Apoptosis detection using permiable cells

- 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

PROTOCOL:

Fixation:
Cold ethanol fixation, as described earlier

Staining:
Buffer for PI staining:
  PBS + Triton X-100 (0.1%) + 0.1 mM EDTA + 50 ug/ml RNase (50 u
Buffer for Hoechst 33342 (or 33258)
  PBS (no additions)
Buffer for Chromomycin A3
  PBS + 5mM MgCl2

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

TIPS:

REFERENCE:

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

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