Antibody staining of cells for surface antigens

This protocol is suitable to label murine or human cells isolated from the blood or from tissues, such as lymph nodes, spleen, liver or lungs, after tissue dissociation and eventual digestion. Please ensure to follow a protocol with optimized procedures for the dissociation of each tissue.

Ensure all antibodies had been previously titrated and the optimal dilution of each one to stain 1 x 10^6 cells is known.

Reagents & Materials

- Trypan blue
- 5-mL round bottom FACS tubes or 96-well U-bottom plate
- [Optional] Viability Dye
- Fc Block (unlabeled CD16/32, clone 2.4G2)
- Fluorescently labeled antibodies (each one at its optimal dilution in FACS Buffer)
- FACS Buffer (1X PBS with 2% FBS and 0.02% NaN_3)

Procedures

1. Count the cells in suspension using an Neubauer hemocytometer.

    1.1. When having several millions of cells, it is more accurate to have cells suspended in at least 5 mL.

    1.2. Take 20-50 μL of cell suspension and make a 1:1 dilution in Trypan Blue to assess only the viable cells.

2. Sediment the cells by centrifugation at 1400 rpm for 5 min and discard the supernatant by decantation.

3. Vortex the pellets and resuspend cells in FACS Buffer at 20 X 10^6 cells/mL.

4. Transfer 100 μL cell suspension (~ 2 X 10^6 cells) per well of a round-bottomed 96-well plate or 500 μL cell suspension (~ 10^7 cells) to 5 mL FACS tubes or Eppendorfs.

5. Add 100 μL FACS Buffer per well (for plates) or 500 μL FACS Buffer to tubes.

6. Sediment the cells by centrifugation at 1400 rpm for 5 minutes (for plates, a quick spin up to 2500 rpm is sufficient) and discard the supernatant by decantation.

7. Vortex the pellets and add 20 μL Fc Block (unlabeled CD16/32, clone 2.4G2, at final concentration) per well (for plates) or 100 μL for tubes.

8. Incubate for 15 minutes at 4ºC.

9. Add 20 μL labeled antibody mix (at final concentration) per well (for plates) or 100 μL for tubes. It is highly recommendable to stain cells also with a Viability Dye.

10. Incubate for 30 minutes at 4ºC.

11. Add 200 μL FACS Buffer per well (for plates) or 1 mL for tubes.

12. Sediment the cells by centrifugation at 1400 rpm for 5 minutes (for plates, a quick spin up to 2500 rpm is sufficient) and discard the supernatant by decantation.

13. Vortex the pellets and repeat steps 11 and 12 to ensure removal of antibody in excess.

14. Vortex the pellets and resuspend in ~300-500 μL FACS Buffer (minimum volume recommended: 200 μL).

15. Filter cells before for flow cytometry analysis using cell-strainers or tubes with filter-caps to remove any clumps that may be present.