Vybrant® Apoptosis Assay Kit #2

V13241 Alexa Fluor® 488 annexin V/propidium iodide, 50 assays

Quick Facts

Storage upon receipt:

- 2–6°C
- Protect from light

Note: Do not freeze Component A

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] In normal live cells, phosphatidylserine (PS) is located on the cytoplasmic surface of the cell membrane. However, in apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, thus exposing PS to the external cellular environment.[6] In leukocyte apoptosis, PS on the outer surface of the cell marks the cell for recognition and phagocytosis by macrophages.[7,8] The human anticoagulant, annexin V, is a 35–36 kD Ca^{2+}-dependent phospholipid-binding protein that has a high affinity for PS.[9] Annexin V labeled with a fluorophore or biotin can identify apoptotic cells by binding to PS exposed on the outer leaflet.[10]

The Vybrant® Apoptosis Assay Kit #2 provides a rapid and convenient assay for apoptosis. The kit contains recombinant annexin V conjugated to one of our best and brightest fluorophores, the Alexa Fluor® 488 dye, to provide the maximum sensitivity. Alexa Fluor® 488 dye is an almost perfect spectral match to fluorescein (FITC), but it creates brighter and more photostable conjugates. In addition, the kit includes a ready-to-use solution of the red-fluorescent propidium iodide (PI) nucleic acid binding dye. PI is impermeant to live cells and apoptotic cells, but stains dead cells with red fluorescence, binding tightly to the nucleic acids in the cell. After staining a cell population with Alexa Fluor® 488 annexin V and PI in the provided binding buffer, apoptotic cells show green fluorescence, dead cells show red and green fluorescence, and live cells show little or no fluorescence (Figure 1).

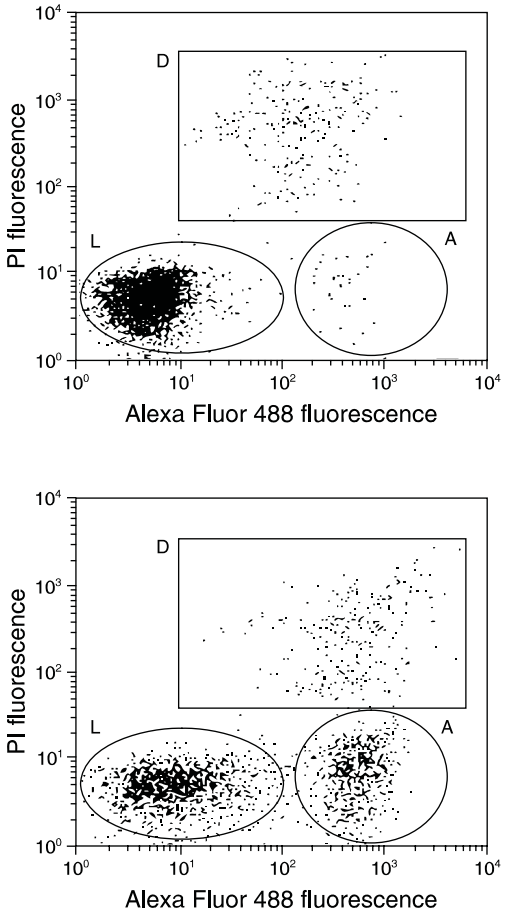

Figure 1. Jurkat cells (T-cell lukemia, human) treated with 10 µM camptothecin for four hours (bottom panel) or untreated (as control, top panel). Cells were then treated with the reagents in the Vybrant® Apoptosis Assay Kit #2, followed by flow cytometric analysis. Note that the camptothecin-treated 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.

These populations can easily be distinguished using a flow cytometer with 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. Molecular Probes offers a wide selection of products for apoptosis research—please refer to our website at probes.invitrogen.com.

Materials

Kit Components

- **Alexa Fluor® 488 annexin V** (Component A), 250 µL of a solution in 25 mM HEPES, 140 mM NaCl, 1 mM EDTA, pH 7.4, plus 0.1% bovine serum albumin (BSA)
- **Propidium iodide** (Component B), 100 µL of a 1 mg/mL (1.5 mM) solution in dH_2O
- **5X annexin-binding buffer** (Component C), 15 mL of 50 mM HEPES, 700 mM NaCl, 12.5 mM $CaCl_2$, pH 7.4

The kit provides sufficient reagents for 50 flow cytometry assays, based on a 100 µL assay volume. Appropriate sample concentrations range from 2×10^5 to 1×10^6 cells per milliliter.

Storage and Handling

Upon receipt, store the kit at 2–6°C, protected from light. The components of the kit should be stable for at least 6 months. DO NOT FREEZE. Alexa Fluor® 488 annexin V and propidium iodide are light sensitive. These compounds may be handled in normal room light, but avoid prolonged exposure to light.

Caution: Propidium iodide is a potential mutagen; use 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.

Flow Cytometry

1.1 Induce apoptosis in cells using the desired method. A negative control should be prepared by incubating cells in the absence of inducing agent.

1.2 Harvest the cells after the incubation period and wash in cold phosphate-buffered saline (PBS).

1.3 Prepare 1X annexin-binding buffer. For example, for ~10 assays, add 1 mL of 5X annexin-binding buffer (Component C) to 4 mL of deionized water (dH_2O).

1.4 Prepare a 100 µg/mL working solution of PI, for example, by diluting 5 µL of the 1 mg/mL PI stock solution (Component B) in 45 µL of 1X annexin-binding buffer. The unused portion of this working solution may be saved for future experiments.

1.5 Re-centrifuge the washed cells (from step 1.2), discard the supernatants and resuspend the cells in 1X annexin-binding buffer. Determine the cell density and dilute in 1X annexin-binding buffer to ~1×10^6 cells/mL, preparing a sufficient volume to have 100 µL per assay.

1.6 Add 5 µL of Alexa Fluor® 488 annexin V (Component A) and 1 µL of the 100 µg/mL PI working solution (prepared in step 1.4) to each 100 µL of cell suspension.

1.7 Incubate the cells at room temperature for 15 minutes.

1.8 After the incubation period, add 400 µL of 1X annexin-binding buffer, mix gently and keep the samples on ice.

1.9 As soon as possible, analyze the stained cells by flow cytometry, measuring the fluorescence emission at 530 nm (e.g. FL1) and >575 nm (e.g. FL3). The population should separate into three groups: live cells will show only a low level of fluorescence, apoptotic cells will show green fluorescence, and dead cells will show both red and green fluorescence (see Figure 1). Confirm the flow cytometry results by viewing the cells under a fluorescence microscope, using filters appropriate for fluorescein (FITC) and tetramethylrhodamine (TRITC) or Texas Red® dye.

Microscopy

This protocol was developed using Jurkat cells treated with camptothecin to induce apoptosis and may be adapted for adherent cell lines.

2.1 Induce apoptosis in cells using the desired method. A negative control should be prepared by incubating cells in the absence of inducing agent.

2.2 After the incubation period, wash the cells in cold PBS.

2.3 Prepare 1X annexin-binding buffer. For example, to make 1 ml of the 1X buffer, add 200 µL of 5X annexin-binding buffer (Component C) to 800 µL of dH_2O.

2.4 Prepare a 100 µg/mL working solution of PI, for example, by diluting 5 µL of the 1 mg/mL PI stock solution (Component B) in 45 µL of 1X annexin-binding buffer. The unused portion of this working solution may be saved for future experiments.

2.5 Re-centrifuge the washed cells (from step 2.2), discard the supernatant and resuspend the cells in 1X annexin-binding buffer. Determine the cell density and dilute in annexin-binding buffer to ~1×10^6 cells/mL, preparing a sufficient volume for deposition on a slide.

2.6 Add 5–25 µL of the annexin V conjugate (Component A) and 1–2 µL of the 100 µg/mL PI working solution (prepared in step 1.4) to each 100 µL of cell suspension. Higher concentrations of the annexin V conjugate tend to produce better results; the optimal staining concentration will need to be determined empirically.

2.7 Incubate the cells at room temperature for 15 minutes.

2.8 Wash the cells with 1X Annexin-Binding buffer.

2.9 Deposit the cells onto slides, mount them using the desired method and observe the fluorescence using appropriate filters. The cells should separate into three groups: live, apoptotic, and dead. Live cells should show only weak annexin V staining of the cellular membrane, while apoptotic cells should show a significantly higher degree of surface labeling. Dead cells will show both membrane staining by annexin V and strong nuclear staining from the propidium iodide.

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 31, 1 (1998); **7.** J Immunol 148, 2207 (1992); **8.** J Immunol 151, 4274 (1993); **9.** J Biol Chem 265, 4923 (1990); **10.** Blood 84, 1415 (1994).

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

Cat #	Product Name	Unit Size
V13241	Vybrant® Apoptosis Assay Kit #2 *Alexa Fluor® 488 annexin V/propidium iodide* *50 assays*	1 kit

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