Basic Antibody Titration Protocol

Reagents and Materials

- Cells to be stained
- FACS buffer – can be cell dependent
  - o 1x Ca/Mg2+ free PBS
  - o Either FBS (1-10%) or BSA (0.1-1%)
  - o EDTA (0.5-5mM) if you have very sticky cells
  - o Commonly used: 1% FBS in 1X PBS (consider filtering FBS to remove debris)
- Antibodies
- Eppendorf or FACS tubes

Procedure

1. Prepare single cell suspension (lyse RBCs if needed)
2. Count cells
3. Adjust cell concentration to 1-5 million cells per 50 µL FACS buffer

a. Try to stain the same number of cells you plan to use for your final experiment

4. Add Fc block to the pooled cells following the manufacturers protocol
   a. Usually 1-5 µl per 100 µl at 4°C for 10-15 minutes or more
5. Make 4 tubes per antibody (1.5 ml Eppendorf or 5 ml FACS tubes) serial dilutions
   a. Tube 1 = 0.3 µg dilution 1/50
   b. Tube 2 = 0.1 µg dilution 1/100
   c. Tube 3 = 0.03 µg dilution 1/400
   d. Tube 4 = 0.01 µg dilution 1/800
   e. You may choose to go higher (3 µg, 1 µg) or lower (0.003 µg, 0.001 µg) depending on the antibody dilution 1/1600 if necessary
   f. Beware: some antibodies are 0.5 mg/ml and others are 0.2 mg/ml. Brillant violet antibodies tend to be odd concentrations. Make sure you check the concentration!
   g. If it is a small population, you may need other antibodies to gate the population
   h. You should have a population with both a positive peak and a negative peak
   i. Other cells can be used if you are struggling to find a clear positive and negative population, but it is ideal to titrate on your specific cells of interest
6. Pipette antibodies to have the appropriate µg in 50 µl FACS buffer
   a. Prepare these either in advance (antibodies can be saved in FACS buffer for several weeks) or while cells are in Fc block
   b. **IMPORTANT**: vortex and quickly spin down the antibody tubes before pipetting to avoid unusual highly fluorescent debris during acquisition on the cytometer
7. After the Fc block incubation, pipette 50 µL of cells into antibody-containing tubes for staining
   a 50 µl of cells + 50 µl antibody mixture = 100 µl total volume
8. Incubate for 30 minutes in the dark at 4°C (cover tubes in alu foil if needed)
9. Wash cells with FACS buffer
   a. 1 ml if staining in Eppendorf tubes
   b. 2 ml if staining in flow tubes
10. Centrifuge
11. Aspirate, pipette, or dump tubes to remove supernatant
    a. Be careful not to disturb the pellet!
12. Resuspend in FACS buffer
    a. Volume depends on the number of cells
    b. At least 350 µl for acquisition on the Fortessa or Symphony