Indirect Flow Cytometry protocol

1. Add 50 µl of EDTA treated blood or cell suspension (1.10^6 cells) in a reagent tube.
2. Add 10 µL of the primary purified mAb tested or the isotype-matched control mAb.
3. Vortex the tube and incubate: for blood 30 mins at room temperature in the dark and for cells 30 mins at 4°C.
4. For whole blood, add 2.5 ml of lysing solution, incubate 10 min at room temperature in the dark.
5. Wash twice with PBS containing 1% BSA. 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 blood 30 mins at room temperature in the dark and for cells 30 mins at 4°C.
8. Wash once with PBS containing 1% BSA. Remove supernatant.
9. Resuspend cells in 200 µl of PBS or 250 µl of PBS 1% paraformaldehyde if required.
10. Analyse by flow cytometry.

DIACLONE SAS - 6 Rue Docteur Jean-François-Xavier Girod - BP 1985- 25020 BESANCON Cedex - France
Tel: +33(0)3.81.41.38.38 - Fax: +33(0)3.81.41.36.36
info@diaclone.com - www.diaclone.com