Flow Cytometry (murine): Polyclonal Antibody

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

1. Wash cells in PBS and resuspend to approx 4 x 10^6 viable cells/ml in PBS/BSA/azide
2. Add 5 μl of murine gamma-globulin (at 10 mg/ml) per 10^6 cells, to block Fc receptors.
3. Incubate at room temperature for at least 20 minutes (during incubation, prepare secondary antibody - see Step 8). There is no need to wash out the gamma-globulin.
4. Aliquot 50 μl of Fc-blocked cells into FACS tube (Falcon 2008)
5. Dilute primary antibody (purified IgG) to an appropriate concentration in PBS/BSA/azide. This will need to be determined by titration.
6. Add 10 μl of primary antibody to cells, pipette up and down to mix, and incubate at room temperature for 30 minutes (during incubation, set centrifuge to cool to 4°C)
7. Wash with 3 ml ice-cold PBS/BSA/azide
8. Add 50 μl of secondary antibody (eg anti-IgG (or anti-IgM) conjugated to a fluorochrome or biotin) to cells. *Secondary antibody should be diluted to an appropriate concentration in PBS/BSA/azide To block non-specific binding, add 2% normal mouse serum and 100 μg/ml mouse gamma-globulin and pre-adsorb for 30 minutes prior to use.*
9. Incubate on ice for 30 minutes.
10. Wash with 3 ml ice-cold PBS/BSA/azide
11. Dilute streptavidin-conjugate to appropriate concentration in PBS/BSA/azide and add 50 μl to cells. Incubate on ice for 30 minutes
12. Wash with 3 ml ice-cold PBS/BSA/azide then with 3 ml ice-cold PBS/azide (no BSA)
13. After final spin, tip off the supernatant, vortex, add 200 – 500 μl of 1% paraformaldehyde and vortex briefly again
14. Store in dark at 4°C until analysis (up to 2 weeks)
15. Note: PBS/BSA/azide = PBS containing 1% BSA and 0.04% sodium azide

Suggested controls*:

1. Cells only
2. Isotype-matched negative control antibodies for each fluorochrome, or FMO controls
3. Compensation controls (ie, a brightly-staining antibody by itself) for each fluorochrome

***NOTE:** See Recommended Controls document for comprehensive details