Basic Titration Staining Protocol

Written by: Laura Johnston
Date: 1/3/18

Materials

- Cells to be stained
- FACS buffer – can be cell dependent
    - 1X Ca/Mg2+ free PBS
    - Either FBS (1-10%) or BSA (0.1-1%)
    - EDTA (0.5-5mM) if you have very sticky cells
    - Commonly used: 1% FBS in 1X PBS (consider filtering FBS to remove debris)
- Antibodies
- Eppendorf or flow 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 on using 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 (1.7 mL Eppendorf or 5 mL flow tubes)
    a. Tube 1 = 0.3 μg
    b. Tube 2 = 0.1 μg
    c. Tube 3 = 0.03 μg
    d. Tube 4 = 0.01 μg
    e. You may choose to go higher (3 μg, 1 μg) or lower (0.003 μg, 0.001 μg) depending on the antibody
    f. Beware: some antibodies are 0.5 mg/mL and others are 0.2 mg/mL. Brilliant 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 so that you 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 your cells are in Fc block
    b. **IMPORTANT**: vortex and quickly spin down the antibody tubes before pipetting, or you may detect unusual highly fluorescent debris on the cytometer
7. Once you have completed 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 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 to not disturb the pellet!
12. Resuspend in FACS buffer
    a. Volume depends on the number of cells
    b. No less than 350 μL