Protocol: Cell Surface FACS

Application:
A method to detect cell surface proteins using flow cytometry.

Procedure:
1. Harvest and process organs or cells of interest per usual protocol and count total cells.
   Ratio of cells to Trypan Blue (TB) for counting:
   - Bone marrow is 1:1 => i.e. 50 µL cells to 50 µL TB
   - Spleen is 1:5 => i.e. 20 µL cells to 100 µL TB
   - Thymus is 1:5 => i.e. 20 µL cells to 100 µL TB
   - Testis is 1:1 => i.e. 50 µL cells to 50 µL TB

2. Spin at 4 °C, 600 x g for 5 minutes (min) and resuspend cells at 20 million (M) cells/mL in FACS buffer (1x PBS + 0.1% BSA).
   Note: Unless otherwise specified, all centrifugation steps performed on a benchtop centrifuge.

3. Mix antibody cocktails in 1x PBS in tissue culture (TC) hood (sterile) without light.

4. Aliquot cells (usually 4-5M cells, 200-250 µL) into 5 mL FACS tubes.

5. Stain with appropriate antibody cocktail for 30 min on ice in the dark.

6. Add 3 mL of FACS buffer to each tube and spin at 4 °C, 600 x g for 5 min.
   Note: Repeat steps 4-5 if performing a multi-step stain (example: Biotin + Streptavidin).

7. Resuspend cells in 250 µL of 1x PBS + 0.4% BSA + 2 mM EDTA.
   Note: if clumping is expected or visible:
   - Strain cells before flow cytometric analysis.

8. Keep cells on ice and in the dark until analysis on flow cytometer.