Flow Cytometry Cytoplasmic Staining Protocol

Reagents required :

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

Flow Cytometry Perm Buffer (10x) (PF00011-C) dilute to 1x concentrate in distilled water prior to use

Flow Cytometry Staining Buffer (1x) (PF00012)

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 4% PFA fix buffer and vortex briefly, incubate for 20 minutes at 4°C in the dark.

4. Centrifuge at 350-500 x g for 5 minutes, discard the supernatant.

5. Resuspend the cells with 2 mL of 1x Flow Cytometry Perm Buffer and incubate at room temperature for 5 minutes in the dark.

6. Centrifuge at 350-500 x g for 5 minutes, discard the supernatant.

7. Resuspend the cells with 100 µL of 1x Flow Cytometry Perm Buffer.

8. Add the recommended amount of primary antibody for detection of intracellular target and incubate for 20-60 minutes at 4°C in the dark.

9. Wash the cells with 1 mL of 1x Flow Cytometry Staining Buffer by centrifugation at 350-500 x g for 5 minutes, discard the supernatant.
Note: If using fluorochrome-conjugated primary antibodies, skip to step 12.

10. Resuspend the cells with diluted fluorochrome-conjugated secondary antibody in 100 µL of 1x Flow Cytometry Perm Buffer and incubate for 15-30 minutes at 4°C in the dark.

11. Wash the cells with 1 mL of 1x Flow Cytometry Staining Buffer by centrifugation at 350-500 x g for 5 minutes, discard the supernatant.

12. Resuspend the cells with 200-500 µL of 1x Flow Cytometry Staining Buffer and analyze on flow cytometer.