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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.

2. Spin at 4 °C, 1600 revolutions per minute (rpm) 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. Aliquot cells (usually 4-5M cells, 200-250 μL) into 5 mL FACS tubes.

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

5. Add 3 mL of FACS buffer to each tube and spin at 4 °C, 1600 rpm for 5 min.
   Note: Repeat steps 4-5 if performing a multi-step stain (example: Biotin + Streptavidin).

6. Resuspend cells in 250 µL of FACS buffer.
   Note: If clumping is expected or visible:
   - Strain cells before flow cytometric analysis or
   - Add EDTA to FACS buffer at a final concentration of 2 mM.

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