Antibody Staining Protocol

Cells should be suspended at approximately 1-10x10^6 cells/ml. Cells should be rinsed with PBS to remove serum proteins prior to antibody staining.

If staining with more than one antibody, prepare a pool of antibodies together.

All incubations should be performed on ice and with minimal light exposure.

Fc Block of Cells from Tissues

Phagocytic cells (such as macrophages) can bind non-specifically to antibodies unless the Fc receptors of these cells are blocked. If the sample is from a tissue homogenization possibly containing macrophages, or from a cell culture line expressing Fc receptors, you can use anti-Fc Receptor non-labeled antibody (anti-CD16/32 in mice) to block the receptors. Samples of cells without Fc Receptors can skip the Fc Block step.

1. Add 2 µl of Fc Block Antibody directly to 100 µl of suspended cells.
2. Incubate on ice for 12-15 minutes.
3. Proceed directly to Primary Antibody Staining (no need to rinse away Fc Block Antibody)

Primary Antibody Staining

1. Add 2 µl of primary antibody directly to 100 µl of suspended cells.
2. Incubate on ice for 12-15 minutes.
3. Add 2 ml PBS to rinse non-bound antibody.
4. Centrifuge at 2000 rpm for 5 minutes. Decant and resuspend cells in residual fluid (~100 µl). If your primary antibody is labeled with a fluorichrome, skip to step 10.

Secondary Antibody Staining

5. Add 2 µl of secondary antibody directly to the 100 µl of rinsed cells.
6. Incubate on ice for 12-15 minutes.
7. Add 2 ml PBS to rinse non-bound antibody.
8. Centrifuge at 2000 rpm for 5 minutes.
9. After centrifugation, decant the supernatant from the cell pellet. Resuspend the cells in residual fluid.

Dilute cells and analyze

10. Add 250 µl of cold Hanks or PBS to dilute cells. Check the sample for cell clumping. Cells that clump must be filtered through a 70 µ mesh filter prior to Flow cytometry to prevent clogging of the instrument. The sample is ready for Flow analysis.

Fixation of cells

If you will not be able to analyze the cells by Flow Cytometry within a few hours of staining, you should fix the cells.

11. Prepare a solution of 1% Paraformaldehyde, 1% sodium azide, in PBS.
12. After resuspending cells in residual fluid (when staining is complete at step 4 or step 9), add 250 µl 1% Paraformaldehyde solution.
13. Incubate for 10-15 minutes.
14. Add 2 ml PBS (preferably with 1% sodium azide).
15. Centrifuge at 2000 rpm for 5 minutes.

16. After centrifugation, decant the supernatant from the cell pellet. Resuspend the cells in residual fluid.
17. Add 250 µl of PBS (preferably with 1% sodium azide) to dilute cells.
18. The sample should be stored in the dark in the refridgerator and can be read at least several days later.
19. Prior to submitting the sample for analysis, check for cell clumping. Cells that clump must be filtered through a 70 µ mesh filter prior to Flow cytometry to prevent clogging of the instrument. The sample is ready for Flow analysis.

PBS solutions may contain 1% Sodium Azide to stabilize the light scatter. However, Sodium Azide is toxic, so it should not be used when cells will be retained for further culture.

Fixation may quench some fluorescent proteins such as GFP.

Be sure to fill out a RIC facility Flow Cytometry Sample submission form.
Be sure to schedule your Flow Analysis at least 24 hours in advance.
Be sure to schedule your Flow Sorting at least 1 week in advance.