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NBP3-00496 Protocol

Flow Cytometry Protocol for Amine Reactive Comp-Bead 2 Population Kit (NBP3-00496)

1. Allow beads to come to room temperature, then vortex briefly.
2. Add 1 drop (about 50 uL) of the High Binding beads to a 1.5 mL microcentrifuge tube.
3. Wash the beads by adding 0.5 mL of PBS (free of surfactant and blocker) to the microcentrifuge tube, centrifuge at 300 x G for 5 minutes, decant and repeat.
4. Decant and resuspend in 50 uL PBS.
5. Prepare the amine reactive dye according to the manufacturer's instructions.
6. Add 1 - 4 uL of the amine reactive dye to the bead suspension and vortex briefly.
7. Incubate for 30 minutes. Protect tube from light.
8. Add 1 mL of PBS to the same tube and vortex briefly.
9. Centrifuge at 300 x G for 5 minutes, decant and repeat.
10. Resuspend the beads in PBS containing 0.05% BSA with brief vortex.
11. Add a drop (about 50 uL) fo the Negative Binding beads to the labeled High Binding beads.
12. Analyze on the flow cytometer using a live gate around the singlet population in the FSC/SSC dot plot.
13. Create a fluorescent histogram for the appropriate detectors and perform compensation to achieve the desired results.