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

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

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. 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. 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.

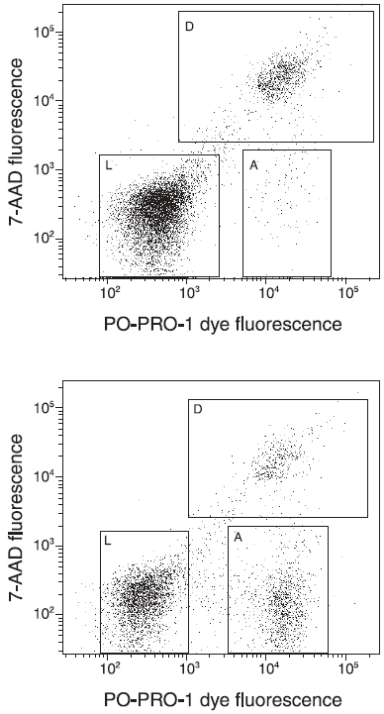

Figure 1 Flow cytometry analysis with the Membrane Permeability/Dead Cell Apoptosis Kit with PO-PRO™-1 and 7-Aminoactinomycin D for Flow Cytometry.
Jurkat cells (human T-cell leukemia) were treated with 10 µM camptothecin for 4 hours (bottom panel) or untreated (as control, top panel). Cells were then 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.

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 **http://thermofisher.com/apoptosis** for a wide selection of products for apoptosis research.

Contents and storage

Component	Amount	Concentration	Storage[1]
PO-PRO™-1 dye (Component A)	500 µL	1 mM solution in DMSO	2°C to 6°C[2] Dessicate Protect from light[3]
7-aminoactinomycin D (7-AAD) (Component B)	200 µL	1 mg/mL solution in DMSO	
Number of assays: 200 flow cytometry assays containing 2 × 10^5 to 1 × 10^6 cells in a 1 mL volume.			
Approximate fluorescence excitation/emission maxima: PO-PRO™-1: 434/456 nm; 7-AAD: 546/647 nm.			

[1] Kit is stable for 6 months when stored as directed.
[2] For long-term storage, store the vial of PO-PRO™-1 dye at ≤–20°C.
[3] 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.

Before you begin

Required materials not supplied

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

Label cells for flow cytometry

The assay is optimized using Jurkat cells (human T-cell leukemia line) 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 incubation, wash in cold phosphate-buffered saline (PBS), and adjust the cell density to approximately 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. Immediately after incubation, 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 3 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.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 29851 Willow Creek Road | Eugene, Oregon 97402 USA

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0002438

Revision	Date	Description
A.0	19 July 2022	The format and content were updated. The version numbering was reset to A.0 in conformance with internal document control.
2.00	12 March 2010	Names were changed.
1.00	17 June 2005	New document for the Membrane Permeability/Dead Cell Apoptosis Kit with PO-PRO™-1 and 7-Aminoactinomycin D for Flow Cytometry.