Flow cytometry (human): Intracellular Cytokine Detection

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

* *This procedure has been optimised for analysis of cytokine production by human cells cultured in an MLR. If cells of interest have not been cultured in an MLR, they may need to be stimulated by alternative means, eg PMA + ionomycin*

1. Add 10 μl of Brefeldin stock solution per 2 ml of cells. Final concentration = 5 μg/ml
2. Return to 37°C incubator for 4 hours
3. Harvest cells by pipetting approx 10 times in the well
4. Top up tube with PBS/AB/az and centrifuge; resuspend in PBS/AB/az to approx 4 x 10^6 viable cells/ml
5. Perform surface labelling as usual: block Fc receptors using human IgG, add antibodies, wash
6. Add 500 μl of paraformaldehyde (1% in PBS) and incubate at 4°C for at least 1 hour.

* If required (for convenience), the cells may be fixed overnight and cytokine labelling performed the next day. However, minimise the time that the cells are in fixative by adding the paraformaldehyde as late as possible on Day 1 and washing the cells as early as possible on Day 2.

7. Add 3 ml ice-cold PBS/AB/az, spin down, tip off supernatant
8. Add 250 μl of saponin buffer to each tube to permeabilise the cell membranes (saponin buffer = PBS/AB/az + 0.1% saponin). Incubate in the dark for 12 minutes at 4°C, mixing half-way through incubation
9. Add 3 ml ice-cold PBS/AB/az, spin down, tip off supernatant and resuspend cells in volume remaining in bottom of tube
10. To block any proteins within the cell capable of binding mouse immunoglobulin, add 10 μg of purified murine IgG per tube (eg 8 μl of 1.5 mg/ml stocks) and incubate at room temperature for 30 minutes
11. Add fluorochrome-labelled anti-cytokine antibodies and incubate at room temperature in the dark for 30 minutes
12. Add 3 ml ice-cold PBS/AB/az, spin down, tip off supernatant
13. Add 3 ml ice-cold PBS/az (no protein), spin down, tip off supernatant
14. Resuspend in 200 - 500 μl of 1% paraformaldehyde and store at 4°C in the dark until analysis

* Brefeldin stock solution: 1 mg/ml in ethanol; store at -70°C and protect from light at all times. The solution is very unstable, so return to -70°C as soon as possible and discard stocks after a few months.