

Direct Immunofluorescence Staining of Immunoglobulin Light Chains on B Lymphocytes in Whole Blood

FC6

For use with Bio-Rad's directly conjugated dual color reagents recognizing human kappa and lambda immunoglobulin light chains.

The immunofluorescent staining of immunoglobulin expression by B lymphocytes in whole blood requires a procedure to remove serum immunoglobulin, which otherwise interferes with staining with immunoglobulin-specific antibodies. 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.

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. Collect blood into an appropriate anticoagulant such as EDTA, heparin or acid citrate dextrose.
2. Aliquot 2-3 ml of whole blood into a 50 ml centrifuge tube.
3. Add 20-25 ml of PBS/BSA, pre-warmed to 37°C and mix well.
4. Centrifuge at 300-400 g for 5 minutes at 37°C. Carefully aspirate the supernatant, taking care not to disturb the cell pellet, and resuspend the pellet in the residual supernatant.
5. Repeat steps 3 and 4 twice more for a total of three washes.
6. Resuspend in 2-3 ml cold (4°C) PBS/BSA. Aliquot 100 µl of the washed blood into the required number of test tubes. Add appropriate volume of antibody at the vendor-recommended dilution and incubate at 4°C for at least 30 minutes, avoiding direct light.
7. Add 2 ml of freshly prepared erythrolyse red cell lysing buffer and mix well.
8. Incubate for 10 minutes at room temperature.
9. Centrifuge at 300-400 g for 5 minutes, discard supernatant.

10. Wash with 2 ml of room temperature PBS/BSA, centrifuge at 300-400 g for 5 minutes at room temperature and discard the supernatant.
11. Resuspend cells in 200 µl cold (4°C) PBS or with 200 µl of 0.5% paraformaldehyde in PBS if required.
12. Acquire data by flow cytometry. Analyze fixed cells within 24 hours.

Reference

Reynolds, WM et al. (1992). A simple technique for the determination of kappa and lambda immunoglobulin light chain expression by B cells in whole blood. *J Imm Meths.* 151:123-129.

Notes:

- The cell washing procedure described above has no effect upon other cell surface antigens, and has been shown not to affect the recovery of individual cell subsets.
- 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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