Immunofluorescence staining of cells in combination with PI staining of cells for cell cycle analysis

Protocol

FC13

This method provides a general procedure for DNA staining for cell cycle analysis using propidium iodide when you need to stain for other intracellular antigens. 70% ethanol is optimal for DNA so if you do not need to stain for other antigens we recommend Protocol FC12 “Propidium iodide staining of cells for cell cycle analysis”. This method will allow you to stain for DNA and cellular antigens, however these are guidelines only and the incubation times may need to be adjusted for different cell types and different antibodies.

Reagents:

- Phosphate buffered saline (PBS) (BUF036A)
- 70% Ethanol in DI water
- 2% (w/v) paraformaldehyde in PBS
- 0.1% saponin (w/v), 1% (w/v) bovine serum albumin in PBS
- Nucleic acid staining solution (1x PBS, 100 µg/ml RNAse A)

Method:

1. Prepare cells appropriately; refer to protocol FC1. ‘Preparation of cells for flow cytometry’ for further information.
2. Fix in 2 mls 2% paraformaldehyde for 30 minutes on ice.
3. Centrifuge at 500 x g for 5 minutes, decant supernatant.
4. Resuspend in 2-5 ml cold (4°C) 70% ethanol. Add drop wise to cell pellet while vortexing. Fix for at least 30 minutes on ice. **Note:** specimens can be left at 4°C for several weeks.
5. Centrifuge at 500 x g for 10 minutes, decant supernatant.
6. Wash twice with 0.1% saponin at 300-400 g for 5 minutes at 4°C, discard the supernatant.
7. Resuspend in 100 µl of 0.1% saponin and add the directly conjugated antibody at the vendor-recommended dilution and incubate for at least 30 minutes at 4°C, avoiding direct light.
8. Wash twice in PBS. Pellet cells at 300-400 g at 4°C for 5 minutes. Discard supernatant.
9. Resuspend in 500 µl nucleic acid staining solution and incubate for 30 min at room temperature avoiding direct light.
10. Add propidium iodide e.g. 1-2 drops of ReadiDrop™ propidium iodide (1351101).
11. Analyze cells by flow cytometry. The propidium iodide should be read on the appropriate channel in the linear scale. Doublets should be gated out using the Area vs Height or Width depending on your instrument.

Notes:

- Appropriate controls should always be carried out, for flow cytometry the following should be considered for inclusion;
  - A known positive sample
  - 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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