



Direct Immunofluorescence Staining of Cells and Blood

This technique is applicable where the fluorochrome is directly linked to the primary antibody e.g. PE, FITC and Alexa Fluor® conjugates.

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

1. Prepare cells appropriately (see here 1). Adjust the cell suspension to a concentration of 1×10^6 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 μ l of cell suspension [whole blood] into as many test tubes as required.
3. Add antibody at the recommended dilution (see specific datasheets). Mix well and incubate at room temperature for 30 minutes.
4. Wash cells with 2 ml of PBS/BSA, centrifuge at 400 g for 5 minutes and discard the resulting 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].

Resuspend cells in 0.2 ml of PBS/BSA or with 0.2 ml of 0.5% paraformaldehyde in PBS/BSA if required.

Acquire data by flow cytometry. Appropriate standards should always be included e.g. an isotype-matched control sample.

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