

# Apoptosis detection using permeable cells

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- For detection of apoptosis in ethanol fixed samples
  - Any DNA fluorochrome can be used
  - Can be used along with immunofluorescence staining
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## REAGENT LIST (Pick one dye):

### Stock solutions

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|----------------------------|---------------------------------|
| - 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 |
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## 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.
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## TIPS:

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## REFERENCE:

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

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