Indirect Flow Cytometry Protocol

Statement: The Flow Cytometry Protocol provided here is intended for reference only. Please design a more appropriate experimental protocol based on actual conditions.

Materials Required

a. Cells: Suspension or adherent cells (harvested using non-enzymatic dissociation if adherent).

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

c. Buffers:

- Staining buffer (Pre-cooled PBS + 1% BSA or 2% FBS).
- Fixation buffer (1-4% paraformaldehyde or commercial fixatives).
- 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, microcentrifuge tubes, ice, pipettes.

Procedure

1. Planning Your Experiment

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

b. Select appropriate fluorochrome-conjugated secondary 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 cells from tissues, use an appropriate method such as enzymatic digestion or mechanical disruption.
- Suspension cells: Collect the cell suspension into the centrifuge tube, centrifuge at 300 × g for 5 minutes, discard supernatant and resuspend in staining buffer.
- Adherent cells: Detach gently (e.g., EDTA or enzyme-free dissociation buffer), collect the cell suspension into the centrifuge tube, centrifuge at 300 × g for 5 minutes, discard supernatant, and resuspend in staining buffer.

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

b. Wash cells with staining buffer, centrifuge, and discard supernatant. Repeat once.

c. Cell count: Adjust cell density to 1 x 10^6 cells/mL in staining buffer.

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 (e.g., 1 µg/10^6 cells anti-CD16/32) to cell suspension.

b. Incubate 10-15 minutes at 4°C.

4. Cell Staining for Surface Markers

a. Transfer 100 µL of cell suspension (approximately 1 x 10^5 cells) into flow cytometry tubes or a 96-well plate.

b. Add the appropriate amount of primary antibody to each tube or well according to the manufacturer's instructions, mix gently. Incubate for 30-60 minutes at 4°C in the dark.

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

d. Resuspend cells in 100 µL fluorochrome-conjugated secondary antibody diluted with staining buffer according to the manufacturer's instructions, mix gently. Incubate for 30-60 minutes at 4°C in the dark.

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

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

5. Fixation (Optional)

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

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

6. Intracellular Staining (If Required)

a. Fix cells with 100-200 µL fixation buffer for 15 minutes at room temperature in the dark.

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

c. Permeabilization: Add 100-500 µL permeabilization buffer, mix gently and incubate at room temperature for 10-15 minutes.

d. Wash cells with permeabilization buffer, centrifuging at 300 × g for 5 minutes at 4°C, discard supernatant. Repeat once.

e. Resuspend cells in 100 µL primary antibody diluted with permeabilization buffer according to the manufacturer's instructions, mix gently. Incubate for 30-60 minutes at 4°C in the dark..

f. Wash cells with permeabilization buffer, centrifuging at 300 × g for 5 minutes at 4°C, discard supernatant. Repeat once.

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

g. Wash cells with permeabilization buffer, centrifuging at 300 × g for 5 minutes at 4°C, discard supernatant. Repeat once.

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

7. Viability Staining (Optional)

Add viability dye (e.g., DAPI or 7-AAD) to cells. Incubate 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).