invitrogen USER GUIDE

Membrane Permeability/Dead Cell Apoptosis Kit with YO-PRO™-1 and PI for Flow Cytometry

Catalog Number V13243

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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 green fluorescent YO-PRO™-1 dye can enter apoptotic cells, whereas other dyes, such as the red fluorescent dye, propidium iodide (PI), cannot. Thus, use of YO-PRO™-1 dye and PI together provide a sensitive indicator for apoptosis.

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

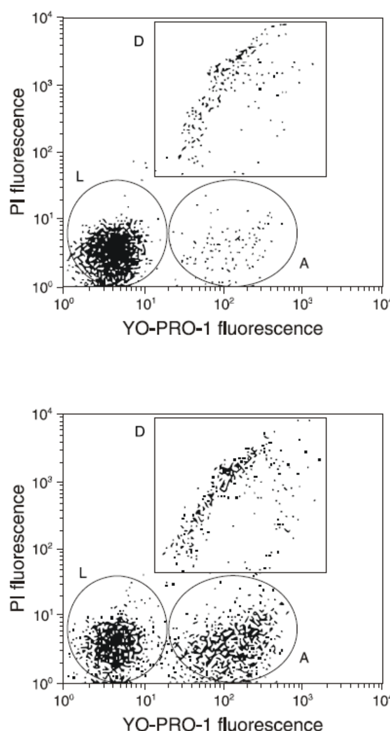

Figure 1 Flow cytometry analysis with the Membrane Permeability/Dead Cell Apoptosis Kit with YO-PRO™-1 and PI for Flow Cytometry.
Jurkat cells (human T-cell leukemia) were treated with 10 µM camptothecin for 4 hours (bottom panel) or untreated (as control, top panel). Cells were then treated with the reagents in the kit and analyzed by flow cytometry using 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). L = live cells, D = dead cells.

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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]
YO-PRO™-1 dye (Component A)	200 µL	100 µM solution in DMSO	2°C to 8°C[2] Desiccate Protect from light[3]
Propidium iodide (PI) (Component B)	200 µL	1 mg/mL (1.5 mM) solution in deionized water	
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: YO-PRO™-1: 491/509 nm, bound to DNA; Propidium iodide: 535/617 nm, bound to DNA.			

[1] Kit is stable for 6 months when stored as directed.
[2] For long-term storage, store the YO-PRO™-1 solution at ≤–20°C.
[3] YO-PRO™-1 and propidium iodide 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
- 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 assay volume.
3. Add 1 µL YO-PRO™-1 stock solution (Component A) and 1 µL PI stock solution (Component B) to each 1 mL of cell suspension.
4. Incubate the cells on ice for 20–30 minutes.
5. Immediately after incubation (within 1–2 hours), analyze the stained cells by flow cytometry using 488 nm excitation with green fluorescence emission for YO-PRO™-1 (i.e., 530/30 bandpass) and red fluorescence emission for propidium iodide (i.e., 610/20 bandpass), gating on cells to exclude debris. Using single-color stained cells, perform standard compensation.

   The population should separate into 3 groups: Live cells show a low level of green fluorescence; apoptotic cells show an incrementally higher level of green fluorescence; and dead cells show both red and green fluorescence (see Figure 1).

Limited product warranty

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

Revision history: Pub. No. MAN0002110

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	17 February 2010	Names were changed.
1.00	12 August 2005	New document for the Membrane Permeability/Dead Cell Apoptosis Kit with YO-PRO™-1 and PI for Flow Cytometry.

The information in this guide is subject to change without notice.

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