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 extra or intracellular. Refer to the preparation section to make sure the appropriate Flow Buffer is used.*

GP_Flow_V1 November 11, 2010

Stemgent
One Kendall Square, B6201
Cambridge, MA 02139
Technical Support: 1.877.783.6911
www.stemgent.com

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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.
13. Add 4 ml of Flow Buffer.
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.
18. Add 4 ml of Flow Buffer.
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.

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