Indirect Immunofluorescence Staining of Surface Epitopes of Cells and Blood

Protocol

FC5

This technique is applicable when using unconjugated or biotin-conjugated monoclonal and polyclonal antibodies recognizing cell surface antigens. A conjugated secondary reagent must be used to visualize the primary antibody, for example streptavidin in the case of biotin.

This method provides a general procedure for use with the majority of Bio-Rad reagents. In some cases specific recommendations are provided on product datasheets, and these methods should always be used in conjunction with the product and batch specific information provided with each vial. A certain level of technical skill and immunological knowledge is required for the successful design and implementation of these techniques; these are guidelines only and may need to be adjusted for particular applications. Specific methodology for blood appears in [ ] brackets.

Reagents:

- Phosphate buffered saline (BUF036A) containing 1% bovine serum albumin (PBS/BSA)
- PBS
- Erythrolyse red blood cell lysing buffer (BUF04)
- Anticoagulant (**Note:** for basic staining any appropriate anticoagulant, such as heparin, EDTA, or acid citrate dextrose, may be used. In some instances specific anticoagulants may be required)
- Optional: 0.5% (w/v) paraformaldehyde in PBS (**Note:** dissolve on heated stirrer and cool before use)

Method:

1. Prepare cells appropriately; refer to protocol FC1. 'Preparation of cells for flow cytometry' for further information. Adjust the cell suspension to a concentration of 1 x 10^7 cells/ml with cold (4°C) PBS/BSA buffer. *[Whole blood samples may be used undiluted unless the cell count is high, e.g. in leukemia. Use appropriate anticoagulant.]*
2. Aliquot 100 µl of the cell suspension [*or whole blood*] into as many test tubes as required.
3. Add primary antibody at the vendor-recommended dilution. Mix well and incubate at 4°C for at least 30 minutes.
4. Wash cells with 2 ml of cold (4°C) PBS/BSA, centrifuge at 300-400 g for 5 minutes and discard the supernatant. *[To the blood suspension add 2 ml of freshly prepared red cell lysis buffer and mix well. Incubate for 10 minutes at room temperature. Centrifuge at 300-400 g for 5 minutes and discard the supernatant. Wash with 2 ml of PBS/BSA, centrifuge at 300-400 g for 5 minutes and discard the supernatant.]*
5. Add an appropriate secondary reagent at the vendor-recommended dilution. Mix well and incubate at 4°C for at least 30 minutes, avoiding direct light.
6. Centrifuge at 300-400 g for 5 minutes at room temperature and discard the supernatant.
7. Resuspend cells in 200 µl of cold (4°C) PBS or with 200 µl of 0.5% paraformaldehyde in PBS if required.
8. Acquire data by flow cytometry. Analyze fixed cells within 24 hours.

Notes:

- To avoid unspecific binding, you also need to block Fc receptors on cell types such as spleen cells, with FcR blocking reagents e.g. Bio-Rad's Mouse Seroblock reagent (BUF041).
- Appropriate controls should always be carried out, for flow cytometry the following should be considered for inclusion;
  - Isotype controls used to determine if the staining is specific
  - Unstained cells should always be included in the experimental set-up to monitor autofluorescence.
- For all multi-color flow cytometry experiments it is advisable to include compensation controls and Fluorescence Minus One (FMO) controls, which assist with identifying gating boundaries.

For more information on flow cytometry resources visit **bio-rad-antibodies.com/applications**

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