

# Worksheet FC4

## *Direct Staining of Intracellular Antigens by Flow Cytometry*

The detection of intracellular antigens requires a cell permeabilisation step prior to staining. The method described below has been found to provide excellent results and may be used with both separated cells and whole blood samples. It may be used with cells of any species.

### Note:

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  $1 \times 10^7$  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. Add 100  $\mu$ l of cell suspension to the appropriate number of test tubes. If required, perform staining of cell surface antigens using appropriate directly conjugated monoclonal antibodies at this stage. Following staining for the recommended period continue with point 3 directly below.
3. Add 100  $\mu$ l "Leucoperm™" solution A (Catalogue number BZL00760). Incubate for 15 minutes at room temperature.
4. Add 3 ml wash buffer, and centrifuge for 5 minutes at 400g. Decant supernatant.
5. Resuspend cells in 100  $\mu$ l of "Leucoperm™" solution B.
6. Immediately add recommended volume of conjugated antibody recognising intracellular antigen to each tube. Mix well, and incubate for 30 minutes. If using an unconjugated primary antibody wash in 3ml of wash buffer as per step 4, and then repeat step 6 using appropriate secondary antibody. There is no requirement to add further Leucoperm.
7. Wash once in wash buffer, and resuspend in 0.25 ml 0.5% paraformaldehyde in PBS. Store at 4°C until acquisition on the flow cytometer, preferably within 24 hours.

### Buffers

**Wash buffer:** PBS containing 1% BSA and 0.09% sodium azide.

**FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE**

---

#### **BIOZOL**

Diagnostica Vertrieb GmbH  
Obere Hauptstr. 10b  
D-85386 Eching  
Postfach 2022  
D-85380 Eching

.. freecall **Bestell-Tel.: 08000-BIOZOL**  
(entspricht der Tel.-Nr. 0800-024 69 65)

Tel.: 089-37 99 666-6  
Fax.: 089-37 99 666-99  
info@biozol.com  
www.biozol.com