Cell Surface Staining Protocol

Materials Required:

- Flow Cytometry Staining Buffer (Stain Buffer) (1X PBS with 2% FBS, 0.09% Na-Azide)
- 12x75 mm round bottom test tubes or 96-well round bottom microtiter plates

Experimental Procedure

1. Prepare cells in a single cell suspension at a concentration of 2 x 10^7- 2 x 10^8 cells/mL in Stain Buffer.
2. Aliquot 50 µL of the cell sample to an individual well or tube.
3. Add the recommended amount of each primary antibody to the sample. The final volume of the cell sample + antibodies should not exceed 100 µL.
4. Incubate at 4°C in the dark for 20-60 minutes.
5. Wash cells in 200-300 µL (for microtiter plates) or 1-2 mL (for tubes) Stain Buffer. Centrifuge at 300-400 x *g* for 5 minutes at room temperature and discard supernatant. Briefly vortex to dissociate the cell pellet.
6. If no second-step reagent is needed, proceed to Step 10.
7. If a second-step reagent is required for detection of purified or biotinylated primary antibody, add 100 µL appropriately diluted secondary reagent to the dissociated cell pellet.
8. Incubate at 4°C in the dark for 20-60 minutes.
9. Wash cells in 200-300 µL (for microtiter plates) or 1-2 mL (for tubes) Stain Buffer. Centrifuge at 300-400 x *g* for 5 minutes at room temperature and discard supernatant. Briefly vortex to dissociate the cell pellet.
10. Stained cells may be resuspended in an appropriate volume of Stain Buffer and acquired on a flow cytometer or further processed for intracellular staining protocols.