

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

Catalog no. V13243

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

Material	Amount	Composition	Storage*	Stability
YO-PRO®-1 dye (Component A)	200 µL	100 µM solution in DMSO	<ul style="list-style-type: none"> <li>• 2–6°C</li> <li>• Desiccate</li> <li>• Protect from light</li> </ul>	When stored as directed this kit is stable for 6 months.
Propidium iodide (PI, Component B)	200 µL	1 mg/mL (1.5 mM) solution in deionized water		
*For long-term storage, store the YO-PRO®-1 solution at ≤–20°C. The YO-PRO®-1 and propidium iodide are light sensitive and may be handled in normal room light, but avoid prolonged exposure to light.				
<b>Number of assays:</b> 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.				
<b>Approximate fluorescence excitation/emission maxima:</b> YO-PRO®-1: 491/509 in nm, bound to DNA; Propidium iodide: 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> 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.<sup>6–9</sup>

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.

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.

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

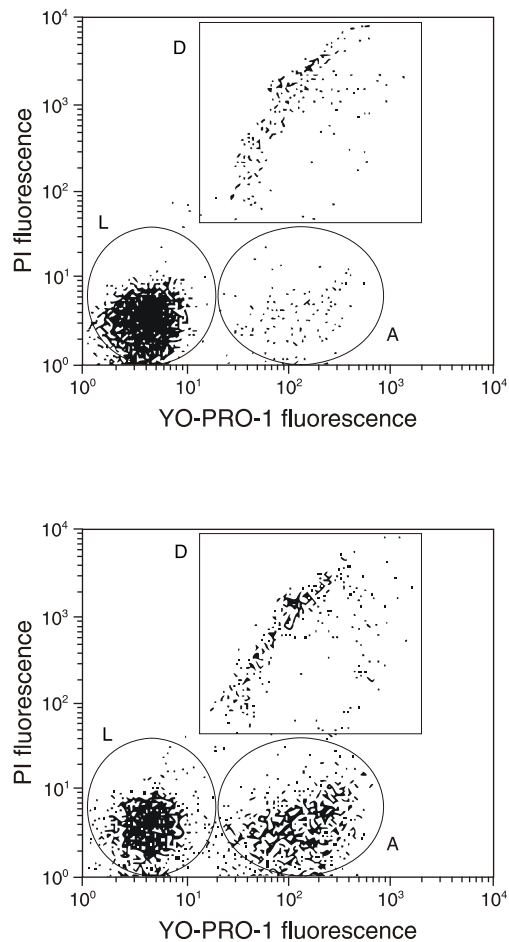
## Before Starting

### Materials Required but Not Provided

- Samples
- Inducing agent
- Phosphate buffered saline (PBS)
- Deionized water
- CountBright™ absolute counting beads (Cat. no. C36950)

### Caution

Propidium iodide is a known mutagen; use 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 (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 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.

## Experimental Protocols

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### Apoptosis Assay

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.

- 1.1 Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.
- 1.2 Harvest the cells after the incubation period, wash 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 assay volume.
- 1.3 Add 1  $\mu$ L YO-PRO<sup>®</sup>-1 stock solution (Component A) and 1  $\mu$ L PI stock solution (Component B) to each 1 mL of cell suspension.
- 1.4 Incubate the cells on ice for 20–30 minutes.
- 1.5 As soon as possible after the incubation period (within 1–2 hours), analyze the stained cells by flow cytometry, using 488 nm excitation with green fluorescence emission for YO-PRO<sup>®</sup>-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 three 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.

### Assay with CountBright™ Absolute Counting Beads

**Note:** The accuracy of cell counts based on CountBright™ absolute counting beads (Cat. no. C36950) depends on sample handling and the precise delivery of the volume of beads. The CountBright™ absolute counting beads must be mixed well to assure a uniform suspension of microspheres; vortex for 30 seconds immediately before removing an aliquot. The microsphere suspension can be pipetted by standard techniques. Cell suspensions may be diluted, but should be assayed without wash steps.

- 2.1 For each assay, prepare a 1 mL volume of sample. Cells may be stained in culture medium or buffer.  
  
**Note:** Cell concentration should be between  $\sim 1 \times 10^6$  cells/mL for each assay. If necessary, adjust concentration and correct for adjustment in the final calculation.
- 2.2 Add 1  $\mu$ L YO-PRO<sup>®</sup>-1 stock solution (Component A) and 1  $\mu$ L PI stock solution (Component B) to each 1 mL of cell suspension.
- 2.3 Incubate the cells on ice for 20–30 minutes.
- 2.4 Allow the CountBright™ absolute counting beads to come to room temperature. Gently vortex the microsphere suspension for 30 seconds to completely resuspend. Immediately after vortexing counting bead suspension, add 50  $\mu$ L of counting beads/mL of sample and vortex.

**Note:** At this dilution, the small amount of Tween 20 and sodium azide contributed by the CountBright™ absolute counting beads has not been noted to affect cell staining or viability.

- 2.5 As soon as possible after the incubation period (within 1–2 hours), analyze the stained cells by flow cytometry, using 488 nm excitation with green fluorescence emission for YO-PRO<sup>®</sup>-1 dye (i.e., 530/30 bandpass) and red fluorescence emission for propidium iodide (i.e., 610/20 bandpass), gating on cells to exclude debris. Set the forward scatter threshold low enough to include the microspheres. Using single-color stained cells, perform standard compensation.

The population should separate into three groups: live cells show only a low level of green fluorescence, apoptotic cells show a higher green fluorescence, and dead cells show both red and green fluorescence. The CountBright™ absolute counting beads can be distinguished from cells for gating (Figure 2).

**Note:** Collect at least 1,000 bead events to assure a statistically significant determination of sample volume.

- 2.6** Gate on the CountBright™ absolute counting beads on the forward scatter versus log side scatter (Figure 3A) or on forward scatter versus linear side scatter making sure the counting bead gate includes the highest side scatter channel (Figure 3B).

**Note:** If the CountBright™ absolute counting beads cannot be resolved from cells in a particular emission parameter combination, use a different combination of emission parameters to gate the counting beads.

Calculation of cell concentration:

$$\frac{A}{B} \times \frac{C}{D} = \text{concentration of sample as cells}/\mu\text{L}$$

Where:

A = number of cell events

B = number of bead events

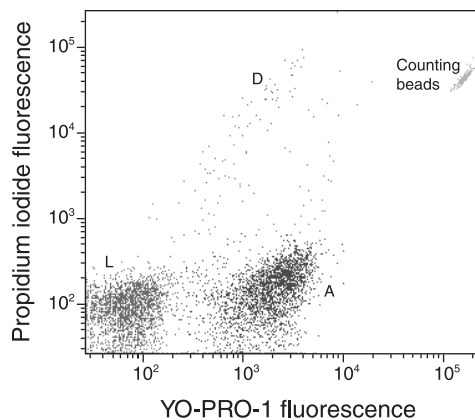
C = assigned bead count of the lot (beads/50  $\mu\text{L}$ )

D = volume of sample ( $\mu\text{L}$ )

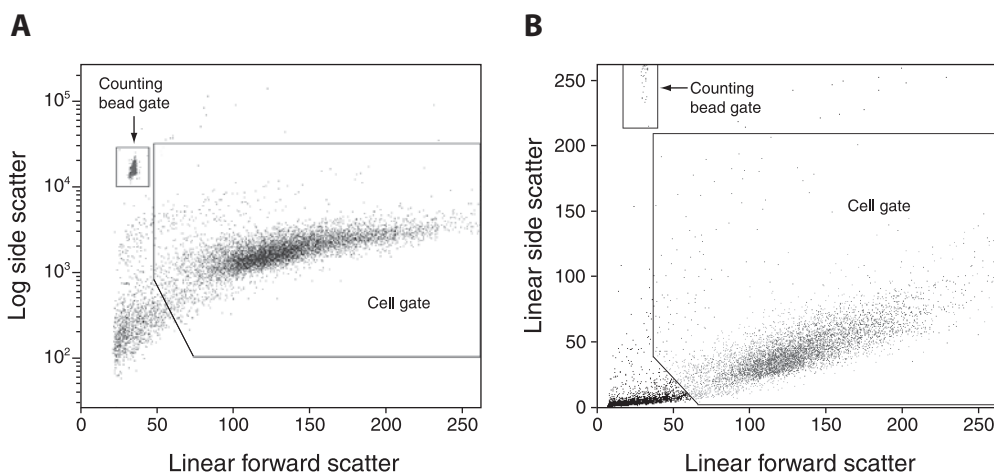
Example calculation: A 1,000  $\mu\text{L}$  volume of cells was stained. Afterwards, 50  $\mu\text{L}$  of CountBright™ absolute counting beads was added.

$$\frac{1,700 \text{ cells}}{1,030 \text{ beads}} \times \frac{49,500 \text{ beads}/50 \mu\text{L}}{1,000 \mu\text{L}} = 81.7 \text{ cells}/\mu\text{L}$$

**Note:** The calculation should be corrected if the sample is diluted or if a different volume of CountBright™ counting beads is used.



**Figure 2.** Plot of YO-PRO®-1 fluorescence collected through a 530/30 bandpass filter vs propidium iodide fluorescence collected through a 585/42 bandpass filter, showing apoptotic (A), live (L), and dead (D) cells, as well as counting beads. Jurkat cells were prepared as in Figure 1 bottom panel. CountBright™ absolute counting beads were added before data acquisition on the flow cytometer. The sample was analyzed using 488 nm excitation.



**Figure 3.** Counting bead gating on forward vs side scatter. Jurkat cells were prepared as in Figure 1 bottom panel, and then CountBright™ absolute counting beads were added prior to data acquisition on the flow cytometer. A) Forward scatter vs logarithmic side scatter shows gating of cells to exclude debris and gating of counting beads. B) Forward scatter vs linear side scatter shows gating of cells to exclude debris as well as gating of counting beads. The counting bead gate is adjusted to include the last channel in side scatter.

## 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
V13243	Membrane Permeability/Dead Cell Apoptosis Kit with YO-PRO®-1 and PI *flow cytometry* *200 assays*.....	1 kit
<b>Related Product</b>		
C36950	CountBright™ absolute counting beads.....	5 mL

## 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:**  
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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

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