Cell cycle and Apoptosis

Cytometry & Cell Sorting
NYULMC Divison of Advanced Research Technologies

- For detection of apoptosis in ethanol fixed samples
- Any DNA fluorochrome can be used
- Can be used along with immunofluorescence staining

Reagent List (pick one dye):
Stock solutions

- Propidium Iodide 0.5 mg/ml in PBS, final 50ug/ml
- Hoechst 33342 (or 33258) 1.0 mg/ml in dH2O, final 1ug/ml
- Chromomycin A3 1.0 mg/ml in PBS, final 20ug/ml
- 80% Ethanol (on ice)
- Buffer for PI staining: PBS + Triton X-100 (0.1%) + 0.1 mM EDTA + 50ug/ml RNase (50 units/mg)
- Buffer for Hoechst 33342: PBS (no additions)
- Buffer for Chromomycin A3:PBS + 5mM MgCl2

Fixation

1. Wash cells into PBS on ice.
2. Add 1ml of iced cell suspension (2x10E6 cells/ml) to 1ml of iced 80% ethanol dropwise while vortexing
3. Allow to incubate a minimum of 30 minutes on ice.

Staining

1. Cells are incubated in respective dye at room temperature, in the dark 30-60 minutes.
2. Cells are analyzed without washing.

Tips

1. Ethanol fixation can decrease cell number.
2. Inadequate RNase digestion can cause broadening of peak distributions (higher CV's)
3. Inconsistant cell number and or dye concentration can cause cell cycle peak position to vary between samples.
4. Cell cycle may be done in conjunction with any green fluorescent compounds (FITC,FDG...)

Reference

Telford,W.G., King, L.E., Fraker, P.J.(1992) Comparative evaluation of several DNA binding dyes in the detection of apoptosis-associated chromatin degradation by flow cytometry. Cytometry 12:137-143