

## Flow Cytometry Cell Surface Staining Protocol

## Reagents required:

Flow Cytometry	Staining	Buffer (1)	k) (PF000	18)
1x PBS				

Flow cytometry antibodies

## **Experiment procedures:**

- 1. Harvest cells and wash them twice with 1x PBS by centrifugation at  $350-500 \times g$  for 5 minutes each time, discard the supernatant.
- 2. Aliquot cell samples to tubes or wells at a cell density of 1 x 10 $^{\circ}$ 6 cells in 100  $\mu$ L of 1x Flow Cytometry Staining Buffer.
- 3. Add the recommended amount of primary antibody and incubate for 20-40 minutes at 4°C in the dark.
- 4. Wash the cells with 1x PBS by centrifugation at 350-500 x g for 5 minutes, discard the supernatant. Note: If using fluorochrome-conjugated primary antibodies, skip to step 7.
- 5. Resuspend the cells in 100  $\mu$ L of diluted fluorochrome-conjugated secondary antibody and incubate for 15-30 minutes at 4°C in the dark.
- 6. Wash the cells with 1x PBS by centrifugation at 350-500 x g for 5 minutes, discard the supernatant.
- 7. Resuspend the cells in 200-500 µL of 1x Flow Cytometry Staining Buffer and analyze on flow cytometer.

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