

Worksheet FC2

Indirect Immunofluorescence Staining for Flow Cytometry

For use with Biozol's unconjugated or biotin conjugated monoclonal and polyclonal antibodies recognising cell surface antigens.

Note:

This method provides a general procedure for use with the majority of Biozol reagents. In some cases specific recommendations are provided on product datasheets, and these methods should always be used in conjunction with product and batch specific information provided with each vial. Please note that 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.

1. Prepare cells in appropriate manner. Adjust cell suspension to a concentration of 1×10^6 cells/ml in PBS/BSA. Whole blood samples may also be used. Biozol recommends the use of EDTA anti-coagulant in these circumstances, although satisfactory results may be obtained using heparin or acid-citrate dextrose.
2. Aliquot 100 μ l of cell suspension/whole blood into required number of test tubes.
3. Add appropriate volume of Biozol primary antibody at recommended dilution (see specific datasheet for details). Mix well and incubate at room temperature for 30 minutes.
4. Add 2 ml of PBS/BSA, centrifuge at 400 x g for 5 minutes and discard supernatant.
5. Add appropriate volume of secondary reagent at recommended dilution (see specific datasheet for details). Mix well and incubate at room temperature for 30 minutes.
6. If using separated cells follow step 7.
If using whole blood samples add 2 ml of freshly prepared Biozol Erythrolyse (Cat. No. BZL05202) and mix well. Incubate for 10 minutes at room temperature. Centrifuge at 400 x g for 5 minutes. Discard supernatant.
7. Wash with 2 ml of PBS/BSA, centrifuge at 400 x g for 5 minutes and discard supernatant.
8. Resuspend cells in 0.2 ml of PBS/BSA or with 0.2 ml of 0.5% paraformaldehyde in PBS if required.
9. Acquire data by Flow Cytometry.

Notes:

Appropriate control samples should always be included. We recommend the inclusion of an isotype-matched control in each case. It may also be useful to include a control in which no primary antibody is used at all, to determine any non-specific binding of the secondary reagent to the target cells.

Please contact Biozol's Technical Services Department for details of recommended isotype controls and secondary reagents for specific applications.

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Solutions used:

PBS/BSA

Phosphate Buffered saline pH 7.4 containing 20 mM glucose and 1% Bovine Serum Albumin.

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