

Worksheet FC8

Whole Blood Protocol for Analysis of Intracellular Cytokines

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.

This method provides a rapid and simple approach to the analysis of intracellular cytokines by flow cytometry. It permits analysis of small samples, and avoids any possibility of generating artefactual results during the separation of peripheral blood cells by density gradient centrifugation.

N.B. All blood samples must be collected into heparin anti-coagulant. EDTA interferes with the cell stimulation process, and must be avoided.

1. Aliquot 0.5 ml blood into 2 tubes, and add 0.5 ml RPMI1640 without any additives.
2. To one tube (the resting population), add monensin to a final concentration of 3mM.
3. To other tube (activated cells), add PMA at a final level of 10 ng/ml, ionomycin at 2 mM and monensin at 3 mM.
4. Incubate for 2-4 hours at 37°C in a 5% CO₂ atmosphere.
5. At the end of the incubation period aliquot 100 µl samples into appropriate tubes.
6. Add required cell surface antibodies (e.g. CD3, CD4, CD8) and incubate for 15 minutes.
7. Add 100 µl Leucoperm™ (Cat. No. BZL00760) reagent A per tube, incubate 15 minutes.
8. Wash twice with PBS containing 0.1% sodium azide and 1% BSA.
9. Add 100 µl Leucoperm™ reagent B and required anti-cytokine antibodies.
10. Incubate for 20 minutes.
11. Wash twice, and analyse by flow cytometry.

Reference

Carrock-Sewell, W.A. *et al.* (1997). Determination of intracellular cytokines by flow cytometry following whole-blood culture. *J. Imm. Meths.* 209: 67-74.

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