Materials

- The fluorochrome-conjugated antibody or antibodies of interest.
- An appropriate DNA binding dye. For single phenotype analysis, **propidium iodide** can be used simultaneously with FITC-conjugated antibodies for analysis on a single laser flow cytometer. Analysis of two phenotypes on a single laser instrument can be accomplished using FITC- and PE-conjugated antibodies and the far red DNA binding dyes **7-aminoactinomycin D** or **LDS-751**. **Hoechst 33342** and **33258** or **DAPI** can be used on dual laser (488 nm/UV) instruments for simultaneous analysis with two or three fluorochrome-conjugated antibodies. RNase should be added for DNA dyes that can bind dsRNA (such as propidium iodide).
- 70% EtOH in H_2O
- 50% fetal bovine serum (FBS) in PBS with 0.1% sodium azide
- 70% EtOH in glycerol
- Staining buffer (2% PBS in PBS with 0.1% sodium azide)

Procedure

1. Prepare the cell type of interest as a single cell suspension and wash once with cold PBS with 0.1% sodium azide. Label with the fluorochrome-conjugated antibody of interest in cold staining buffer.

2. Wash cells once with staining buffer and once with PBS/azide alone. Decant the supernatant, shake tube gently to resuspend pellet and add 1 part (i.e. 0.3 ml) 50% FBS in PBS. While gently mixing, add 3 parts (i.e. 0.9 ml) cold 70% EtOH in H_2O drop-wise. Some precipitation of the PBS proteins will occur during addition of ethanol; this can be disregarded, as these precipitates will be removed during subsequent washes. Incubate for at least two hours or overnight at 4°C.

3. Wash the cells twice with cold PBS/azide to remove EtOH and precipitated protein and add propidium iodide at 50 mg/ml in PBS with 100 U/ml DNase-free RNase. Other DNA content dyes can be substituted for propidium iodide at this point. 7-aminoactinomycin D can be used at concentrations between 10 and 25 mg/ml, and Hoechst 33342 and 3258 and DAPI at 1 to 2 mg/ml, for example. Analyze using the appropriate instrument.

4. Preliminary evidence suggests that 70% EtOH in glycerol can be substituted for 50% PBS followed by 70% EtOH addition with similar preservation of surface markers.