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: Fluorochrome-conjugated primary antibodies (e.g., FITC, PE, APC), isotype controls.

c. Buffers:

- Staining buffer (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 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: Centrifuge at 300 × g for 5 min, discard supernatant.
- Adherent cells: Detach gently (e.g., EDTA or enzyme-free dissociation buffer), centrifuge, and resuspend in staining buffer.

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

b. Wash cells with staining buffer (cold PBS containing 1% BSA or 2% PBS), 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 min at 4°C.

4. Cell Staining for Surface Markers

a. Transfer 100 µL of cell suspension (approximately 1 x 10^6 cells) into flow cytometry tubes or a 96-well plate.
b. Add the appropriate amount of antibody to each tube or well according to the manufacturer's instructions. Mix gently.
c. Incubate cells with antibodies for 30-60 min at 4°C in the dark.
d. Wash cells with staining buffer, centrifuging at 300 × g for 5 min at 4°C, discard supernatant. Repeat once. Resuspend cells in 200-500 µL staining buffer for analysis.

Note: If you are using an unlabeled primary antibody and a labeled secondary antibody, please refer to the indirect staining protocol.

5. Fixation (Optional)

a. Fix cells with 100-200 µL fixation buffer for 15 min at room temperature.
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 min at room temperature.
b. Permeabilization: Add 1-2 mL permeabilization buffer, centrifuge, discard supernatant.
c. Staining: Add intracellular antibodies diluted in permeabilization buffer. Incubate 30-60 min at 4°C in the dark.
d. Wash: Centrifuge and resuspend cells in 200-500 µL staining buffer.

7. Viability Staining (Optional)

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

8. Data Acquisition

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.

9. Data Analysis

a. Use flow cytometry software (e.g., FlowJo, FCS Express).
b. Exclude debris and doublets using FSC-A vs. SSC-A and FSC-H vs. FSC-W gating.
c. Analyze specific marker expression within gated populations.