Labelling Human Cells for Flow Cytometry: 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 at least once and resuspend to approx 4 x 10^6 viable cells/ml in staining buffer.
2. Add purified human IgG to block Fc receptors. Use 5 μl of 10 mg/ml stock per million cells. Incubate at room temperature for 30 minutes
3. Aliquot 50 μl of Fc-blocked cell suspension into FACS tubes (Falcon 2008)
4. 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.
5. Incubate at room temperature for 30 minutes *(during incubation, set centrifuge to cool to 4°C and dilute streptavidin conjugate if required... see Step 7)*
6. Add 3 ml of ice-cold staining buffer, spin down, tip off supernatant *(allow to drain briefly after tipping off, and be consistent between tubes, as different amounts of liquid left in bottom of tube may affect subsequent steps)*
7. If biotinylated antibodies are used, then follow these steps. If all antibodies are directly labelled with fluorochromes, go straight to Step 13.
8. Dilute streptavidin-fluorochrome conjugate to recommended concentration in staining buffer
9. If using a cyanine-containing dye such as PECy5, add human IgG to 1% and allow to pre-adsorb for at least 20 minutes at room temperature
10. Add 50 μl of streptavidin conjugate to all tubes containing biotinylated mAb. Vortex for 3 seconds
11. Incubate on ice 30 minutes
12. Add 3 ml of ice-cold PBS/azide (only to those tubes that received the conjugate), spin down, tip off supernatant, vortex briefly.
13. Add 3 ml ice-cold PBS/azide to ALL tubes. Spin down, tip off supernatant
14. Resuspend in 200 – 500 μl 1% paraformaldehyde (depending on number of cells) to fix
15. Store at 4°C in the dark until analysis

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