Membrane Permeability/Dead Cell Apoptosis Kit with PO-PRO®-1 and 7-aminoactinomycin D for Flow Cytometry

Catalog no. V35123

Table 1. Contents and storage information.

Material	Amount	Composition	Storage*	Stability
PO-PRO®-1 dye (Component A)	500 µL	1 mM solution in DMSO	• 2–6˚C • Dessicate • Protect from light	When stored as directed this kit is stable for 6 months.
7-aminoactinomycin D (7-AAD, Component B)	200 µL	1 mg/mL solution in DMSO		
*For long-term storage, store the vial of PO-PRO®-1 dye at ≤–20˚C. The PO-PRO®-1 dye and 7-AAD 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 2×10^5 to 1×10^6 cells in a 1 mL volume.				
Approximate fluorescence excitation/emission maxima: PO-PRO®-1**:** 434/456 in nm; 7-AAD: 546/647 in nm.				

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] Furthermore, during apoptosis the cytoplasmic membrane becomes slightly permeant. Certain dyes, such as the violet fluorescent PO-PRO™-1 dye can enter apoptotic cells, whereas other dyes, such as the red fluorescent dye, 7-aminoactinomycin D (7-AAD), cannot. The use of YO-PRO®-1 dye (closely related to PO-PRO™-1 dye) with propidium iodide provides a sensitive indicator for apoptosis.[6–9] PO-PRO™-1 dye and 7-AAD provide analogous performance. In addition, annexin V gives poor results with trypsinized cells while PO-PRO™-1 dye provides the same efficiency with trypsinized cells as it does with suspension cells.

The Membrane Permeability/Dead Cell Apoptosis Kit with PO-PRO®-1 and 7-aminoactinomycin D for flow cytometry provides a rapid and convenient assay for apoptosis. The kit contains ready-to-use solutions of both PO-PRO®-1 and 7-AAD dyes. After staining a cell population with PO-PRO®-1 dye and 7-AAD, apoptotic cells show violet fluorescence, dead cells show violet and red fluorescence, and live cells show little or no fluorescence (Figure 1). These populations can easily be distinguished by a flow cytometer that uses both a violet laser and the 488 nm line of an argon-ion laser for excitation.

We have optimized this assay using Jurkat cells, a human T-cell leukemia line, treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types. Because no single parameter defines apoptosis in all systems, we strongly suggest using a combination of different measurements for reliable detection of apoptosis. Refer to

our website at probes.invitrogen.com for a wide selection of products for apoptosis research.

Before Starting

Materials Required but Not Provided

- Samples (appropriate sample concentrations range from 2×10^5 to 1×10^6 cells/mL)
- Inducing agent
- 2 mM hydrogen peroxide
- Phosphate buffered saline (PBS)
- Deionized water

Caution

No data are available addressing the mutagenicity or toxicity of 7-AAD or PO-PRO®-1 dye. Because these reagents bind to nucleic acids, treat them as potential mutagens and handle with appropriate care. Handle the DMSO stock solutions with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

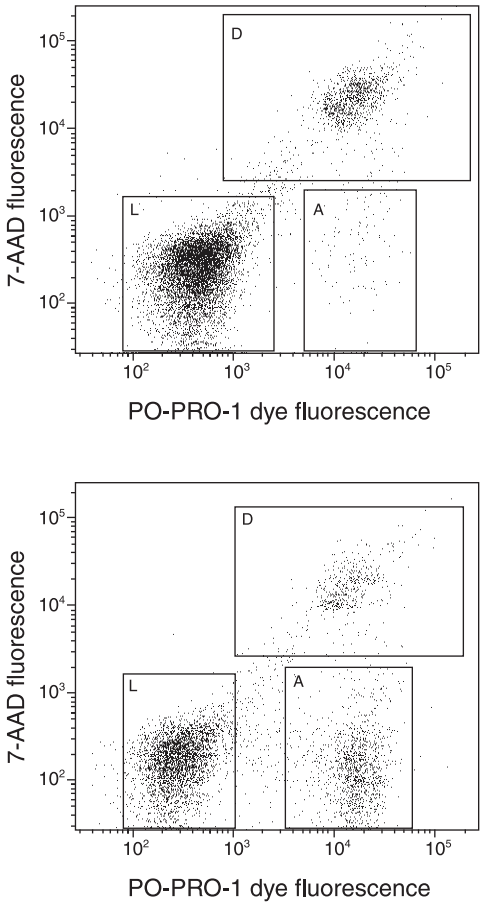

Figure 1. Jurkat cells (human T-cell leukemia) treated with 10 µM camptothecin for four hours (bottom panel) or untreated (as control, top panel). Cells were then treated with the reagents in the kit and analyzed by flow cytometry using 405 nm and 488 nm excitation. Note that the camptothecin-treated cells have a higher percentage of apoptotic cells (indicated by an "A") than the basal level of apoptosis seen in the control cells. L = live cells, D = dead cells.

Experimental Protocol

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, use a 1 mL volume.

3. Add 2.5 µL PO-PRO®-1 stock solution (Component A) and 1 µL 7-AAD stock solution (Component B) to each 1 mL of cell suspension.

4. Incubate the cells on ice for 30 minutes.

5. As soon as possible after the incubation period, analyze the stained cells by flow cytometry, using violet and 488 nm excitation and measuring the fluorescence emission using 440 nm and 670 nm bandpass filters (or their near equivalents). The population should separate into three groups: live cells will show only a low level of fluorescence, apoptotic cells will show violet fluorescence and necrotic cells will show both red and violet fluorescence (see Figure 1). Confirm the flow cytometry results by viewing the cells under a fluorescence microscope using the appropriate filters.

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.** Cancer Res 57, 3804 (1997); **7.** Blood 87, 4959 (1996); **8.** J Exp Med 182, 1759 (1995); **9.** J Immunol Methods 185, 249 (1995).

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

Cat. no.	Product Name	Unit Size
V35123	Membrane Permeability/Dead Cell Apoptosis Kit with PO-PRO®-1 and 7-aminoactinomycin D *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.

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License No. 223: Labeling and Detection Technology
The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402, Tel: (541) 465-8300. Fax: (541) 335-0354.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.