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Flow Cytometry (FCM)

I. Preparation of Solutions and Reagents

1. **PBS Buffer:** Prepared using PBS powder and ddH_2O. Cool to 4°C before use.
2. **Fixative (optional):** 4% Paraformaldehyde.
3. **Permeabilization Solution (optional):** 0.1% Triton X-100, prepared with Triton X-100 and PBS buffer. Store at 4°C.
4. **Blocking Solution:** 5% BSA, prepared with BSA and PBS buffer. Prepare fresh before use and store at 4°C.

II. Experimental Procedure

A. Sample Preparation

Cell Collection

(1) Suspension cells: Centrifuge the cell culture medium directly and collect the cells.

(2) Adherent cells: Discard the medium, wash once with PBS, digest the cells with trypsin, and collect the cells.

(3) Tissue samples: Mince the tissue and digest with collagenase/trypsin, filter through a cell strainer to remove tissue clumps, and collect the cells.

Cell Counting and Suspension Preparation

(1) Resuspend cells in 1 mL PBS, centrifuge at 1500 rpm for 5 minutes, discard the supernatant, and repeat the wash step 1–2 times.

(2) Take a small amount of cell suspension, stain with trypan blue for viability assessment, and adjust the cell concentration to 1×10^6–5×10^6 cells/mL.

B. Cell Surface Antigen Staining (Permeabilization Not Required)

1. Blocking: Resuspend 1×10^6 cells in 300 µL of blocking buffer. Incubate at room temperature for 15 minutes and protect from light. Centrifuge at 1500 rpm for 5 minutes, discard the supernatant, and wash the cells once with PBS buffer.
2. Antibody Incubation: Add an appropriate amount of fluorophore-conjugated antibody and mix gently. Incubate at 4°C for 20–30 minutes in the dark. (Refer to the product website for recommended antibody dilutions.)
3. Washing: Add 1 mL of PBS, centrifuge at 1500 rpm for 5 minutes, discard the supernatant, and repeat once.
4. Resuspension: Resuspend the cells in 500 µL of PBS buffer. Filter through a 35–40 µm cell strainer to remove aggregates, then transfer the cells to a flow cytometry tube.

C. Intracellular Antigen Staining (Membrane Permeabilization Required)

1. Fixation: Take 1×10^6 cells and add 1 mL of 4% paraformaldehyde. Incubate at room temperature in the dark for 15-20 minutes. Centrifuge at 1500 rpm for 5 minutes, discard the supernatant, and wash the cells twice with PBS buffer.
2. Permeabilization: Add 500 µL of 0.1% Triton X-100, vortex gently to mix, and incubate on ice in the dark for 10-20 minutes. Centrifuge at 1500 rpm for 5 minutes, discard the supernatant, and wash the cells once with PBS buffer containing 0.1% Triton X-100.
3. Blocking: Resuspend the cells in 300 µL of blocking buffer and incubate at room temperature in the dark for 20 minutes. Centrifuge at 1500 rpm for 5 minutes, discard the supernatant, and wash the cells once with PBS buffer containing 0.1% Triton X-100.
4. Antibody Incubation: Add an appropriate amount of fluorescently labeled antibody, gently flick to mix, and incubate at 4 °C in the dark for 30-60 minutes (refer to our website for antibody dilution).
5. Washing: Resuspend the cells in 1 mL of PBS buffer containing 0.1% Triton X-100, centrifuge at 1500 rpm for 5 minutes, and discard the supernatant. Repeat the wash once with PBS buffer containing 0.1% Triton X-100, followed by one final wash with PBS buffer.

6. Resuspension: Resuspend the cells in 500 µL of PBS buffer. Filter through a 35–40 µm cell strainer to remove cell clumps, and transfer the cell suspension to a flow cytometry tube.

D. Flow Cytometry Analysis

Set the parameters for the flow cytometer and perform the analysis.

III. Experimental Notes

1. When using fluorescently labeled primary antibodies, secondary antibodies are not required. However, if the primary antibody is unlabeled, a fluorescently labeled secondary antibody (specific to the host species of the primary antibody) must be used.
2. For cell permeabilization, either 0.1% Triton X-100 or 90% methanol can be used. Triton X-100 is suitable for most intracellular antigens, while methanol provides more thorough permeabilization but may disrupt certain antigen conformations. It is recommended to perform a preliminary experiment to optimize conditions.

IV. Recommended Experimental Products

1. Fluorescently Labeled Antibodies for Flow Cytometry:
https://www.targetmol.com/antibodies/flow_cytometry_antibodies
Bovine Serum Albumin (BSA), T5664
Triton X-100, T64297