Direct Immunofluorescence Staining of Intracellular Antigens: Digitonin Method

Protocol

FC10

Alternative method for cell permeabilization required prior to intracellular staining that does not require the use of Leucoperm™ Accessory Reagent.

The detection of intracellular antigens requires a cell permeabilization step prior to staining. This method provides an alternative procedure for use when protocol FC7. “Direct staining of intracellular antigens: Leucoperm Accessory Reagent method” does not provide the desired results. In some cases specific recommendations are provided on product datasheets, and these methods should always be used in conjunction with the product and batch specific information provided with each vial. 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.

Note: This procedure causes a reduction in Forward Scatter (FSC) signal, so the flow cytometer set up may need to be adjusted to compensate.

Reagents:

- Phosphate buffered saline (BUF036A) containing 1% bovine serum albumin (PBS/BSA)
- PBS
- 0.5% (w/v) paraformaldehyde in PBS (**Note:** dissolve on heated stirrer and cool before use)
- 0.05% (v/v) Tween 20 in PBS
- 10 µg/ml digitonin in PBS

Method:

1. Prepare cells appropriately; refer to protocol FC1 cells. ‘Preparation of cells for flow cytometry’ for further information. Adjust the cell suspension to a concentration of 1 x 10^7 cells/ml with cold PBS/BSA.
2. Aliquot 100 µl of the cell suspension into required number of tubes.
3. If required perform staining of cell surface antigens using appropriate directly conjugated monoclonal antibodies at 4°C, avoiding direct light.
4. Wash twice in cold (4°C) PBS/BSA by pelleting cells at 300-400 g and 4°C for 5 minutes. Discard supernatant.
5. Add 50 µl PBS/BSA to each tube, followed by 100 µl 0.5% paraformaldehyde.
6. Incubate for 20 minutes at room temperature.
7. Wash twice with 3ml 0.05% Tween 20. Pellet cells at 300-400 g and 4°C for 5 min.
8. Decant supernatant. Add 100 µl of cold 10 µg/ml digitonin and the directly conjugated antibody at the vendor-recommended dilution and incubate for at least 30 minutes at 4°C, avoiding direct light.
9. Wash twice with 3 ml 0.05%Tween 20. After each wash step pellet cells at 300-400 g and 4°C for 5 minutes.
10. Resuspend in 200 µl PBS.
11. Acquire data by Flow Cytometry. Analyze fixed cells within 24 hours.

Notes:

- The following should be considered when designing your Flow Cytometry experiments:
  - To avoid unspecific binding, you also need to block Fc receptors on cell types such as spleen cells, with FcR blocking reagents e.g. Bio-Rad Mouse Seroblock reagent (BUF041).
  - Appropriate controls should always be carried out, for flow cytometry the following should be considered for inclusion;
    - Isotype controls used to determine if the staining is specific
    - Unstained cells should always be included in the experimental set-up to monitor autofluorescence.
- For all multi-color Flow Cytometry experiments it is advisable to include compensation controls and Fluorescence Minus One (FMO) controls, which assist with identifying gating boundaries.

For more information on Flow Cytometry resources visit **www.abdserotec.com/applications**

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