Chromatin Condensation/Dead Cell Apoptosis Kit with Hoechst 33342 and PI for Flow Cytometry

Catalog no. V13244

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Hoechst 33342 (Component A)	200 µL	5.0 mg/mL solution in water	• 2–6°C • Protect from light	When stored as directed, kit components should be stable for at least 6 months.
Propidium iodide (PI, Component B)	200 µL	1.0 mg/mL solution in water		

* Hoechst 33342 and propidium iodide may be handled in normal room light, but avoid prolonged exposure to light.

Number of assays: The kit provides sufficient reagents for 200 flow cytometry assays, each having 2×10^5 to 1×10^6 cells in a 1 mL volume.

Approximate fluorescence excitation/emission maxima, when bound to DNA: ~350/461 nm Hoechst dye; ~535/617 nm propidium iodide

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.[1-5]

The Chromatin Condensation/Dead Cell Apoptosis Kit with Hoechst 33342 and PI provides a rapid and convenient assay for apoptosis based upon fluorescence detection of the compacted state of the chromatin in apoptotic cells. The kit contains ready-to-use solutions of the blue-fluorescent Hoechst 33342 dye (excitation/emission maxima ~350/461 nm, when bound to DNA), which stains the condensed chromatin of apoptotic cells more brightly than the chromatin of normal cells, and the red-fluorescent propidium iodide dye (excitation/emission maxima ~535/617 nm, when bound to DNA), which is permeant only to dead cells. The staining pattern resulting from the simultaneous use of these dyes makes it possible to distinguish normal, apoptotic, and dead cell populations by flow cytometry (Figure 1).[2,6,7] The 351 nm line of an argon-ion laser or other suitable UV source is required for excitation of the Hoechst 33342 dye, whereas propidium iodide may be excited with the 488 nm line of an argon-ion laser. We have optimized this assay using Jurkat cells, a human T-cell leukemia clone, treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types.

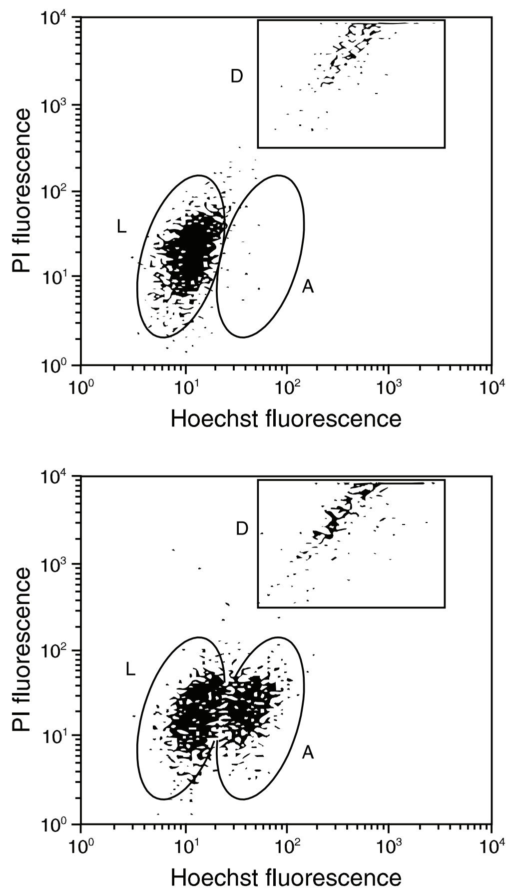

Figure 1. Jurkat cells (T-cell leukemia, human) treated with 10 µM camptothecin for four hours (bottom panel) or untreated (as control, top panel). Cells were then treated with the reagents in the kit and analyzed by flow cytometry using UV/488 nm dual excitation. Note that the campthothecin-treated 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). L = live cells, D = dead cells.

Before You Begin

Materials Required but Not Provided

- Phosphate-buffered saline (PBS)
- Camptothecin or other agent to induce apoptosis

Caution

Propidium iodide and Hoechst 33342 are known or suspected mutagens and should be used with appropriate precautions.

Experimental Protocols

We have optimized this assay using Jurkat cells treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types.

1. Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.

2. Harvest the cells after the incubation period, wash in cold phosphate-buffered saline (PBS) and adjust the cell density to ~1×10^6 cells/mL in PBS. For each assay, a 1 mL volume will be used.

3. Add 1 µL of the Hoechst 33342 stock solution (Component A) and 1 µL of the PI stock solution (Component B) to each 1 mL of cell suspension.

4. Incubate the cells on ice for 20–30 minutes.

5. As soon as possible after the incubation period, analyze the stained cells by flow cytometry, using UV/488 nm dual excitation and measuring the fluorescence emission at ~460 nm and >575 nm. The population should separate into three groups: live cells will show only a low level of fluorescence, apoptotic cells will show a higher level of blue fluorescence, and dead cells will show both blue and red fluorescence (see Figure 1). Confirm the flow cytometry results by viewing the cells under a fluorescence microscope, using filters appropriate for Hoechst or DAPI 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); **6.** Cytometry 17, 59 (1994); **7.** Exp Cell Res 211, 322 (1994).

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

Cat. no.	Product Name	Unit Size
V13244	Chromatin Condensation/Dead Cell Apoptosis Kit with Hoechst 33342 and PI *for flow cytometry* *200 assays*	1 kit

Contact Information

Molecular Probes, Inc.
29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:
6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:
Order Phone: (800) 438-2209
Order Fax: (800) 438-0228

Technical Service:
8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

For country-specific contact information, visit www.invitrogen.com.

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