

Cell Viability Analysis using Propidium Iodide

1. Test principle

Propidium Iodide can only pass through disordered areas of membrane of dead cells and intercalates with the DNA of the nuclei, emitting red fluorescence light.

2. Specimen

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

3. Materials and reagents

- 5ml Falcon polypropylene Round-Bottom test tubes (12x75 mm)
- cooling centrifuge
- sterile-filtered Phosphate-buffered saline (PBS)
- PBS + 2% FBS or PBS + 0.1 % BSA
- 1×10^6 cells / ml cell suspension
- Propidium iodide (PI) solution:
Dissolve PI (Sigma, P 4170 or Invitrogen # P3566) in dH₂O at 1 mg/ml.
Store aliquots at -20°C for up to 2 years. Aliquots that are frequently used can be stored at 4°C for up to 2 months. Discard solutions of PI that have been exposed to room temperature for more than 48 hr, or if they appear dark red.
- Trypsin for adherent cells (instead of trypsin, you can use Accutase Enzyme Cell Detachment Medium by eBioscience, Catalog number 00-4555)

4. Controls

- Negative control - cells without PI staining
- Positive control - cells stained with PI

5. Procedure

Adherent Cells: step 1 – step 9 (or using Accutase cell detachment solution)

Suspension Cells: step 6 – step 9

1. trypsinize for 10 minutes at 37 ° C ; it varies
2. add the medium with serum to stop the trypsinization
3. pipette the cell suspension up and down to separate cell clumps
4. transfer the cell suspension to the tube and spin
5. wash cells 2 times with PBS + 2% FBS
6. resuspend and aliquot 1×10^6 cells in 1 ml PBS + 2 % FBS in the 12 x 75 mm polypropylene tube
7. add final concentration of 2 µg/ml PI to cell suspension
8. incubate 15 min on ice in the dark. (Avoiding strong lights is recommended from step 7.)
9. analyze by flow cytometer

Note: PI is a nucleic acid-specific, red-fluorescent dye. It is also a suspected carcinogen, so employ appropriate safety precautions when working with this dye.
