Propidium Iodide Staining of Cells for FACS Analysis

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[Abstract] Fluorescence Activated Cell Sorting (FACS) is used to study DNA cell content. Propidium iodide (PI) intercalates into double-stranded nucleic acids and fluoresces. It is excluded by viable cells but can penetrate cell membranes of dying or dead cells. Thus PI staining is included in immunofluorescent staining protocols to identify dead cells. DNA staining can be used to study the cell cycle. Relative DNA content shows the proportion of cells in G1, G2 and S phases. Apoptotic cells show characteristic smear on DNA staining. Here a protocol to stain cells by PI is described.

Materials and Reagents

1. Triton X-100 (Sigma-Aldrich, catalog number: T9284)
2. Propidium iodide (PI) (Sigma-Aldrich, catalog number: P4170)
3. RNase A (Sigma-Aldrich, catalog number: R4642)
4. Phosphate buffered saline (PBS)
5. 70% EtOH
6. 10 % Triton X-100 (see Recipes)
7. PI stock solution (see Recipes)

Equipment

1. Standard table-top centrifuges
2. FACS machine
3. Incubator

Procedure

1. Trypsinize and harvest cells, fix cells into 0.5 ml 70% EtOH (pre-cooled to -20 °C overnight).
2. Store fixed cells on ice at least 1 h and for up to several days.
3. Spin down cells for 2 min at 4,000 rpm.

4. Resuspend cell pellet in 0.5 ml PBS containing 0.25% Triton X-100 and incubate on ice for 15 min.
5. Spin down the cells for 2 min at 4,000 rpm.
6. Discard supernatant and resuspend cell pellet in 0.5 ml PBS containing 10 µg/ml RNase A and 20 µg/ml PI stock solution, transfer to FACS tubes and incubate at room temperature (RT) in the dark for 30 min.
7. Ready for FACS.

Recipes

1. 10% Triton X-100
   1 ml Triton X-100
   9 ml ddH_2O
2. 1 mg/ml PI stock solution
   10 mg PI
   10 ml ddH_2O

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