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.

Material Required

- Flow Buffer
- Trypan blue
- 10% formalin solution
- PBS
- 15 mL conical tubes
- 12 × 75 mm round-bottom tubes

Material Preparation

Flow Buffer

- 99 mL PBS (1×)
- 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 hemocytometer.
3. Centrifuge the cell suspension at 300 × 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 minutes at room temperature.

Note: *Formalin should be added slowly while agitating the tube to avoid cell clumping*.

7. Centrifuge the cell suspension at 300 × 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 2×10^6 to 1×10^7 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*.

10. For each sample, add 100 µL of the cell suspension to a 12 × 75 mm round-bottom tube.
11. Add the appropriate amount of primary antibody and/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 × 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 × 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.

REPROCELL USA Inc
9000 Virginia Manor Road
Suite 207
Beltsville, MD 20705
USA

Tel: +1 301 470 3362
Email: info-us@reprocell.com

REPROCELL Europe Ltd
Thomson Pavilion, Todd Campus
West of Scotland Science Park
Acre Road Glasgow
G20 0XA
UK

Tel: +44 (0)141 465 3460
Email: info-emea@reprocell.com

REPROCELL India Ltd
3-1-135/1A, CNR Complex
Mallapur
Hyderabad 500 076
Telangana
India

Tel: +44 (0)141 465 3460
Email: Bhargavi.Gurram@reprocell.com

REPROCELL Inc (Japan)
MetLife Shin-yokohama 381, Bldg. 9F
3-8-11, Shin-yokohama Kohoku-ku,
Yokohama
Kanagawa 222-0033
Japan

Tel: +81 45 475 3887
Email: info-asia@reprocell.com

www.reprocell.com