Flow Cytometry Methanol Permeabilization Protocol

Reagents required:

Methanol, pre-cooled at -20°C

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. Resuspend the cells at a density of 1 x 10^6 cells in 100 µL of 90% methanol. Mix well to dissociate the precipitate.

3. Fix and permeate cells for about 15 minutes at room temperature (20-25°C).

4. Centrifuge at 350-500 x g for 5 minutes, discard the supernatant. Wash the cells 3 times 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 100 µL of 3% BSA (or serum), incubate for 30-60 minutes at 4°C in the dark.

6. (Optional) Centrifuge at 350-500 x g for 5 minutes, discard the supernatant. Wash the cells with enough 1x PBS by centrifugation at 350-500 x g for 5 minutes. Resuspend the cells with 1x PBS.

7. 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.

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

9. Wash the cells with 1x PBS by centrifugation at 350-500 x g for 5 minutes, discard the supernatant. Repeat.
Note: If using fluorochrome-conjugated primary antibodies, skip to step 13.

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

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

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

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