Immunofluorescent Staining of Mouse and Rat Leukocytes

I. Procedure

1. Harvest cells from tissue, preparing a single cell suspension. Red blood cells may be removed by lysis or density gradient: Red blood cells from murine peripheral blood or a spleen cell suspension can be lysed using BD Biosciences Pharmingen's PharM Lyse™ (Cat. No. 555899) solution. Add 2.0 ml of 1X Lysing Solution to the spleen cell suspension or per 200 µl of murine peripheral blood. Gently vortex immediately after adding the lysing solution. Incubate at room temperature, protected from light, for 3-4 min for mouse cells or 5-6 min for rat cells. Centrifuge 200 x *g* for 5 min. Carefully aspirate and dispose of supernatant, without disturbing the pellet. Resuspend pellet in 1X cold wash buffer (PBS/0.1% NaN3/1.0% fetal bovine serum). Centrifuge at 350 x *g* for 5 min. Finally, resuspend cell pellet to a concentration of 2×10^7 cells/ml (i.e., 10^6 cells per 50 µl).
2. Dilute primary mAbs (e.g., unconjugated, biotinylated, or fluorochrome-conjugated mAbs) to predetermined optimal concentrations in wash buffer and deliver to the wells of a U-bottom microtiter plate in a volume of 50 µl.
3. Deliver 10^6 cells in 50 µl to each well already containing 50 µl of mAb (or 50 µl wash buffer for negative controls). Mix by gently vortexing or tapping.
4. Incubate at 4°C for 20-40 min in the dark.
5. Wash 2X with 200 µl wash buffer (or 3X if a biotin-conjugated primary antibody is used). After each centrifugation, 350 x *g* for 5 min, aspirate wells or flick plate to remove supernatant. Vortex gently or tap plate to loosen pellet prior to adding next wash or diluted secondary reagent.
6. If a second-step reagent is needed, resuspend cell pellet in 100 µl of appropriately diluted secondary reagent (e.g., fluorochrome-conjugated avidin, streptavidin, anti-Ig allotype, anti-Ig isotype, polyclonal anti-Ig). For example, dilute antibody to ~1 µg per 100 µl in wash buffer and add this to each well containing the loosened cell pellet.
7. Incubate at 4°C for 20-40 min in the dark.
8. Wash 2X with 200 µl wash buffer, as in Step 5. Use 100 µl wash buffer to transfer cell pellets to 0.4 ml aliquots of wash buffer (final concentration ~10^6 cells in 0.5 ml) in tubes appropriate for flow cytometer. Acquire sample data on flow cytometer as soon as possible after staining.