

# Chromatin Condensation/Membrane Permeability/Dead Cell Apoptosis Kit with Hoechst 33342/YO-PRO®-1 and PI for Flow Cytometry

Catalog no. V23201

**Table 1.** Contents and storage information.

Material	Amount	Composition	Storage*	Stability
Hoechst 33342 (Component A)	200 µL	5 mg/mL (8.1 mM) solution in water	<ul style="list-style-type: none"> <li>• 2–6°C</li> <li>• Dessicate</li> <li>• Protect from light</li> <li>• DO NOT FREEZE</li> </ul>	When stored as directed this kit is stable for 6 months.
YO-PRO®-1 (Component B)	200 µL	100 µM solution in DMSO		
Propidium iodide (PI, Component C)	200 µL	1 mg/mL (1.5 mM) solution in water		

\*For long-term storage, store the vial of YO-PRO®-1 dye at ≤–20°C. All three dyes included in the kit are light sensitive and may be handled in normal room light, but avoid prolonged exposure to light.

**Number of assays:** Sufficient material is supplied for 200 flow cytometry assays each having  $2 \times 10^5$  to  $1 \times 10^6$  cells in a 1 mL volume.

**Approximate fluorescence excitation/emission maxima:** YO-PRO®-1: 491/509 in nm; Hoechst 33342: 350/461 in nm, bound to DNA; PI: 535/617 in nm, bound to DNA.

## Introduction

Apoptosis is a carefully regulated process of cell death that occurs as a normal part of development. Inappropriately regulated apoptosis is implicated in disease states, such as Alzheimer’s disease and cancer. Apoptosis is distinguished from necrosis, or accidental cell death, by characteristic morphological and biochemical changes, including compaction and fragmentation of the nuclear chromatin, shrinkage of the cytoplasm and loss of membrane asymmetry.<sup>1–5</sup>

The Chromatin Condensation/Membrane Permeability/Dead Cell Apoptosis Kit with Hoechst 33342/YO-PRO®-1 and PI for flow cytometry provides a rapid and convenient assay for apoptosis. The kit contains ready-to-use solutions of three nucleic acid stains—YO-PRO®-1 dye, propidium iodide, and Hoechst 33342. Green-fluorescent YO-PRO®-1 dye can enter apoptotic cells, whereas red-fluorescent propidium iodide (PI) cannot. Thus, after staining with YO-PRO®-1 dye and PI, apoptotic cells show green fluorescence and dead cells show primarily red fluorescence and some green fluorescence (Figure 1). Blue-fluorescent Hoechst 33342 brightly stains the condensed chromatin of apoptotic cells and more dimly stains the normal chromatin of live cells (Figure 2). The staining pattern resulting from the simultaneous use of these three dyes makes it possible to distinguish normal, apoptotic and dead cell populations by flow cytometry or fluorescence microscopy.

The three stains are supplied as separate solutions to facilitate optimization of the assay for the cell line under study and the equipment available. However, once optimized, the assay can be completed by simultaneously staining the cells with a mixture of the three nucleic acid stains. All three dyes can then be excited using a combination of UV excitation for the

Hoechst 33342 dye, and 488 nm excitation for the YO-PRO®-1 and propidium iodide dyes. Differences in dye-staining intensity may make it difficult to simultaneously photograph the live, apoptotic and dead cells by microscopy.

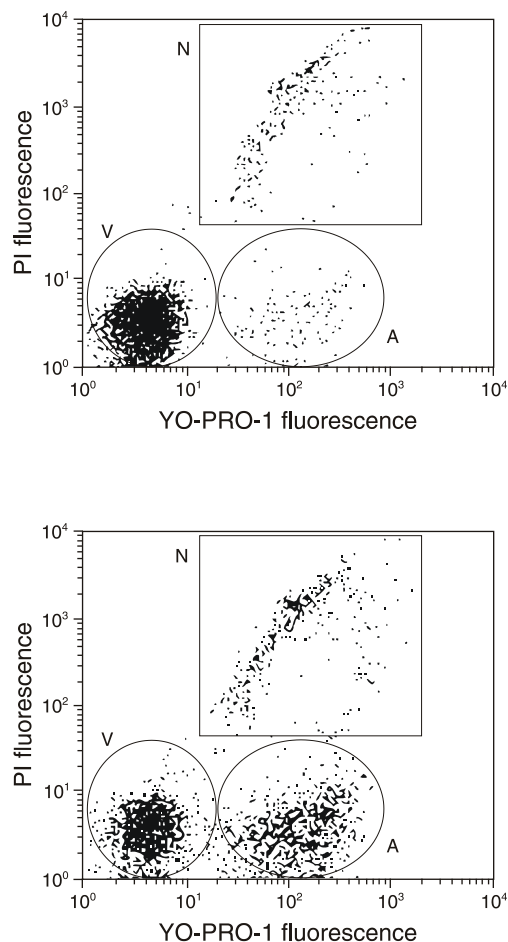
## Before Starting

### Materials Required but Not Provided

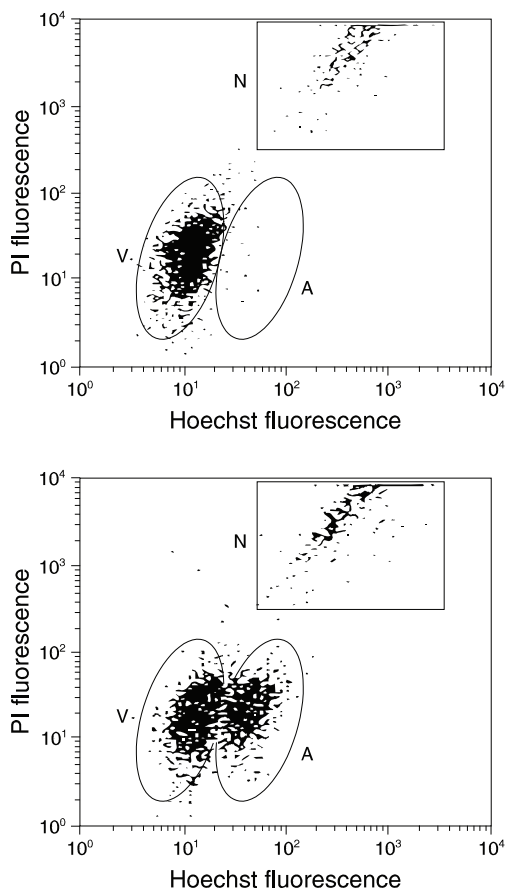
- Samples (appropriate sample concentrations range from  $2 \times 10^5$  to  $1 \times 10^6$  cells/mL)
- Inducing agent
- Phosphate buffered saline (PBS)
- Deionized water

### Caution

Propidium iodide and Hoechst 33342 are potential mutagens; use with appropriate precautions. No data are available addressing the mutagenicity or toxicity of YO-PRO®-1 dye. Because this reagent binds to nucleic acids, treat it as a potential mutagen and handle with appropriate care. Handle the DMSO stock solution with particular caution, as DMSO is known to facilitate the entry of organic molecules into tissues.



**Figure 1.** Jurkat cells (T-cell leukemia, human) treated with 10  $\mu$ M camptothecin for 4 hours (bottom panel) or untreated (as control, top panel). Cells were then stained with YO-PRO®-1 dye and propidium iodide and analyzed by flow cytometry using 488 nm excitation. Note that the camptothecin-treated cells (bottom panel) have a higher percentage of apoptotic cells (indicated by an "A") than the basal level of apoptosis seen in the control cells (top panel). V = viable cells, N = necrotic cells.



**Figure 2.** Jurkat cells (T-cell leukemia, human) treated with 10  $\mu$ M camptothecin for 4 hours (as control, top panel). Cells were then stained with Hoechst 33342 dye and propidium iodide and analyzed by flow cytometry using UV/488 nm dual excitation. Note that the camptothecin-treated cells (bottom panel) have a higher percentage of apoptotic cells (indicated by an "A") than the basal level of apoptosis seen in the control cells (top panel). V = viable cells, N = necrotic cells.

## Experimental Protocol

The following is a suggested protocol for this assay. Some modifications may be required depending on the particular cell type used.

1. Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of an inducing agent.
2. Harvest the cells after the incubation period.
3. Wash the cells in cold phosphate-buffered saline (PBS) and adjust the cell density to  $\sim 1 \times 10^6$  cells/mL in PBS. For each assay, use a 1 mL volume.
4. Add 1  $\mu$ L of the Hoechst 33342 stock solution (Component A), 1  $\mu$ L of the YO-PRO<sup>®</sup>-1 stock solution (Component B), and 1  $\mu$ L of the propidium iodide stock solution (Component C) to each 1 mL of cell suspension.
5. Incubate the cells on ice for 20–30 minutes.
6. As soon as possible after the incubation period, analyze the stained cells by flow cytometry, measuring the fluorescence emission at  $\sim 460$  nm,  $\sim 530$  nm, and  $>575$  nm. Use UV/488 nm dual excitation: UV excitation for detection of the  $\sim 460$  nm emission of Hoechst 33342 dye

and 488 nm excitation for detection of the ~530 nm emission of YO-PRO®-1 dye and the >575 nm emission of propidium iodide.

The population separates into three groups; live cells show only a low level of blue fluorescence, apoptotic cells show bright green and blue fluorescence, and necrotic cells show bright red fluorescence, as well as green and blue fluorescence (see Figures 1 and 2). Confirm the flow cytometry results by viewing the cells under a fluorescence microscope using filters appropriate for Hoechst or DAPI, fluorescein (FITC) and rhodamine (TRITC) or Texas Red® dye.

## References

1. Immunol Cell Biol 76, 1 (1998); 2. Cytometry 27, 1 (1997); 3. J Pharmacol Toxicol Methods 37, 215 (1997); 4. FASEB J 9, 1277 (1995); 5. Am J Pathol 146, 3 (1995).

## Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
V23201	Chromatin Condensation/Membrane Permeability/Dead Cell Apoptosis Kit with Hoechst 33342/YO-PRO®-1 and PI *flow cytometry* *200 assays* .....	1 kit

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