

Worksheet FC7

Protocol for Intracellular Staining with Leucoperm™

METHANOL MODIFICATION FOR DETECTION OF CELL CYCLE ANTIGENS SUCH AS Ki-67, PCNA AND BrdU

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. Add 100 µl of cells to each tube.
2. Add 100 µl of Leucoperm™ reagent A (Cat. No. BZL00760), and incubate for 3 minutes at room temperature.
3. Whilst vortexing gently add 2-4 ml of cold (0-4°C) absolute methanol.
4. Incubate for an additional 10 minutes at 0-4°C.
5. Centrifuge for 5 minutes at 350 x g, and wash once with PBS containing 1% BSA.
6. To the cell pellet add 100 µl of Leucoperm™ reagent B, and add appropriate volume of FITC conjugated antibody.
7. Vortex briefly at low speed, and incubate for 30 minutes.
8. Wash once in PBS/1% BSA and analyse by flow cytometry.

N.B. This protocol is not recommended for the simultaneous staining of cell surface antigens using RPE conjugated antibodies.

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