [Freeze this stock at -20^{0}C as 20µl-50µl aliquots in amber eppendorf tubes. Thawed 'in-use' aliquot can stay at 4^{0}C for a week]

Warm the sterile PBS and RPMI-1640 + 10% FCS (Complete) to 37^{0}C before use for all the procedures listed below.

CFSE Staining Procedure

1. Wash the cells twice with sterile PBS
2. Resuspend the cells in sterile PBS at 2 x 10^6 cells/ml in a 15ml or 50ml Polypropylene Falcon tube such that the cell suspension occupies less than ¼ the total volume of the tube
3. Add 5µl of CFSE from the stock to 1ml of cell suspension (5µM CFSE Final concentration in cell suspension)
4. Swirl the cell suspension to mix gently while adding the CFSE
5. Gently mix the cell suspension by swirling till the dense settled DMSO (CFSE) is no longer visible
6. Loosen the cap of the tube and stand it upright in a 37^{0}C CO_2 incubator for 20 minutes while swirling every 5 minutes
7. After incubation, top off the tube with RPMI-1640 + 10% FCS (Complete) and pellet the cells
8. Wash the cells 4 times with RPMI-1640 + 10% FCS (Complete)
9. Count the cells by trypan blue exclusion – Expect to loose about ¼ of the cells that you started with
10. Resuspend the cells in RPMI-1640 + 10% FCS (Complete) at 1 x 10^6 cells/ml and proceed with proliferation assays