Protocol: Antibody Labeling with Indirect Fluorochrome Conjugation

1. Prepare a single cell suspension from cell source (culture, tissue, etc.).
2. Ascertain cell viability using Trypan Blue dye exclusion.*
3. Adjust the cell concentration to $10x10^6$ cells/ml (this is maximum concentration, can be less) in 1X PBS containing blocking serum** or 2% BSA.
4. Aliquot $1x10^6$ cells into each labeled tube (100ul of cell suspension).
5. Add primary antibody to cells, vortex.
   Note: *Amount of antibody added will depend on manufacturer instructions and/or titration results.*
6. Incubate antibody/cell suspension in the dark for 30 minutes at 4°C.
7. Following incubation, wash cells twice in 1 ml of PBS wash solution^{++} to remove excess antibody (add 1 ml wash solution, vortex, spin at 1500RPM for five minutes, aspirate supernatant, vortex in residual fluid).
8. Add appropriate volume of secondary antibody; incubate for 30 min. in the dark, at 4°C.
9. Wash cells twice in 1ml of PBS wash solution as in step 7.
10. Resuspend cells in 0.5ml of 0.5% paraformaldehyde and vortex. (Optional: Instead of paraformaldehyde, resuspend in 0.5ml PBS and acquire on flow cytometer within one hour).
11. Store samples at 4°C until flow cytometric acquisition/analysis.

* Viability can be checked using various dyes on the flow cytometer if no fixative is added at the end of the procedure–see discussion under "*Viability Assessment"* in *"Technical Considerations".*

** See discussion re: use of blocking serum under *"Technical Considerations"* in this section. For indirect labels, blocking serum should be from the species of the secondary antibody.

$^{++}$1X PBS w/2% FCS and 0.1% Sodium Azide.

Reference:

Stewart CC, Stewart SJ: Immunophenotyping. In: *Current Protocols in Cytometry,* Robinson JP, Managing Editor, Unit 6.2, 2001.