IF Imaging of Intracellular Epitopes

Cell Plating

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

Antibody Staining

1. Pellet and wash cells 2x with PBS +/+
2. Dilute Cell Surface Bio Primary Antibody in blocking buffer (10% NGS recommended) to desired concentration (1ug/mL recommended)
3. Use 1-4% Paraformaldehyde (PFA) to fix the cells
4. Pellet and wash cells 3x with PBS +/+
5. Incubate cells with 0.1% Tritonx100 diluted in PBS-/- for 15 minutes to permeabilize cells
6. Pellet and wash cells 3X with PBS +/+
7. Incubate cells with Primary Antibody for 1 hour
8. Pellet and wash cells 2X with PBS +/+
9. Dilute secondary antibody conjugated with fluorophore of your choice in blocking buffer.
10. Incubate cells with diluted secondary for 30 minutes
11. Pellet and wash cells 3X with PBS +/+
12. Use 1-4% paraformaldehyde (PFA) to fix the cells
13. Pellet and wash cells 3x with PBS+/+
14. Add DAPI and incubate for 5 minutes
15. Pellet and wash with PBS +/+ 2X
16. Add PBS +/+ and Image using fluorescent imager

Material

- Cell Surface Bio primary antibody
- Cells
- Blocking buffer (NGS)
- PBS +/+
- Tritonx100
- Fluorophore conjugated anti-mouse secondary antibody
- Paraformaldehyde (PFA)
- DAPI
- Fluorescent imager