CYTOMETRY RESEARCH, LLC

FACS Staining

Necessary controls:

Cells only

1° Ab only

2° Ab only

1. If adherent cells, trypsinize, scrape, or treat with EDTA to get single cell suspension.
2. Count cells, at least 2 x 10^6 cells will be needed for each sample.
3. Spin cells down (5 min @ 100 x g in tabletop centrifuge).
4. Resuspend cells to 2 x 10^6 cells/200 µl in 2% FBS/PBS (FACS buffer).
5. Transfer 200-µl volumes to individual 1.5 mL microcentrifuge tubes.
6. Add 10 µl 1° Ab at the desired dilution to appropriate tubes. Incubate 1 hr at 4°C. Optimal concentration for each antibody will need to be determined by the end user.
7. Add 1 ml FACS buffer and spin down. (3 min @ 800 x g in Eppendorf microcentrifuge)
8. Aspirate the supernatant.
9. Wash with 1 ml FACS buffer, spin down again.
10. Aspirate the supernatant.
11. Add 2° Ab at desired concentration in 100 µl of FACS buffer + 1% BSA to appropriate tubes.
12. Incubate at 4°C for 30 minutes.
13. Repeat steps 7-10.
14. Resuspend in 500 µl FACS buffer.
15. Transfer into 12 x 75 mm polystyrene FALCON # 2024 snap-cap, 5 ml test tubes. If fixing cells before analysis, do not add propidium iodide.