Protocol: Flow Cytometric Staining with Cell Surface Abs

Steps:

1. Sample collection and dispensing:
    a. Whole blood: dispense 50-200 μL per sample.
    b. Fresh PBMC: resuspend at 5-10 x 10^6 viable lymphocytes per mL in wash buffer or cRPMI, dispense 50-200 μL per sample.
    c. Cryopreserved PBMC: Follow PBMC Cryopreservation protocol, resuspend at 5-10 x 10^6 viable lymphocytes per mL in warm cRPMI, dispense 50-200 μL per sample.

2. Staining:
    1. Add appropriate titers of all antibodies, incubate for 30-60 m at room temperature in the dark. If possible, vortex or otherwise agitate occasionally during staining.

3. Washing:
    1. Whole blood: add appropriate volume of an erythrocyte lysing buffer (e.g., BD PharmLyse or BD FACS Lysing Solution). Follow manufacturer's directions for incubation and washing.
    2. PBMC: add wash buffer to at least 200 μL, centrifuge at 250 x G for 5 m at room temperature. Repeat wash a second time for optimal results.

4. Final fixation (optional):
    a. For assays with Cy7 tandems, but NOT AmCyan: Resuspend in at least 200 μL of BD Stabilizing Fixative and store at 4C in the dark. Acquire within 24 h if possible.
    b. For other assays: Resuspend in at least 200 μL of 0.5-2% paraformaldehyde in PBS and store at 4C in the dark. Acquire within 24 h if possible.

Notes:

1. Staining can be done in 5-mL FACS tubes, or various types of 96-well conical or round-bottom plates.

2. Antibodies should be titered for the intended application (sample type, surface vs intracellular staining, number of cells, staining vessel/volume, time, and temperature). Choose the titer point that maximizes signal:noise.

3. It can be convenient to create a master mix of all antibodies in wash buffer so that, for example, 50 μL are added to each 50 μL cell sample (for a total staining volume of 100 μL).

4. Most antibodies work well when stained for 30 m at room temperature. Longer times (up to 60 m) can improve weak staining, as can agitation during Ab incubation. Occasional Abs may work better when stained on ice.

5. After fixation, cells become more bouyant, and need to be centrifuged at higher G force (500 x G).

6. Fixation of stained samples can degrade staining of some sensitive markers. Resuspending in wash buffer is preferable, even if cells will be stored overnight prior to acquisition (keep dark at 4C).

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Stock Solution Recommendations:

Solution	**Stock Concentration**	**Intermediate Dilution**	**Final Concentration**
Wash Buffer	0.5% BSA+0.1% NaN_3 in PBS	None	Use neat
Final fixative	0.5% paraformaldehyde in PBS	None	Use neat