Indirect Immunofluorescence Staining of Cells and Blood

This technique is applicable where using unconjugated or biotin-conjugated monoclonal and polyclonal antibodies. A secondary reagent must be used to visualize the primary antibody e.g. avidin in the case of biotin.

Note. Specific methodology for blood appears in *[ ] brackets.*

1. Prepare cells appropriately (see here 1). Adjust the cell suspension to a concentration of 1 × 10e6 cells/ml with PBS/BSA buffer (phosphate buffered saline pH 7.4 and 1% BSA).
   [Whole blood samples may be used undiluted unless the cell count is high e.g. as in leukemia. EDTA and heparin are preferred anti-coagulants].
2. Aliquot 100 µl of cell suspension [whole blood] into as many test tubes as required.
3. Add primary antibody at the recommended dilution (see specific datasheets). Mix well and incubate at room temperature for 30 minutes.
4. Add 2 ml of PBS/BSA buffer, centrifuge at 400 *g* for 5 minutes and discard the resulting supernatant.
5. Add an appropriate secondary reagent at the recommended dilution (see specific datasheets). Mix well and incubate at room temperature for 30 minutes.
6. Wash cells with 2 ml of PBS/BSA, centrifuge at 400 *g* for 5 minutes and discard the supernatant.
   [To the blood suspension add freshly prepared red cell lysis buffer e.g. 2 ml of AbD Serotec's Erythrolyse and mix well. Incubate for 10 minutes at room temperature. Centrifuge at 400 g for 5 minutes and discard the supernatant].
7. Resuspend cells in 0.2 ml of PBS/BSA or with 0.2 ml of 0.5% paraformaldehyde in PBS/BSA if required.
8. Acquire data by flow cytometry. Appropriate standards should always be included e.g. an isotype-matched control sample.

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