

Immunophenotyping Protocol:

1. Prepare cells in a manner that generates a single cell suspension. Place $1 \times 10^5 - 10^6$ in a 12 x 75 mm tube or a 96-well round bottom plate.
2. **Optional!** Fc γ II (CD32) and Fc γ III (CD16) are low-affinity receptors that bind complexed IgG (Fc receptors or FcR). CD32 and CD16 are expressed on the following cell types: B lymphocytes, NK cells, granulocytes, monocytes, macrophages, and platelets. Some antibodies bind these receptors, generating high background staining. This binding can be blocked by incubating the cells with unlabeled anti-CD32 and anti-CD16 for 30 min on ice prior to labeling. After this incubation, wash the cells.
3. Resuspend cells in 50-100 μ L staining buffer.
4. Add the antibody or antibodies (this protocol assumes the user has titrated the antibodies to the proper concentration for the designated cell number). Incubate at least 30 min at 2-8°C or on ice. Protect from light. **Note:** Some antibody protocols require room temperature incubations; if so incubate at room temperature (usually for peripheral blood mononuclear cells).
5. Wash the cells with staining buffer. For 12 x 75 mm tubes, add 2 ml buffer and centrifuge the cells. Aspirate the supernatant. For 96-well plates, add 100-200 μ l buffer, centrifuge, and aspirate supernatant. Repeat for a total of three times.
6. If the antibody is not directly conjugated to a fluorochrome, repeat the staining and wash steps (steps 4 and 5) for the secondary reagent.
7. Cells can be re-suspended in staining buffer and run on the flow cytometer. Alternatively, cells can be fixed in 0.5%-2% paraformaldehyde and run within a few days.

Materials:

Tubes and Plates:

12 x 75 mm Falcon tubes, or USA Scientific 1.2 ml tubes in 96-well plates, catalog # 1412-1410, or 96-well round bottom plates

Staining buffer:

Phosphate buffered saline with 0.1% BSA or 1% fetal calf serum

Blocking reagents:

Becton Dickinson Fc Block rat anti-mouse CD16/CD32, catalog # 553141 and 553142

eBioscience Anti-Mouse CD16/CD32, catalog # 14-0161-82

eBioscience Human Fc Receptor Binding Inhibitor, catalog # 14-9161-71

Biolegend TruStain fcX™ (anti-mouse CD16/32) Antibody, catalog # 101319

Biolegend Human TruStain FcX™ (Fc Receptor Blocking Solution), catalog # 422301