Indirect Flow Cytometry Protocol

Statement: The flow cytometry protocol provided is for reference only. A more suitable protocol should be designed based on the actual experimental context.

Materials Required

a. Samples: Cell lines or single-cell suspensions from tissues.

b. Antibodies: Un-conjugated primary antibodies, fluorochrome-conjugated secondary antibodies (e.g., FITC, PE, APC), and isotype control antibodies.

c. Buffers:

- Staining buffer (Pre-cooled PBS + 1% BSA or 2% FBS).
- Fixation buffer (1-4% paraformaldehyde or commercially available fixation reagents).
- Permeabilization buffer (e.g., 0.1% Triton X-100 or commercial kits for intracellular staining).

d. Other reagents: Fc receptor blocking solution (e.g., anti-CD16/32), viability dye (e.g., DAPI, 7-AAD).

e. Equipment: Flow cytometer, centrifuge, refrigerator.

f. Other materials: Centrifuge tubes/flow cytometry tubes, ice, pipettes.

Procedure

1. Planning Your Experiment

a. Determine the specific cell populations and markers of interest.

b. Select appropriate fluorochrome-conjugated antibodies that match with your flow cytometer's laser and filter setup.

2. Cell Preparation

a. Collect cells from culture or tissue as needed.

- If isolating the cells from tissues, use an appropriate method such as enzymatic digestion and mechanical disruption.
- Suspension cells: Collect the cell suspension into the centrifuge tube, centrifuge at 300 × g for 5 minutes, discard the supernatant and resuspend in staining buffer (remove the culture medium).
- Adherent cells: Gently detach the cells from the culture flask or dish (trypsin-free cell dissociation solution is recommended), collect the cell suspension into the centrifuge tube, centrifuge at 300 × g for 5 minutes, discard the supernatant, and resuspend in staining buffer (remove the culture medium).

Note: Please process the sample into a single-cell suspension.

b. Wash the cells with staining buffer, centrifuge at 300 × g for 5 minutes, and discard the supernatant.

c. Count cell density and viability (with viability guaranteed to be at least 90%).

d. Adjust cell density to 1 x 10^7 cells/mL in staining buffer.

e. Transfer 100 µL of cell suspension (approximately 1 x 10^6 cells) into flow cytometry tubes.

3. Fc Receptor Blocking (Optional)

If the samples you are testing are cells with high FcR expression, please proceed with this step.

a. Add Fc blocking reagent to the cell suspension.

b. Incubate 10-15 minutes at 4°C.

4. Cell Surface Staining

a. Add the appropriate amount of the primary antibody to each tube according to the manufacturer's instructions. Mix gently.

b. Incubate for 30-60 minutes at 4°C in the dark.

c. Wash the cells with staining buffer, centrifuge at 300 × g for 5 minutes at 4°C, discard the supernatant. Repeat once.

d. Resuspend the cells in 100 µL staining buffer, add the appropriate amount of the secondary antibody to each tube according to the manufacturer's instructions, mix gently. Incubate for 30-60 minutes at 4°C in the dark.

e. Wash the cells with staining buffer, centrifuge at 300 × g for 5 minutes at 4°C, discard the supernatant. Repeat once.

f. Resuspend the cells in 200-500 µL staining buffer for analysis.

5. Fixation (Optional)

a. Fix the cells with 100-500 µL fixation buffer for 15 minutes at room temperature in the dark.

b. Centrifuge and resuspend the cells in staining buffer for analysis.

6. Intracellular Staining (If Required)

a. Fix the cells with 100-500 µL fixation buffer for 15 minutes at room temperature in the dark.

b. Wash the cells with staining buffer, centrifuge at 300 × g for 5 minutes, discard the supernatant. Repeat once.

c. Permeabilization: Add 1-2 mL permeabilization buffer, mix gently and incubate at room temperature for 10-15 minutes in the dark.

d. Wash the cells with permeabilization buffer, centrifuge at 300 × g for 5 minutes, discard the supernatant. Repeat once.

e. Resuspend the cells in 100 µL permeabilization buffer, add the appropriate amount of the primary antibody to each tube according to the manufacturer's instructions. Mix gently and incubate for 30-60 minutes at 4°C in the dark.

f. Wash the cells with permeabilization buffer, centrifuge at 300 × g for 5 minutes, discard the supernatant. Repeat once.

g. Resuspend the cells in 100 µL permeabilization buffer, add the appropriate amount of the secondary antibody to each tube according to the manufacturer's instructions, mix gently. Incubate for 30-60 minutes at 4°C in the dark.

h. Wash the cells with staining buffer, centrifuge at 300 × g for 5 minutes at 4°C, discard the supernatant. Repeat once.

i. Resuspend the cells in 200-500 µL staining buffer for analysis.

7. Viability Staining (Optional)

Add viability dye (e.g., DAPI or 7-AAD) to the cells. Incubate for 10-15 minutes in the dark.

8. Data Acquisition and Analysis

a. Prepare the flow cytometer by setting up voltage setting.

b. Use unstained and single-stained controls for compensation.

c. Collect ≥10,000 events per sample.

d. Analyze with the flow cytometry software (e.g., FlowJo, FCS Express).