Flow Cytometry Triton X-100 Permeabilization Protocol

Reagents required :

4% PFA Fix Buffer (4% paraformaldehyde dissolved in 1x PBS, adjust pH to 7.4)

0.1% Triton X-100 (in 1x PBS)

1x PBS

Flow cytometry antibodies

Experiment procedures:

1. Harvest cells and wash them twice with 1x PBS by centrifugation at 350-500 x g for 5 minutes each time, discard the supernatant.

2. (Optional) Perform cell surface staining with recommended amount of fluorochrome-conjugated primary antibody, wash the cells with 1 mL staining buffer by centrifugation at 350-500 x g for 5 minutes, discard the supernatant.

3. Resuspend the cells with 200 µL of 4% PFA Fix Buffer and vortex briefly, incubate for 20 minutes at room temperature in the dark.

4. Centrifuge at 350-500 x g for 5 minutes, discard the supernatant. Wash the cells twice with 1x PBS by centrifugation at 350-500 x g for 5 minutes each time.

5. Resuspend the cells at a cell density of approximately 1 x 10^6 cells in 1 mL of 0.1% Triton X-100, incubate for 15 minutes at room temperature in the dark.

6. Centrifuge at 350-500 x g for 5 minutes, discard the supernatant. Wash the cells twice with 1x PBS by centrifugation at 350-500 x g for 5 minutes each time.

7. Resuspend the cells with 1x PBS.

8. Incubate the cells with the primary antibody in each 100 µL of cell resuspension. The concentration of the primary antibody is based on the recommendations or the results of titration.

9. Incubate for 45-60 minutes at 4°C in the dark.

10. Wash the cells with 1 mL staining buffer by centrifugation at 350-500 x g for 5 minutes, discard the supernatant. Repeat.
Note: If using fluorochrome-conjugated primary antibodies, skip to step 14.

11. Resuspend the cells with diluted fluorochrome-conjugated secondary antibody in 100 µL 1x PBS (use recommended concentration for secondary antibody dilution).

12. Incubate for 45-60 minutes at 4°C in the dark.

13. Wash the cells with 1x PBS by centrifugation at 350-500 x g for 5 minutes, discard the supernatant.

14. Resuspend the cells with 200-500 µL 1x PBS and analyze on flow cytometer.