

Flow Cytometry Foxp3 / Transcription Factor Staining Protocol

Reagents required:

Foxp3 / Transcription Factor Staining Buffer Kit (PF00011) , including **PF00011-A, PF00011-B, PF00011-C**
Foxp3 / Transcription Factor Fix/Perm Concentrate (4X) (PF00011-A), dilute to 1X concentrate with
Foxp3 / Transcription Factor Fix/Perm Diluent (1X) (PF00011-B) prior to use
Flow Cytometry Perm Buffer (10X) (PF00011-C), dilute to 1x concentrate with distilled water prior to use

Flow Cytometry Staining Buffer (1X) (PF00018)

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 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.
3. (After staining cell surface markers, wash and remove the supernatant.) For each tube of 1×10^6 cells, add 1 mL 1X Fix/Perm Buffer. Gently pipette to fully suspend cells. Incubate for 45 minutes at room temperature in the dark.
4. Centrifuge at 350-500 x g for 5 minutes, discard the supernatant. Add 2 mL 1X Flow Cytometry Perm Buffer. Gently pipette to fully resuspend cells. Centrifuge at 350-500 x g for 5 minutes, discard the supernatant.
5. Resuspend cell pellet in 100 μ L 1X Flow Cytometry Perm Buffer and incubate for 10-15 minutes at room temperature in the dark.
6. Add the recommended amount of primary antibody and incubate for 30-45 minutes at 4°C in the dark to stain intracellular targets.
7. Wash the cells with 2 mL 1X Flow Cytometry Perm Buffer. Centrifuge at 350-500 x g for 5 minutes. Discard the supernatant. Repeat.
Note: If using fluorochrome-conjugated primary antibodies, skip to step 10.
8. Resuspend the cells with diluted fluorochrome-conjugated secondary antibody in 100 μ L of 1X Flow Cytometry Perm Buffer and incubate for 15-30 minutes at 4°C in the dark.
9. Wash the cells with 2 mL of 1X Flow Cytometry Perm Buffer by centrifugation at 350-500 x g for 5 minutes, discard the supernatant.
10. Resuspend the cells with 200 μ L of 1X Flow Cytometry Perm Buffer and analyze on flow cytometer.