

Cell Division Analysis Using CFSE (CD4/CD8)

Cells should be labeled with CFSE prior to plating in order to visualize cell division upon stimulation. Cells can be analyzed with or without counterstaining to delineate specialized groups of cells such as CD4 and CD8 T cells.

1. Incubate activated cells for 72 hours and then pool for flow cytometry.
2. Centrifuge samples at 1000rpm for 10 minutes and resuspend in appropriate amount of FACS buffer.
3. Add Fc Block (anti-CD16/32, BD Pharmingen) at a concentration of 0.8 μ g/mL and incubate the cells at 4°C for 5 minutes.
4. Add PE IgG2a and PE anti-CD4/CD8 at 0.8 μ g/mL to appropriate tubes and incubate at 4°C for 30 minutes with occasional mixing.
5. Wash cells twice with FACS buffer and resuspend in 400 μ L of FACS buffer for analysis.
6. Centrifuge cell at 4000rpm for 5 minutes.
7. Resuspend cells in FACS buffer (PBS).