Labelling Human Cells for Flow Cytometry: Indirect Protocol (1-3 colour)

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 unlabelled primary antibody and mix. For all primary antibodies the optimal concentration should be determined by titration. If also using a directly-labelled antibody/s (ie multicolour staining), **DO NOT** add at this step.
5. Incubate at room temperature for 30 minutes *(during incubation, set centrifuge to cool to 4°C and dilute secondary antibody - see Step 7. Also dilute streptavidin conjugate, if required - see Step 10)*
6. Add 3 ml 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. Dilute secondary antibody (eg anti-IgG (or anti-IgM) conjugated to a fluorochrome or biotin) to appropriate dilution in staining buffer, and add 1% human IgG. Allow to pre-adsorb for at least 20 minutes at room temperature.
8. Add 50 μl of pre-diluted secondary antibody to tubes and vortex for 3 seconds. Incubate on ice 30 minutes
9. Add 3 ml ice-cold staining buffer, spin down, tip off supernatant, vortex for 3 seconds
10. If doing multicolour labelling:

- add 10 μl of purified IgG corresponding to the host of the primary antibody at 2 mg/ml per tube (this is CRITICAL to block free binding sites on the secondary)
- incubate for 20 minutes at room temperature
- add directly labelled primary antibody/s (10 μl at 50 μg/ml)
- if secondary antibody is biotinylated instead of directly labelled, then also add 50 μl of streptavidin-fluorochrome conjugate (diluted appropriately, with 1% huIgG if appropriate)
- incubate at room temperature for 30 minutes
- add 3 ml ice-cold staining buffer, spin down, tip off supernatant, vortex for 3 seconds

11. Add 3 ml ice-cold PBS/azide (NO serum) to ALL tubes. Spin down, tip off supernatant

    Resuspend in 200 – 500 μl 1% paraformaldehyde and 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