Flow Cytometry for Intracellular Epitopes

Cell Plating

Begin with a cell suspension of 0.5e6 cells/mL - 1.0e6 cells/mL

Antibody Staining

1. Dilute Cell Surface Bio Primary Antibody in blocking buffer with 0.2% Saponin (10% NGS recommended) to desired concentration (1ug/mL recommended)
2. Monodisperse cells and then fix using 1-4% paraformaldehyde (PFA)
3. Pellet and wash the cells 2X with PBS -/-
4. Incubate cells with 0.2% Saponin diluted in PBS-/- for 20 minutes to permeabilize cells
5. Incubate cells with Cell Surface Bio Primary Antibody for 1 hour
6. Pellet and wash cells 2X with PBS -/-
7. Dilute Anti-mouse secondary antibody conjugated with fluorophore of your choice in 0.2% saponin blocking buffer at a 1:400 dilution
8. Incubate cells with diluted secondary for 30 minutes
9. Pellet and wash cells 3X with PBS -/-
10. Fix cells using 1-4% paraformaldehyde (PFA)
11. Pellet and wash 3X in PBS -/-
12. Resuspend in reading buffer and read on cytometer

Materials

- Cell Surface Bio primary antibody
- Cells
- Saponin
- Blocking buffer (NGS)
- PBS -/-
- Fluorophore conjugated anti-mouse secondary antibody
- Paraformaldehyde (PFA)
- Cytometer