Protocol

Immunofluorescence Staining of Cells for Flow Cytometry

OVERVIEW
The following is a general cell staining protocol for analysis by flow cytometry. It is recommended that a separate isotype control be tested in parallel as a negative control.

MATERIALS REQUIRED
- Flow Buffer
- Trypan blue
- 10% formalin solution
- PBS
- 15 ml conical tubes
- 12 x 75 mm round-bottom tubes

MATERIAL PREPARATION
- Flow Buffer
  99 ml PBS (1X)
  1 ml FBS
  0.1 ml Sodium Azide (100%)
  - If detecting extracellular antigens, the Flow Buffer is ready for use.
  - If detecting intracellular antigens, add Saponin to a final concentration of 0.1%.
  Store Flow Buffer at 4°C.

STAIN CELLS FOR FLOW CYTOMETRY
1. Isolate and dissociate the cells to a single cell suspension. Collect the cells in a 15 ml conical tube.
2. Remove a sample of the cell suspension and count live cells using trypan blue and a hemacytometer.
3. Centrifuge the cell suspension at 300 x g for 5 minutes at 4°C.
4. Aspirate the medium and flick the tube to disrupt the cell pellet.
5. If detecting extracellular antigens, resuspend the cell pellet in 3 ml of PBS.
6. If detecting intracellular antigens, resuspend the cell pellet in 2 ml 10% formalin solution and incubate for 15 min at room temperature.
   - Note: Formalin should be added slowly while agitating the tube to avoid cell clumping.
7. Centrifuge the cell suspension at 300 x g for 5 minutes at 4°C.
8. Aspirate the medium and flick the tube to disrupt the cell pellet.
9. Add enough Flow Buffer to bring the cell suspension to a concentration of 2 x 10^6 to 1 x 10^7 cells per ml. Keep the cells on ice.
   - Note: The Flow Buffer formulation depends on if the antigen is extracellular or intracellular. Refer to the preparation section to make sure the appropriate Flow Buffer is used.
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10. For each sample, add 100 μl of the cell suspension to a 12 x 75 mm round-bottom tube.
11. Add the appropriate amount of primary antibody and/or isotype control to each sample.
12. Incubate on ice for 30 minutes to 1 hour, protecting the samples from light if using a conjugated antibody.
14. Centrifuge at 300 x g for 5 minutes at 4°C.
15. Aspirate the supernatant and flick the tube to disrupt the cell pellet.
    **Note:** If using a conjugated antibody, skip steps 16 through 20 and go directly to step 21. If using a purified antibody, continue with step 16.
16. Add the appropriate amount of secondary conjugated antibody to each sample.
17. Incubate on ice for 30 minutes to 1 hour, protecting the samples from light.
19. Centrifuge at 300 x g for 5 minutes at 4°C.
20. Aspirate the supernatant and flick the tube to disrupt the cell pellet.
21. Add appropriate volume of Flow Buffer to each tube.
    **Note:** For this step, use Flow Buffer without 0.1% Saponin for both extra and intracellular antigen detection.
22. Analyze the cells by flow cytometry within 4 hours.