

## Indirect Flow Cytometry (FACS) protocol

- 1/ Add 50 µl of EDTA treated blood or cell suspension (1.10<sup>6</sup> cells) to a reagent tube.
- 2/ Add 10 µL of primary purified mAb tested or the isotype-matched control mAb.
- 3/ Vortex the tube and incubate for 15 min at room temperature in the dark.
- 4/ For whole blood, add 2 ml of lysing solution, incubate 10 min at room temperature in the dark.
- 5/ Wash twice with PBS containing 1% BSA and 0.1% NaN<sub>3</sub>. Remove supernatant and gently vortex the cell pellet.
- 6/ Dilute the fluorochrome conjugated secondary antibody at the optimal dilution (see manufacturer's instructions) and add to the cells.
- 7/ Vortex the tube and incubate for 15 min at room temperature in the dark.
- 8/ Wash twice with PBS containing 1% BSA and 0.1% NaN<sub>3</sub>. Remove supernatant.
- 9/ Resuspend cells in 200 µl of PBS, or 250 µl of 1% paraformaldehyde if required.
- 10/ Analyse by flow cytometry.