Vybrant® Apoptosis Assay Kit #6 (V23200)

Biotin-X annexin V/Alexa Fluor® 350 streptavidin/propidium iodide, 50 assays

Quick Facts

Storage upon receipt:

- 2–6ºC
- Do not freeze
- Protect from light

Ex/Em:

- 345/442 nm for Alexa Fluor® 350 streptavidin
- 535/617 nm for PI bound to nucleic acids

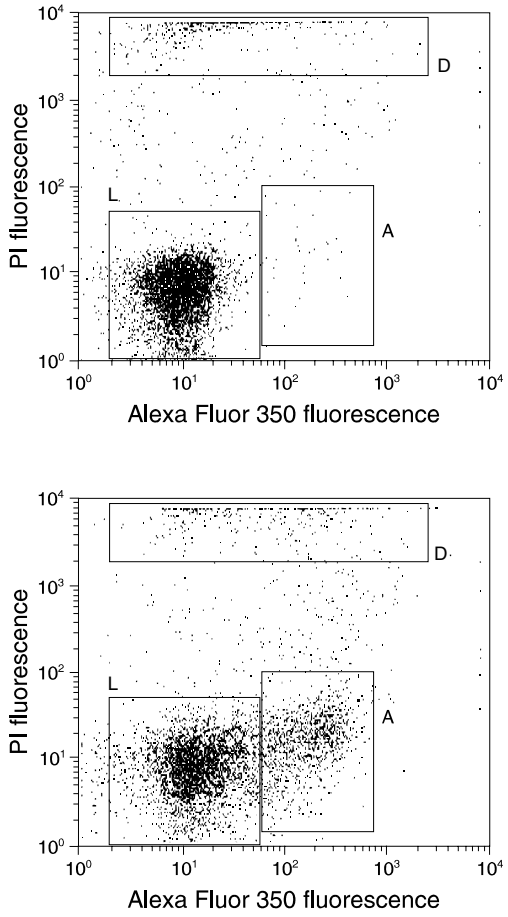

Figure 1. Jurkat cells (T-cell lukemia, human) treated with 10 µM camptothecin for four hours (bottom panel) or untreated (as control, top panel). Cells were then treated with the reagents in the Vybrant® Apoptosis Assay Kit #6, followed by flow cytometric analysis. Note that the camptothecin-treated 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.

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 viable 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 #6 provides a rapid and convenient assay for apoptosis. The kit contains recombinant annexin V conjugated to biotin-X, as well as an Alexa Fluor® 350 streptavidin conjugate for the secondary detection of the biotin-X annexin V. 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 biotin-X annexin V in the provided binding buffer, Alexa Fluor® 350 streptavidin is added to fluorescently label the bound annexin V. Finally, PI is added to detect dead cells. Apoptotic cells show blue fluorescence, dead cells show red and blue fluorescence, and live cells show little or no fluorescence. These populations can easily be distinguished using a flow cytometer with UV excitation for the Alexa Fluor® 350 fluorophore and 488 nm excitation for PI (Figure 1). With the Vybrant® Apoptosis Assay Kit #6, fluorescence in the green channel (FL1) is minimal. In the same experiment for apoptosis detection, the researcher can apply a green-fluorescent probe, for example an antibody labeled with fluorescein or Alexa Fluor® 488 dye.

Because no single parameter defines apoptosis in all systems, we strongly suggest using a combination of different measurements for reliable assessment of apoptosis. Molecular Probes offers a wide selection of products for apoptosis research. Please visit our website (probes.invitrogen.com) for more information.

Materials

Kit Components

- **Biotin-X annexin V** (Component A), 250 µL of a solution in 25 mM HEPES, 140 mM NaCl, 1 mM EDTA, pH 7.4
- **Alexa Fluor® 350 streptavidin** (Component B), 200 µg
- **Propidium iodide** (Component C), 100 µL of a 1 mg/mL (1.5 mM) solution in dH_2O
- **5X annexin-binding buffer** (Component D), 28 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 (each assay contains ~1×10^6 cells).

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® 350 streptavidin 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 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. A negative control should be prepared by incubating cells in the absence of inducing agent.

2. Harvest the cells after the incubation period and wash in cold phosphate-buffered saline (PBS). Approximately 1×10^6 cells will be required for each sample to be tested.

3. Prepare 1X annexin-binding buffer. For example, for ~5 assays, add 2 mL of 5X annexin-binding buffer (Component D) to 8 mL of deionized water (dH_2O).

4. Prepare a 1 mg/mL working solution of Alexa Fluor® 350 streptavidin by adding 200 µL of PBS to the vial containing Alexa Fluor® 350 streptavidin (Component B). For long-term storage, this working solution should be stored in aliquots at ≤–20°C.

5. Re-centrifuge the washed cells (from step 2), discard the supernatants and resuspend the cells in a small volume of 1X annexin-binding buffer (prepared in step 3). Determine the cell density, and then adjust the density to have ~1×10^7 cells/mL in 1X annexin-binding buffer. A 100 µL volume will be used for each assay.

6. Add 5 µL of Biotin-X annexin V (Component A) to each 100 µL of cell suspension.

7. Incubate the cells at room temperature for 15 minutes.

8. After the incubation period, centrifuge the cells, resuspend the cell pellet in 1 mL of 1X annexin-binding buffer, recentrifuge the sample and then resuspend the washed cells in 100 µL of 1X annexin-binding buffer.

9. Add 1 µL of the 1 mg/mL Alexa Fluor® 350 streptavidin solution (prepared in step 4) and gently mix.

10. Incubate the cells at room temperature, or on ice, for 30 minutes.

11. After the incubation period, centrifuge the cells and resuspend the cell pellet in 1 mL of 1X annexin-binding buffer.

12. Add 1 µL of the 1 mg/mL PI stock solution (Component C).

13. Incubate the cells at room temperature, or on ice, for 5–10 minutes.

14. Analyze the stained cells by flow cytometry. For measuring Alexa Fluor® 350, use UV excitation (e.g. 365 nm) and fluorescence detection at ~440 nm (e.g. FL5). For PI, use 488 nm excitation and fluorescence detection at >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 blue fluorescence, and dead cells will show both red and blue fluorescence (see Figure 1). Confirm the flow cytometry results by viewing the cells under a fluorescence microscope, using filters appropriate for DAPI and rhodamine (TRITC) or Texas Red® dye.

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
V23200	Vybrant® Apoptosis Assay Kit #6 *biotin-X annexin V/Alexa Fluor® 350 streptavidin/propidium iodide* *50 assays*	1 kit

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