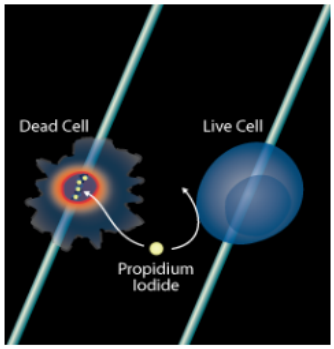
Flow Cytometry Protocol for Analysis of Cell Viability using Propidium Iodide



Flow cytometry provides a rapid and reliable method to quantify viable cells in a cell suspension. Determination of cell viability is critical when evaluating the response to cytotoxic drugs or other environmental factors. In addition, it is often necessary to detect dead cells in a cell suspension in order to exclude them from the analysis. Dead cells can generate artifacts as a result of nonspecific antibody binding or through unwanted uptake of fluorescent probes. One method to assess cell viability is through the use of dye exclusion. Live cells have intact membranes that exclude a variety of dyes that easily penetrate the damaged, permeable membranes of non-viable cells.

Propidium iodide (PI) is a membrane impermeant dye that is generally excluded from viable cells. It binds to double stranded DNA by intercalating between base pairs. PI is excited at 488 nm and, with a relatively large Stokes shift, emits at a maximum wavelength of 617 nm. Because of these spectral characteristics, PI can be used in combination with other fluorochromes excited at 488 nm such as fluorescein isothiocyanate (FITC) and phycoerythrin (PE). The following protocol has been developed and optimized by R&D Systems Flow Cytometry Laboratory for the staining of non-viable cells with PI.

Note: PI is a suspected carcinogen and should be handled with care. The dye must be disposed of safely and in accordance with applicable local regulations.

Please read the protocol in its entirety before starting.

Reagents Required

- PBS (1X): 0.137 M NaCl, 0.05 M NaH_2PO_4, pH 7.4 or Hank's Balanced Salt Solution (HBSS; 1X)
- Flow Cytometry Staining Buffer (R&D Systems, Catalog # FC001, or an equivalent solution containing BSA and sodium azide)
- PI Staining Solution: 10 µg/mL PI in PBS stored at 4 °C in the dark
- Detection Antibodies (optional)
- Isotype Control Antibodies (optional)

Materials Required

- FACS™ Tubes (5 mL round-bottom polystyrene tubes)
- Pipette Tips and Pipettes
- Centrifuge
- Vortex

Procedure

Note: Staining of surface antigens with antibodies may be done at this point. PI cannot be used when labeling intracellular molecules.

Note: Use the FL-2 channel if staining only with PI. Collect PI fluorescence in the FL-3 channel if the cells have been stained with an FITC- or a PE-conjugated antibody.

Note: Do not wash cells after the addition of the PI staining solution.

1. Harvest cells and aliquot up to 1 x 10^6 cells/100 µL into FACS tubes. Wash the cells 2 times by adding 2 mL of PBS (or HBSS), centrifuging at 300 x g for 5 minutes, and then decanting the buffer from the pelleted cells.
2. Resuspend cells in 100 µL of Flow Cytometry Staining Buffer.
3. To adjust flow cytometer settings for PI, add 5 - 10 µL of PI staining solution to a control tube of otherwise unstained cells. Mix gently and incubate for 1 minute in the dark.
4. Determine PI fluorescence (using the FL-2 or FL-3 channel) with a FACScan™ instrument.
5. Acquire data for unstained cells and single-color positive controls.
6. Add 5 - 10 µL of PI staining solution to each sample just prior to analysis. Set the stop count on the viable cells from a dot-plot of forward scatter versus PI.

Flow Cytometry

Related Information

- Flow Cytometry Protocols
- Flow Cytometry Protocols
- Flow Cytometry Supplemental reagents

Literature
Product Categories
Protocols

Chromatin Immunoprecipitation (ChIP) Protocol

Flow Cytometry Protocols

Protocol For Analysis of Cell Viability using 7-Amino Actinomycin D (7-AAD)

Protocol For Analysis of Cell Viability using Propidium Iodide

Protocol For Staining Intracellular Molecules using Alcohol to Permeabilize the Cell Membrane

Protocol For Staining Intracellular Molecules using Detergents to Permeabilize the Cell Membrane

Protocol For Staining Membrane-associated Proteins in Suspended Cells

IHC/ICC Protocols

Proteome Profiler Arrays