Protocol Make Flow Compensation Beads

Supplies

1. 1.5 mL centrifuge tubes (one for each fluorochrome)
2. Positive and negative BD CompBeads
3. flow tubes (one for each fluorochrome)
4. Microcentrifuge
5. Wash buffer

Protocol

1. Place 1 drop of positive and negative Comp Beads in each 1.5 mL centrifuge tube
2. Place 5 mcL of each mAb in separate tubes.
    - Use same amount of mAb that use in master mix. For example, if you place only 2 mcL of the mAb in the staining master mix, then use only 2 mcL of the mAb for the compensation.
3. Incubate x 15 minutes at room temperature in the dark.
4. Add 1 mL of washing buffer to each 1.5 mL centrifuge tube.
5. Centrifuge in microcentrifuge for 3-4 minutes.
6. Vacuum out supernatant.
7. Resuspend in 200 mcL of wash buffer in flow tube.
8. Keep at 4 C and in the dark until ready to acquire on the cytometer.