Direct Immunofluorescence of Intracellular Antigens: Paraformaldehyde/ Saponin Method

Protocol

FC11

Alternative protocol for cell permeabilization required prior to intracellular staining, that does not require the use of Leucoperm™.

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 and Cytokines by flow cytometry using Leucoperm” does not provide the desired results. This method is particularly suitable for use with directly conjugated FITC monoclonal antibodies. 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.

Reagents:

- Phosphate buffered saline (BUF036A) containing 1% bovine serum albumin (BSA)
- PBS
- 4% (w/v) paraformaldehyde in PBS (**Note:** dissolve on heated stirrer and cool before use)
- 0.1% (w/v) saponin in PBS
- Optional: 0.5% (w/v) paraformaldehyde in PBS (**Note:** dissolve on heated stirrer and cool before use)

Method:

1. Prepare cells appropriately; refer to protocol FC1. ‘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 (4°C) PBS/BSA.
2. If required, perform staining of cell surface antigens using appropriate directly conjugated monoclonal antibodies at 4°C, avoiding direct light.
3. Following staining, wash cells once in 3 ml PBS/BSA pellet cells at 300-400 g and 4°C for 5 minutes and discard the supernatant.
4. Resuspend cells in 100 µl 4% paraformaldehyde per 1×10^6 cells. Incubate for 20 minutes at room temperature. Wash once in 3 ml PBS/BSA.
5. Resuspend cells in 100 µl 0.1% saponin per 1 × 10^6 cells. Incubate for 15 minutes at room temperature.
6. Aliquot 100 µl of cell suspension into the required number of tubes containing directly conjugated antibody at the vendor-recommended dilution. Incubate for at least 30 minutes at 4°C, avoiding direct light.
7. Wash once in 3 ml 0.1% saponin and resuspend in 200 µl 0.5% paraformaldehyde.
8. 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’s 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 **bio-rad-antibodies.com/applications**

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