Propidium Iodide Staining for DNA Content in Ethanol Fixed Cells

The most frequently used method for measuring DNA content of cells involves the fixation and permeabilization of the cells with ethanol. The ethanol treatment provides access of the dye to allow the intercalation of the dye into the DNA grooves. Formaldehyde treatment of the cells, while adequately fixing and permeabilizing the cell interferes with access of the dye to the DNA. This is apparently due to the fixation of chromatin proteins onto the DNA leading to steric hindrance of the dye access to the DNA grooves.

The cells should be prepared into a single cell suspension. For adherent cell lines the cells will need to be released from the culture dish. Trypsin with or without EDTA is usually sufficient but there are other methods. Scraping cells off culture surfaces is **NOT** recommended.

Solid tissues will need to be disaggregated using enzyme treatment usually with collagenase and proteinases. After the cells are prepared into a single cell suspension they should be washed with PBS and 1-2 x 106 cells placed in 5ml of PBS

1. Prepare the ethanol (70%) and bring to 4°C or colder (place in a -20°C freezer for several hours). Place 4.5ml into each polypropylene centrifuge tube. Prepare one tube of ethanol for each 5ml of cell suspension. Keep ethanol on ice at all times.

2. Prepare the staining solution. To 10ml of 0.1% TritonX-100 in PBS add 2mg DNase-free RNase and 200ug of 1mg/ml propidium iodide in water. RNase may be purchased DNase free or made DNase free by placing a small volume in a 100°C water bath for 5 min.

3. Place 5ml of cell suspension in a polypropylene test tube (round bottom is best) and centrifuge to pellet the cells. Decant supernatant immediately after centrifugation stops.

4. Break up cell pellet and then add 0.5ml of PBS and completely resuspend the cells.

5. Add the cell suspension to one tube of ethanol and mix thoroughly.

6. Keep the cells in the ethanol for at least 2 hours.

7. Centrifuge the ethanol suspension of cells and decant the ethanol completely.

8. Suspend the cells in 5ml of PBS and wait 1-2 minutes. Centrifuge cells. Some cell types do not like to centrifuge well and the pellet almost will disappear. It sometimes helps to include 0.5% BSA in the PBS.

9. Break up the cell pellet and then resuspend the cells in 1ml of the staining solution.

10. Keep at 37°C for 15 min. or at room temp for 30 min. Place on ice and keep covered with foil until analyzed on the flow cytometer.