Cell Surface Immunofluorescence Staining Protocol

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.

Stop cell lysis by adding 10 ml Cell Staining Buffer to the tube. Centrifuge for 5 minutes at 350 x g and discard supernatant.

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.

Add Cell Staining Buffer at 510 x 10^6 cells/ml and centrifuge at 350 x g for 5 minutes.

Centrifuge at 15-20 x g for 5 minutes, discard supernatant.

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Repeat wash as in step 2.
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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.

Wash 2X with at least 2 ml of Cell Staining Buffer by centrifugation at 350 x 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.

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7. Repeat step 5.
8. Resuspend cells in 0.5 ml Cell Staining Buffer or 0.5 ml 2% paraformaldehyde-PBS fixation buffer