CYTOMETRY RESEARCH, LLC

Direct Immunofluorescence Labeling

1. Prepare single-cell suspension with staining buffer at a cell concentration of 1-2 million per ml. (Note: Staining buffer = PBS, 2% FCS, 0.1% azide. The sodium azide assists in preventing capping and shedding or internalization of the antibody-antigen complex after the antibodies bind to the receptors.)
2. Place 1 ml of the cell suspension into each of the 12 x 75 polypropylene tubes.
3. Centrifuge at 250-300 x g for 5 minutes at 4°C. Use a pipet to remove the liquid. Be careful not to disturb the pellet. A slight amount of liquid can remain.
   Optional blocking step: If cells express high levels of Fc receptors that may contribute to non-specific binding and background fluorescence, do the following: Add 50 microliters/tube of blocking antibody, vortex and incubate for 2 min at room temperature. Have questions? Give us a call!
4. Add the appropriate amount of monoclonal antibody or control. The amount is usually given by the manufacturer. If not, it should have been determined previously by titration, using target cells with a large number of receptors. Cytometry Research can provide this service for you—just call for a quote.
5. Vortex. Keep the tubes on ice in a covered bucket (in the dark) for approximately 30 minutes.
6. First wash - Add 2 ml of staining buffer. Vortex.
7. Centrifuge and remove liquid; see Step 3.
8. Second wash - Add 2 ml of staining buffer. Vortex.
9. Centrifuge and remove liquid; see Step 3.
10. Add 1 ml of staining buffer. Vortex.

Keep cells on ice and covered; maintain in culture medium supplemented with antibiotics and 2-5% fetal bovine serum. If viability is less than 90%, consider adding another fluorochrome to identify dead cells during analysis. If cells cannot be analyzed within a few hours, cells may be centrifuged and fixed in 1 ml of 1% paraformaldehyde (in PBS) at 4°C for analysis the next day.