Flow Cytometry (murine): Direct Protocol

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 30 minutes, then place on ice. There is no need to wash out the gamma-globulin.
4. Aliquot 50 μl of Fc-blocked cells into ice-cold FACS tube (Falcon 2008)
5. Add 10 μl of each primary antibody and mix. For all primary antibodies the optimal concentration should be determined by titration. Each antibody must be directly labelled with a fluorochrome or biotin, and all antibodies that are to be used together in one tube must be labelled with *different* conjugates.
6. Incubate on ice 30 minutes *(during incubation, set centrifuge to cool to 4°C)*
7. Add 3 ml PBS/BSA/azide, spin down, tip off supernatant
8. If one antibody is biotinylated:
   - dilute streptavidin-conjugate to a suitable concentration in PBS/BSA/azide
   - add 50 μl to cells.
   - incubate on ice for 30 minutes.
9. Wash with 3 ml ice-cold PBS/BSA/azide.
10. Wash all tubes with 3ml PBS/azide (NO BSA).
11. Resuspend in 200 – 500 μl paraformaldehyde (depending on number of cells) to fix
12. Store at 4°C in the dark until analysis (up to 1 week).

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