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Flow Cytometry Protocol for Staining of Colonocytes Intracellular Antigen

Protocol:

1. Prepare 25,000 to 50,000 cells (5-8µm) per reaction.

2. Wash with 2 ml PBS.

3. Centrifuge at 2000 rpm for 5 minutes, 4 ºC, remove the supernatant using pasteur pipette.

4. Fix cells with 2 ml 1% paraformaldehyde in PBS for 30 minutes.

5. Centrifuge at 2000 rpm for 5 minutes, 4 ºC, remove the supernatant using pasteur pipette.

6. Add 1 ml PBS, suspend them gently by tapping tube.

7. Centrifuge at 2000 rpm for 5 minutes, 4 ºC, remove the supernatant using pasteur pipette.

8. Incubate the cells with 2 ml of 1% BSA, 0.1% Saponin in PBS for 10 minutes

9. Repeat steps 2-3 twice.

For Direct Staining:

10. Add primary antibody with conjugate, usually 5ul/50,000 cells (1mg/ml).

11. Incubate the cells at room temperature for 15 minutes or 4 ºC for 60 minutes

12. Add 1ml 1% BSA in PBS, suspend them gently by tapping tube.

13. Centrifuge at 2000 rpm for 5 minutes, 4 ºC, remove the supernatant using pasteur pipette.

14. Suspend cells in 1ml 1%BSA in PBS.

15. Analyze cells with flow cytometer.

For Indirect Staining:

10. Add primary antibody without conjugate, usually 5ul/50,000 cells (1mg/ml).

11. Incubate the cells at room temperature for 15 minutes or 4 ºC for 60 minutes

12. Add 1ml 1% BSA in PBS, suspend them gently by tapping tube.

13. Centrifuge at 2000 rpm for 5 minutes, 4 ºC, remove the supernatant using pasteur pipette. (CONTINUED)

14. Suspend cells in 1ml 1%BSA in PBS.

15. Repeat step 13-14 twice.

16. Adding secondary antibody with conjugate, usually 5ul/50,000 cells (1mg/ml).

17. Incubate the cells at room temperature for 15 minutes or 4 ºC for 60 minutes.

18. Add 1ml 1% BSA in PBS, suspend them gently by tapping tube.

19. Centrifuge at 2000 rpm for 5 minutes, 4 ºC, remove the supernatant using pasteur pipette.

20. Suspend cells in 1ml 1%BSA in PBS.

21. Analyze cells with Flow cytometer.

Flow Cytometer Detectors/Amps setting:

Run FASCComp with Lysis/No wash to reduce signal from debris.

Detectors	Voltage	Mode
FSC	E-0	Log
SSC	280-360	Log
FL1	425-475	Log
FL2	450-500	Log

Notes:

1. First choice of antibody conjugate is PE, ideal for low expression proteins. Second choice is the FITC. Third choice is PerCP, PE-Cy5 or APC.

2. Colonocytes have cell size range from 2 to 8 µm, hence the FSC has 16 levels of differences. It is very difficult to use Quadrant gate to get data. A Regulate gate is a better choice.