Basic Flow Cytometry Staining Protocol
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Date: 12/20/17

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
- Live/dead stain

Procedure
1. Prepare single cell suspension
2. Lyse RBCs (optional)
3. Count cells
4. Adjust cell concentration to 1-5 million cells per 50 µL FACS buffer
5. Pipette 50 µL cells into Eppendorf or flow tubes for staining
   a. Need one tube per sample
6. Pool remaining cells for unstained and FMO controls
   a. If you have 10 colors, make sure you have 10 x 50 = 500 µL pooled cells
   b. Ideally keep concentration the same as sample concentration, but you could use less cells in the FMOs if you really can’t avoid it. Could also eliminate some FMOs that have clear positive populations.
7. Add Fc block
   a. 1 µL per sample, but it’s easier to make a 1:10 dilution in FACS buffer and add 10 µL to each sample
   b. Don’t forget to add Fc block to pooled samples! For 500 µL pooled cells, add 10 µL undiluted Fc block
8. Make sure samples are mixed by gently flicking the tube or pipetting up and down
9. Incubate at 4°C for 10-15 minutes
10. Add antibody – 50 µL per tube
    a. For samples: make master mix of all antibodies so that appropriate amount is in 50 µL (when added to cells, final staining volume is 100-110 µL, calculate for 100µL final volume)
    b. For FMOs: combine 50 µL FMO antibody mix with 50 µL pooled cells
11. Incubate for 30 minutes in the dark at 4°C (cover tubes in foil if needed)
12. Wash cells with FACS buffer
    a. 1 mL if staining in Eppendorf tubes
    b. 2 mL if staining in flow tubes
13. Centrifuge
14. Aspirate, pipette, or dump tubes to remove supernatant
    a. Be careful to not disturb the pellet!
15. Resuspend in FACS buffer
    a. Volume depends on the number of cells
    b. No less than 350 µL
16. A few minutes before running samples, add live/dead stain (see manufacturers protocol)