Protocol: Antibody Labeling with Direct Fluorochrome Conjugate

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 directly conjugated 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, repeat).
8. Resuspend suspension 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).
9. 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.
†† 1X PBS w/2% FCS and 0.1% Sodium Azide.

Reference: