Best in Class

Protocol for cell viability

Propidium iodide for flow cytometry

Background information

Flow cytometry is 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 fluorescent dye that intercalates into double-stranded nucleic acid. It is excluded from viable cells, but can penetrate cell membranes of dead or dying cells. Therefore, it is widely used for evaluation of cell death and apoptosis or for determination of DNA content in cell cycle analysis. The fluorescence emission maximum for DNA-bound PI is about 615–620 nm. When excited by a 488 nm laser, PI can therefore be detected in both, the red fluorescence channel commonly used for R-phycoerythrin (PE)-Cy5 tandem dye detection as well as the yellow fluorescence channel commonly used for PE detection. PI can be used in combination with other fluorochromes excited at 488 nm such as fluorescein isothiocyanate (FITC).

Caution: Propidium iodide is a suspected carcinogen; contact with eyes, skin, and mucous membranes should be avoided. Always wear proper protective clothing and gloves when handling the solution.

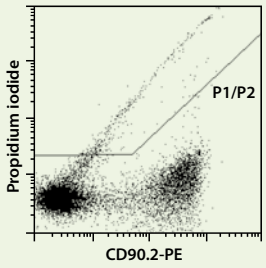

Figure 1: Shortly before flow cytometric analysis 10 µL of Propidium Iodide Solution (# 130-093-233) was added to 1 mL of cell suspension.

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