



Flow Cytometry Protocol for Staining of Colonocytes - Surface Antigens

Protocol:

1. Prepare 25,000 to 50,000 cells (5-8 μ m) per reaction.
2. Suspend the colonocytes in 1 ml of PBS. (If the cells are frozen, wash once with 1 ml of PBS).
3. Wash with 2 ml 1% BSA in PBS.
4. Centrifuge at 2000 rpm for 5 minutes, 4 °C and remove the supernatant using pasteur pipette.
5. Repeat step 3 and 4 once more.
6. Aliquot 25,000-50,000/100 μ l of 1% BSA in PBS per antigen into each 12x75mm tube.

For Direct Staining:

7. Add 5 μ l of primary antibody with conjugate (1mg/ml) / 50,000 cells.
8. Incubate the cells at room temperature for 15 minutes or 4 °C for 60 minutes.
9. Add 1 ml of 1% BSA in PBS, suspend the cells gently by tapping the tube.
10. Centrifuge at 2000 rpm for 5 minutes at 4 °C and remove the supernatant using pasteur pipette.
11. Repeat step 9-10 once more.
12. Suspend the cells in 0.5 ml of 1% BSA in PBS.
13. Analyze using flow cytometer.

For Indirect Staining:

7. Add 5 μ l of primary antibody (1mg/ml) / 50,000 cells.
8. Incubate the cells at room temperature for 15 minutes or 4 °C for 60 minutes.
9. Add 1 ml of 1% BSA in PBS, suspend the cells gently by tapping the tube.
10. Centrifuge at 2000 rpm for 5 minutes at 4 °C and remove the supernatant using pasteur pipette.
11. Repeat step 9 and 10 once more.
12. Suspend the cells gently in 100 μ l of 1% BSA in PBS by tapping the tube.
13. Add 5 μ l of secondary antibody with conjugate (1mg/ml) / 50,000 cells.
14. Incubate the cells at room temperature for 15 minutes or 4 °C for 60 minutes.
15. Add 1ml 1% BSA in PBS, suspend them gently by tapping tube.



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16. Centrifuge at 2000 rpm for 5 minutes at 4 °C and remove the supernatant using pasteur pipette.
17. Repeat step 15 and 16 once more.
18. Suspend the cells in 0.5 ml of 1%BSA in PBS.
19. Analyze using flow cytometer.

Flow Cytometer Detectors/Amps setting:

Run FASCComp with Lysis/No wash (even if cells were washed) to reduce the signal from debris.

Detectors	Voltage	Mode
FSC	E-0	Log
SSC	320	Log
FL1	450	Log
FL2	450	Log

Notes:

1. First choice of antibody conjugate is PE, ideal for low expression protein (because PE is brightest). Second choice is the FITC. Third choice is PerCP, PE-Cy5 or APC.
2. Colonocytes have cell size range from 2 to 8 μ m, hence the FSC has 16 levels of differences. It is very difficult to use Quadrant gate to get data. A Region gate as shown below is recommended, based on the unstained autofluorescence plot (top row, right). The second row shows a FITC stained antibody and the third row shows a PE stained antibody:

