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 Perm/Wash Buffer (1X). Around 7 mL Perm/Wash Buffer (1X) are needed per tube of 1x10^6 cells or 2mL per well in a 96-well plate.

(Optional) Perform cell viability staining with one of Phantom Dyes (PD00001~ PD00009) 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 400-600 g for 5 minutes, discard the supernatant.

2. Wash cells with PBS. Add 500 uL Fixation Buffer (PF00019-A) per tube or 200 uL Fixation Buffer per well. Mix and incubate at RT in the dark for 15 minutes. Centrifuge at 400-600 g for 5 minutes. Aspirate the supernatant.

3. Wash the cells: add 1 mL Perm/Wash Buffer (1X) per tube or 200 uL per well in a 96-well plate. Mix well by pipetting. Centrifuge at 400-600 g for 5 minutes. Aspirate the supernatant. Repeat.

4. Resuspend cells in 100 uL Perm/Wash Buffer (1X).

5. Stain cells with primary antibodies at the optimal titration according to vendor recommendations.

6. After incubation with primary antibody, wash cells twice as in step 3.

7. If staining with secondary antibody, continue to the next step. If not, skip to step 10.

8. Dilute secondary antibody to the optimal titration with Perm/Wash Buffer (1X). Add 100 uL diluted secondary antibody to the cells.

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

10. Resuspend cells in 200 uL Perm/Wash Buffer (1X) and acquire samples on a flow cytometer.