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Experimental Procedure

Instructions

1. Aliquot cell samples to tubes in a volume and at a cell concentration suitable for staining.
2. Stain cell surface antigen(s) with the recommended optimal concentration of labeled antibodies.
3. Incubate for 20-30 minutes at 4°C or room temperature. Samples are light sensitive and should be protected from light.
4. Wash cells with 1-2 mL Flow Staining Buffer (1X).
5. Centrifuge samples at 300-400 x g at room temperature for 5 minutes, discard the supernatant.
6. Vortex sample (<5 seconds) to completely dissociate the cell pellet.
7. Add 1 mL Transcription Factor Fixation/Permeabilization working solution to each tube and pulse vortex (< 5 seconds).
8. Incubate at 4°C or room temperature for 30-60 minutes in the dark.
9. Centrifuge samples at 300-400 x g at room temperature for 5 minutes, discard the supernatant.
10. Wash cells with 1-2 mL Flow Cytometry Perm Buffer working solution.
11. Centrifuge samples at 300-400 x g at room temperature for 5 minutes, discard the supernatant.
12. [Optional] Block with 2% normal mouse/rat serum by adding 2 µL directly to the cells. Incubate at room temperature for 15 minutes.
13. Without washing, add the recommended amount of fluorochrome-conjugated antibody for detection of intracellular antigen to cells and incubate in the dark at room temperature for at least 30 minutes.
14. Wash cells with 1-2 mL Flow Cytometry Perm Buffer working solution.
15. Centrifuge samples at 300-400 x g at room temperature for 5 minutes, discard the supernatant.
16. Wash cells with 1-2 mL Flow Staining Buffer (1X).
17. Centrifuge samples at 300-400 x g at room temperature for 5 minutes, discard the supernatant.
18. Resuspend stained cells in an appropriate volume of Flow Staining Buffer (1X) and acquire data on a flow cytometer.