

# Worksheet FC5

## Direct Staining of Intracellular Antigens by Flow Cytometry - Methanol Modification

### Note:

The detection of intracellular antigens requires a cell permeabilization step prior to staining. The method described below has been found to provide excellent results in our hands; however other permeabilisation techniques have been published, and may also be successfully used in this application. This modification is particularly suitable for the detection of some nuclear antigens, such as PCNA and Ki67. In some cases specific recommendations are provided on product datasheets, and this method 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. Harvest cells and determine total number present. Wash twice in wash buffer. (If required, this procedure may also be used with whole blood samples)
2. If required perform staining of cell surface antigens using appropriate directly conjugated monoclonal antibodies at this stage. Following staining wash cells once in PBS and discard supernatant.

**N.B.** Phycoerythrin conjugates are not suitable for the detection of cell surface antigens using this method.

3. Resuspend cells in cold (2-8°C) Leucoperm™ solution A (Catalogue number BZL00760), using 50 µl per 5x10<sup>5</sup> cells. Incubate for 10 minutes at 2-8°C.
4. Add 500 µl cold absolute methanol, vortex and incubate for 10 minutes at 2-8°C.
5. Wash once in wash buffer
6. Resuspend cells in Leucoperm™ solution B, using 50 µl per 5 x 10<sup>5</sup> cells.
7. Aliquot 50 µl of cell suspension into the required number of tubes containing directly conjugated antibody. Incubate for 30 minutes at room temperature.
8. Wash once in wash buffer, and resuspend in 0.25 ml 0.5% paraformaldehyde in PBS.
9. Store at 4°C until acquisition on the flow cytometer, preferably within 24 hours

### Buffers

**Wash buffer** PBS containing 1% BSA and 0.1% 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