PROPIDIUM IODIDE STAINING OF DEAD CELLS FOR FLOW CYTOMETRY

Propidium iodide (PI) intercalates into double-stranded nucleic acids. It is excluded by viable cells but can penetrate cell membranes of dying or dead cells.

I. MATERIALS:

1. Propidium iodide (e.g., Cat #537059, EMD Millipore, MA)

2. Buffer: 1 X PBS (Ca^{2+} and Mg^{2+} free, e.g., Cat #9240, Irvine Scientific, CA)
+2% newborn calf serum (or 0.2% BSA)
+0.1% sodium azide

A) PI buffer:

Dissolve PI in buffer at a concentration of 1 µg/mL. Keep the solution tightly closed at 4°C protected from light. Discard after 1 month.

B) PI stock buffer:

Dissolve PI in buffer at a concentration of 500 µg/mL. Keep the solution tightly closed at 4°C protected from light. We have kept this solution for several months and did not observe loss in staining activity.

II. METHOD:

Stain your cells as outlined in the protocol for single-color staining with FITC-labeled monoclonal antibodies.

A) After the last washing step resuspend your cell pellet in the PI buffer and keep your samples in that solution at 4°C protected from light until analysis on the flow cytometer.

B) After the last washing step resuspend your cells as usual for analysis. If you want to assess viability of your samples add 2 µL of the PI stock solution to each tube and mix well. Keep the samples in this solution at 4°C protected from light until analysis on the flow cytometer.

NOTE: This method cannot be used on formaldehyde-fixed samples. It is possible to use it on samples that are stained with PE (phycoerythrin)-conjugated antibodies according to a method by Sasaki *et al.* (*Cytometry* 8:413, 1987). However, because of the extensive overlap of the emission spectra of PI into the PE channel and other fluorescence channels, it is preferable to use dead cell discrimination with 7-amino-actinomycin D (see the appropriate protocol) or other commercially available dyes that are suitable.