WEBSITE www.immunoway.com
E-MAIL order@ immunoway.com
tech@ immunoway.com

FLOW CYTOMEYRT PROTOCOL

Indirect flow cytometry requires two incubation steps, firstly with a primary antibody then with a compatible secondary antibody. The secondary antibody has the fluorescent dye (FITC, PE, Cy5, etc.) conjugated.

A. Fixation

1. Harvest and wash the cells then determine the total cell number.
2. Resuspend the cells to approximately 1-5 x 10^6 cells/ml in ice cold PBS.
3. Resuspend cells briefly in 0.5-1 ml PBS. Add formaldehyde to a final concentration of 2-4% formaldehyde.
4. Fix for 10 minutes at 37°C.
5. Chill tubes on ice for 1 minute.

Note: For extracellular staining with antibodies that do not require permeabilization; for intracellular staining, proceed to permeabilization.

B. Permeabilization

1. Permeabilize cells by adding ice-cold 100% methanol slowly to pre-chilled cells, while gently vortexing, to a final concentration of 90% methanol. Alternatively, to remove fix prior to permeabilization, pellet cells by centrifugation and resuspend in 90% methanol.
2. Incubate 30 minutes on ice.
3. Proceed with staining or store cells at -20°C in 90% methanol.

C. Immunostaining

1. Add 0.1-10 µg/ml of the primary antibody. Dilutions, if necessary, should be made in 3% BSA/PBS.
2. Incubate for at least 30 min at room temperature or 4°C in the dark.
3. Wash the cells 3-times by centrifugation at 400 g for 5 min and resuspend them in ice cold PBS. You may need to adjust the conditions of the centrifugation for the cell types used.
4. Dilute the fluorochrome-labeled secondary antibody in 3% BSA/PBS at the optimal dilution and then resuspend the cells in this solution.
5. Incubate for at least 20-30 minutes at room temperature of 4°C. This incubation must be done in the dark.
6. Wash the cells 3 X by centrifugation at 400 g for 5 min and resuspend them in ice cold PBS, 3% BSA, 1% sodium azide.
7. Store the cell suspension immediately at 4°C in the dark.

D. Optional DNA Stain

1. Resuspend cells in 0.5 ml of DNA dye.
2. Incubate for at least 5 minutes at room temperature.
3. Analyze cells in DNA stain on flow cytometer.