

Propidium Iodide (PI) DNA Labeling following Ethanol Fixation

The following outlines the procedure for quickly fixing and labeling cells for flow cytometric analysis of cellular DNA content. Several points should be considered before labeling:

Viability -- Low viability can dramatically affect the quality of results, due to added sensitivity to handling and the potential for increased subcellular debris. Also, free DNA fragments can lead to increased clumping and noise, thus confounding resultant histograms. If problems with viability or debris are concerns, adding a DNASE to the wash buffer BEFORE fixation with ethanol can improve histogram quality.

Cell number -- Cell concentration can affect PI signals. This procedure is appropriate for the indicated cell number. Counts dramatically disparate from this (e.g., a factor of 5) will change critical dye:cell dynamics, possibly resulting in artifactual peak shifts. Accurate knowledge of FINAL cell counts (following all treatments/manipulation) is recommended.

Internal standards (IS) -- Controls used in peak positioning and ploidy determination should be processed the same as experimental specimens. When adding IS (e.g., trout or chicken red cell nuclei), be sure to maintain a reasonable cell:IS balance; too many IS cells may overwhelm and confound the analysis.

Note: Cultured cells and ficoll separated nucleated cells from whole blood should be washed 1x before fixation to remove serum-based proteins that may precipitate in ethanol.

1) Count cells. Pellet $0.5-1 \times 10^6$ cells per sample. Resuspend in 0.5 ml PBS.

Note: never add fixative to a cell pellet -- may cause clumping and loss of sample.

2) Add 0.5ml 100% cold ethanol, drop-wise while vortexing. Incubate for a minimum of 20 minutes.

3) Pellet cells (1000 rpm for 5-7 minutes) then carefully invert tubes to decant ethanol. While inverted, gently blot tubes to wick away excess ethanol.

Note: Removing all of the ethanol is not essential -- trace remaining will be diluted.

4) Add 0.5ml PI-RNase solution (final concentrations: $50\mu\text{g/ml}$ PI + $100\mu\text{g/ml}$ RNase Type I-A in PBS). Mix well, then incubate (room temperature in the dark) for a minimum of 20 minutes.

5) Analyze by flow cytometry. Acquire a minimum of 5×10^3 cells in a histogram, if possible.