Procedure

1. Add 0.5 x 10^6 - 1 x 10^6 cells to polystyrene tubes.
2. Spin down cells at 300 x g for 10 minutes at 4°C.
3. Remove supernatant.
4. Resuspend cell pellet in 50-100 µl of staining buffer containing conjugated monoclonal antibodies each at 1µg x 10^6 cells (or use dilution determined by titration for optimal staining). If using biotin conjugate, always stain with this antibody first and by itself.
5. Incubate on ice 20-30 minutes (Keep away from the light).
6. Wash once by adding 1ml 1X PBS / 1% FCS
7. Spin down cells at 300 x g for 10 minutes at 4°C.
8. If using biotin conjugate, resuspend cell pellet in 50µl of staining buffer (1X PBS / 1% FCS) containing streptavidin conjugated dye plus other directly conjugated antibodies, repeat steps 6 through 8.
9. Resuspend cell pellet in Hank's Buffered saline, PBS or fixative (analyze unfixed cells on the same day). Adjust concentration of cell suspension to at least 10^6 cells/ml
10. add Propidium Iodide or other available viability dye to unfixed stained cells to discriminate live from dead cells
11. An unstained sample or negative Ig control and single stained samples for each conjugated dye are also necessary for setting up the flow cytometer.

Reagents

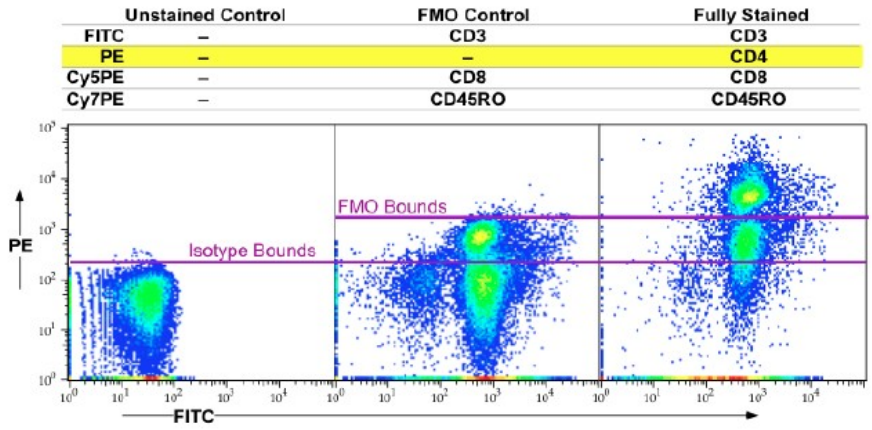
Staining buffer

1X PBS / 1% FCS or 1%BSA

Controls that are necessary:

a. Unstained cells: show the background or autofluorescence of the system.
b. Isotype control: to identify non-specific binding. Isotype controls are good staining controls to identify potential problems in staining, particularly if a primary and secondary antibody are used.
c. Controls for indirect staining are represented by a sample incubated with secondary reagent only and a sample incubated with non-specific primary antibody followed by secondary reagent, ideally of the same isotype as the specific antibody you will be using.
d. Negative and Positive controls for each different primary antibody and each different cell population used in your experiment are strongly recommended, when possible.
e. FMO (Fluorescence Minus One) control: the cells were stained with all reagents except one. FMO control is essential when antigen expression is low or variable.

   The following figure shows how a gate on an unstained or isotype would be set incorrectly as compared to an FMO control. Furthermore, the second panel demonstrates how FMO controls can resolve gating boundaries even with improper compensation:



References

https://www.amrepflow.org.au/public/documents/FCMcontrolsJTrotter2006.pdf