Basic Flow Cytometry Staining Protocol with FMOs

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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%)
    - *Optional:* 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 (this protocol is for unfixable stains such as DAPI, PI, and the Sytox dyes)
- 5 mL flow tubes: Falcon #352008

Procedure

1. Prepare single cell suspension
2. lyse RBCs (optional)
3. Count cells
4. Adjust cell concentration to 0.1-5 million cells per 50 μL FACS buffer
    a. Number of cells needed depends on your experiment. General starting point is often 1 million cells.
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. 1-5 μL per sample following manufacturer's protocol
        i. Anytime you need to pipette 1 μL 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!
8. Make sure samples are mixed by gently vortexing or pipetting up and down
9. Incubate at 4°C for 10-15 minutes
10. Add antibody cocktail – 50 μL total volume per tube
    a. For example if you are adding 2 μL of antibody per tube, you would first combine 2 μL antibody and 48 μL FACS buffer, then add the 50 μL of antibody cocktail to the 50 μL of cells for a total staining volume of 100 μL
    b. For samples: make master mix (example below of 10X 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)
        i.

	1X	10X
FITC	1	10
APC	5	50
PE	2	20
FACS Buffer	42	420
TOTAL	50	500

        ii. Always add an extra 1-2 to your master mix to account for pipetting error, so a 10x master mix will be good for 8-9 samples
    c. 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. If cells were stained in Eppendorf tubes, transfer to 5 mL FACS tubes
17. A few minutes before running samples on the cytometer, add live/dead stain (see manufacturers protocol)