

## Compensation Bead Staining Procedure

1. Vortex CompBeads thoroughly before use.
2. Label a separate 1.5mL Eppendorf tube for each flurochrome-conjugated mouse Ig, k antibody to be used on a given experiment.
3. Add 1 full drop (approximately 60uL) of CompBeads Negative Control to the unstained tube and 1 full drop of CompBeads anti-mouse Ig k to each additional tube.
4. Centrifuge @ 200g/10min. Carefully decant supernatant to ensure a “dry pellet”.
5. Sonicate each tube for 10sec in a water bath sonicator (NEY Ultrasonik).
6. Add 2uL of each antibody directly to beads and gently reflux.
7. Incubate for 15min @ RT in the dark.
8. Add 1uL of mouse serum; incubate 5min @RT.
9. Add 100uL of IFA and reflux.
10. Sonicate each tube for 10sec.
11. Add 1mL of staining buffer. For manual compensation, add 1 drop of negative beads to each test tube that contains an antibody. **\*NOTE:** Negative beads are not required for automated compensation (ADC) on the Beckman-Coulter XL or FC500.
12. Centrifuge for 10min. @ 200g.
13. Decant and blot.
14. Resuspend in 0.5mL of staining buffer.
15. Transfer to 12 x 75mm tubes for flow cytometry.
16. Sonicate for 10sec.
17. Acquire.

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BD CompBeads, Anti-mouse Ig κ  
BD Biosciences # 552843

*Adapted from procedures, complements of Donnenberg Lab, University of Pittsburgh*