Technical Resources DSF·UNIPD

Flow cytometry and cell sorting facility
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Cell Cycle Analysis by Propidium Iodide Staining

Background

Propidium iodide (PI), a fluorescent dye that intercalates with DNA, is used to stain each cell. The fluorescence intensity of PI dye integrated into cells is directly proportional to the amount of DNA in cells. Because the dye cannot penetrate living cells, the latter are treated with a fixative before staining. This approach works well in assessing the cell cycle in cells fixed with 70% ethanol; other fixatives like aldehydes give unacceptably high coefficients of variations (CV) for the G1/G0 peaks.

Specimen

Cells in suspension, from cell culture, whole blood or bone marrow

Reagents and materials

- 5 ml flow cytometry test tubes
- refrigerate centrifuge
- sterile-filtered phosphate-buffered saline (PBS)
- 70 % ethanol, ice-cold
- 100 μg/ml RNAse A solution in PBS
- 50 μg/ml propidium iodide (PI) solution in distilled water

Controls

Untreated cells stained with PI

Procedure

1. Harvest cells in the appropriate manner, the objective is to get approximately 10^6 cells in each test tube.

2. Centrifuge cells at the appropriate conditions (likely 300 x g for 5 min), discard supernatant, resuspend in 3 ml PBS and centrifuge again.

3. Discard the supernatant and resuspend the pellet in 400 µl PBS.

4. Fix in 1ml cold 70 % ethanol. Add drop wise to cell pellet while vortexing. This should ensure fixation of all cells and minimise clumping. Place on ice for 30 min. Once fixed, cells may be stored for months in 70 % ethanol at 4°C prior to PI staining.

5. Centrifuge cells at higher speed compared to live cells for 5 minutes (ethanol-fixed cells become more floating), discard the supernatant being careful not to aspire the pellet.

6. Wash twice with 3 ml PBS.

7. To ensure DNA staining only, add 50 µl of RNase A solution directly to the pellet.

8. Add 400 µl of PI and mix well.

9. Incubate at room temperature for 5 to 10 minutes. For optimal staining, certain cells, such as fibroblasts, may require an extended incubation time, typically overnight.

10. Analyse samples by flow cytometry. Remember to analyse PI in a linear scale. Record at least 10,000 doublets-excluded events. Use a low flow rate to reduce CV of the peaks.