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## NB100-78041 Protocol

# Cell Surface Immunofluorescence Staining Protocol

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Harvest Tissue or Cells:
1. Obtain desired tissue (e.g. spleen, lymph node, thymus, bone marrow) and prepare a single cell suspension in Cell Staining Buffer. If using resuspend previously activated cultures in Cell Staining Buffer and proceed to Step 2.
2. Add Cell Staining Buffer up to ~15 ml and centrifuge at 350 x g for 5 minutes, discard supernatant.
Lyse Red Cells:
3. If necessary (e.g. spleen), dilute 10X Red Blood Cell (RBC) Lysis Buffer to 1X working concentration with DI water and resuspend pellet in 3 ml 1X RBC Lysis Buffer. Incubate on ice for 5 minutes.
4. Stop cell lysis by adding 10 ml Cell Staining Buffer to the tube. Centrifuge for 5 minutes at 350 x g and discard supernatant.
5. Repeat wash as in step 2.
6. Count viable cells and resuspend in Cell Staining Buffer at 510 x 10^6 cells/ml and distribute 100 ul/tube of cell suspension (5-10 x 10^5 cells/tube) into 12 X 75 mm plastic tubes.
Block Fc-Receptors:
7. Reagents that block Fc receptors may be useful for reducing nonspecific immunofluorescent staining. In the mouse, purified anti-mouse CD16/CD32 antibody specific for Fc clone 93) can be used to block nonspecific staining of antibodies. In this case, block Fc receptors by pre-incubating cells with 5-10 ug/ml purified anti-CD16/32 on ice for 10 minutes. In the absence of an effective/available blocking antibody pre-block cells with excess irrelevant purified Ig from the same species and same isotype as the antibodies used for immunofluorescent staining.
Cell-Surface Staining with Antibody:
8. Add appropriately conjugated fluorescent, biotinylated, or purified primary antibodies at predetermined optimum concentrations (e.g. anti-CD3-FITC, anti-CD4-Biotin, and anti-CD8-APC) and incubate on ice for 15-20 minutes in the dark.
9. Wash 2X with at least 2 ml of Cell Staining Buffer by centrifugation at 350 x g g for 5 minutes.
10. If using a purified primary antibody, resuspend pellet in residual buffer and add previously determined optimum concentrations of anti-species immunoglobulin fluorochrome conjugated secondary antibody (e.g. FITC anti-mouse Ig) and incubate in the dark for 15-20 minutes.
If using a biotinylated primary antibody, resuspend cell pellet in residual buffer and add previously determined optimum concentrations of fluorochrome conjugated Streptavidin (SAv) reagent (PE) and incubate on ice for 15-20 minutes in the dark.
11. Repeat step 9.
12. Resuspend cell pellet in 0.5 ml of Cell Staining Buffer and add 5 ul (0.25 ug)/million cells of 7-ADD Viability Staining Solution to exclude dead cells. Note, Novus Biologicals does notrecommend use of 7-AAD with either PE-Cy5 or PE-Cy7 antibody conjugates.
13. Incubate on ice for 3-5 minutes in the dark.
14. Analyze with a Flow Cytometer.

Immunofluorescent Staining of Whole Blood:
1. Add predetermined optimum concentrations of desired fluorochrome conjugated, biotinylated, or purified primary antibodies to 100 ul of anti-coagulated whole blood.
2. Incubate at room temperature for 15
3. Dilute 10X Red Blood Cell (RBC) Lysis Buffer to 1X working concentration with DI water. Warm to room temperature prior to use. Add 2 ml of 1X RBC lysis solution to whole blood/antibody mixture. Incubate at room temperature for 10 minutes.
4. Centrifuge at 350 X g for 5 minutes, discard the supernatant.
5. Wash 1X with at least 2 ml of Cell Staining Buffer by centrifugation at 350 x g for 5 minutes.
6. If using a purified primary antibody, resuspend pellet in residual buffer and add a previously determined optimum concentration of anti-species immunoglobulin fluorochrome conjugated secondary antibody (e.g. FITC anti-mouse Ig) and incubate in the dark for 15-20 minutes.
If using a biotinylated primary antibody, resuspend cell pellet in n residual buffer and add a previously determined optimum concentration of fluorochrome conjugated Streptavidin (SAv) reagent (PE) and incubate for 15-20 minutes

in the dark.
7. Repeat step 5.
8. Resuspend cells in 0.5 ml Cell Staining Buffer or 0.5 ml 2% paraformaldehyde-PBS fixation buffer
9. Analyze with a Flow Cytometer.

Key Reference: Current Protocols in Cytometry (John Wiley & Sons, New York), Unit 6 Phenotypic Analysis.