Propidium iodide staining of cells for cell cycle analysis

Protocol

FC12

This method provides a general procedure for DNA staining for cell cycle analysis using propidium iodide (PI). These are guidelines only and the incubation times may need to be adjusted for different cell types.

Reagents:

- Phosphate buffered saline (PBS) (BUF036A)
- 70% Ethanol in DI water
- 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 2-5 ml cold (4ºC) 70% ethanol. Add drop wise to cell pellet while vortexing. This should ensure fixation of all cells and minimize clumping.
3. Fix for at least 30 minutes on ice. **Note:** specimens can be left at 4ºC for several weeks.
4. Centrifuge at 500 x g for 10 minutes, decant supernatant.
5. Wash twice with 3 ml PBS at 300-400 g for 5 minutes at 4ºC and discard supernatant.
6. Resuspend cell pellet in 500 µl nucleic acid staining solution. Mix well.
7. Incubate for 30 minutes at room temperature.
8. Add propidium iodide e.g. 1-2 drops of ReadiDrop™ propidium iodide (1351101)
9. Analyze 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;
  - Unstained cells should always be included in the experimental set-up to monitor autofluorescence.

For more information on flow cytometry resources visit **bio-rad-antibodies.com/applications**

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