

Flow Cytometry Cytoplasmic Staining Protocol

Reagents required :

Intracellular Flow Cytometry Fixation & Permeabilization Buffer Kit ([PF00019](#))

Deionized water

1X PBS

Flow cytometry antibodies

Experiment procedures:

1. Dilute 1 part Perm/Wash Buffer (10X) (PF00019-B) with 9 parts deionized water to make 1X Perm/Wash Buffer. Around 7 mL 1X Perm/Wash Buffer are needed per tube of 1×10^6 cells or 2 mL per well in a 96-well plate.

(Optional) Perform cell viability staining with one of Phantom Dyes ([PD00001~PD00007](#)) before fixation.

(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.

2. Wash cells with PBS. Add 500 μ L Fixation Buffer (PF00019-A) per tube or 200 μ L Fixation Buffer per well. Mix and incubate at room temperature in the dark for 15 minutes. Centrifuge at 350-500 x g for 5 minutes. Aspirate the supernatant.

3. Wash the cells: add 1 mL 1X Perm/Wash Buffer per tube or 200 μ L per well in a 96-well plate. Mix well by pipetting. Centrifuge at 350-500 x g for 5 minutes. Aspirate the supernatant. Repeat.

4. Resuspend cells in 100 μ L 1X Perm/Wash Buffer.

5. Add the recommended amount of primary antibody and incubate for 20-40 minutes at 4°C in the dark.

6. Wash cells twice as in step 3.

Note: If using fluorochrome-conjugated primary antibodies, skip to step 9.

7. Resuspend the cells with diluted fluorochrome-conjugated secondary antibody in 100 μ L of 1X Perm/Wash Buffer and incubate for 15-30 minutes at 4°C in the dark.

8. After incubation, wash cells twice as in step 3.

9. Resuspend cells in 200 μ L 1X Perm/Wash Buffer and analyze on flow cytometer.