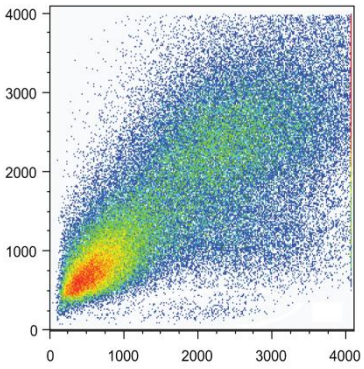
Flow Cytometry Protocol (Flow)

General procedure for flow cytometry using a primary antibody and conjugated secondary antibody. Please note that this is a general protocol and you may need to adapt it for your applications. You may find more antibody application protocols and tips at NovoPro official website (http://www.novoprolabs.com/).



A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS)**
2. **16% Formaldehyde (methanol free).**
3. **100% methanol.**
4. **Incubation Buffer:** Dissolve 0.5 g Bovine Serum Albumin (BSA) in 100 ml 1X PBS. Store at 4°C.
5. **Secondary Antibodies:** Anti-mouse, Anti-rabbit, Anti-rat

Step 1. Fixation

1. Collect cells by centrifugation and aspirate supernatant.
2. Resuspend cells in 0.5–1 ml 1X PBS. Add formaldehyde to obtain a final concentration of 4%.
3. Fix for 10 min at 37°C.
4. Chill tubes on ice for 1 min.
5. For extracellular staining with antibodies that do not require permeabilization, proceed to immunostaining (Step 3) or store cells in PBS with 0.1% sodium azide at 4°C; for intracellular staining, proceed to permeabilization (Step 2).

Step 2. Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, remove fix prior to permeabilization by centrifugation and resuspend in 90% methanol as described above.

2. Incubate 30 min on ice.
3. Proceed with immunostaining (Step 3) or store cells at -20°C in 90% methanol.

Step 3. Immunostaining

NOTE: Account for isotype matched controls for monoclonal antibodies or species matched IgG for polyclonal antibodies. Count cells using a hemocytometer or alternative method.

1. Aliquot 0.5–1 x 106 cells into each assay tube (by volume).
2. Add 2–3 ml incubation buffer to each tube and wash by centrifugation. Repeat.
3. Resuspend cells in 100 µl of primary antibody (prepared in incubation buffer at the recommended dilution). See individual antibody datasheet or product webpage for the appropriate dilutions.
4. Incubate for 1 hr at room temperature.
5. Wash by centrifugation in 2–3 ml incubation buffer.
6. If using a fluorochrome-conjugated primary antibody, resuspend cells in 0.5 ml 1X PBS and analyze on flow cytometer; for unconjugated or biotinylated primary antibodies, proceed to immunostaining (Step 9).
7. Resuspend cells in fluorochrome-conjugated secondary antibody or fluorochrome-conjugated avidin, diluted in incubation buffer at the recommended dilution.
8. Incubate for 30 min at room temperature.
9. Wash by centrifugation in 2–3 ml incubation buffer.
10. Resuspend cells in 0.5 ml PBS and analyze on flow cytometer; alternatively, for DNA staining, proceed to optional DNA stain (Step 4).

Step 4. Optional DNA Dye

1. Resuspend cells in 0.5 ml of DNA dye.
2. Incubate for at least 30 min at room temperature.
3. Analyze cells in DNA staining solution on flow cytometer.

Good Luck and Enjoy Your Immunoprecipitation!