

## Vybrant® Apoptosis Assay Kit #14 (V35124)

Pacific Blue™ annexin V/7-aminoactinomycin D, 50 assays

### Quick Facts

#### Storage upon receipt:

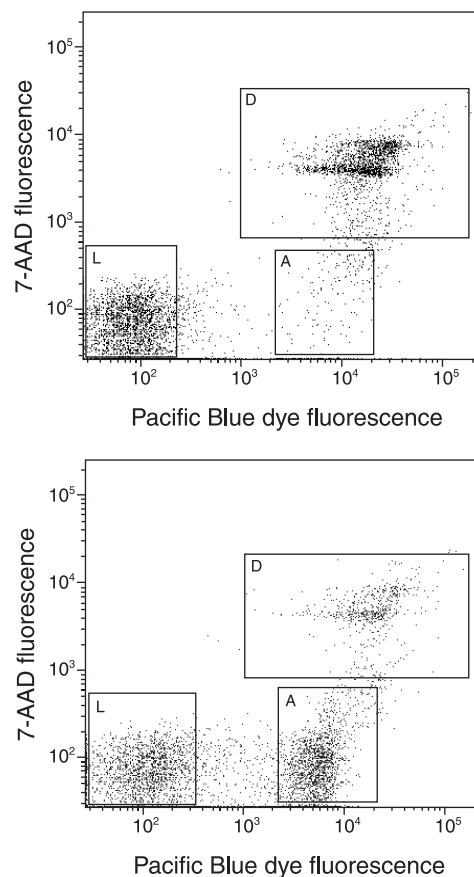
- 2–6°
- Protect from light

**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.<sup>1-5</sup> 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.<sup>6</sup> In leukocyte apoptosis, PS on the outer surface of the cell marks the cell for recognition and phagocytosis by macrophages.<sup>7,8</sup> The human vascular anticoagulant, annexin V, is a 35–36 kD Ca<sup>2+</sup>-dependent phospholipid-binding protein that has a high affinity for PS.<sup>9</sup> Annexin V labeled with a fluorophore or biotin can identify apoptotic cells by binding to PS exposed on the outer leaflet.<sup>10</sup>

The Vybrant® Apoptosis Assay Kit #14 provides a rapid and convenient assay for apoptosis. The kit contains recombinant annexin V conjugated to the violet-excitable fluorophore, Pacific Blue™ dye, to provide the maximum sensitivity. Pacific Blue™ dye absorbs maximally at 415 nm with fluorescence emission ~460 nm, which makes this a good choice for violet diode laser excitation in flow cytometry. In addition, the kit includes a ready-to-use solution of 7-aminoactinomycin D (7-AAD). 7-AAD is a fluorescent intercalator that undergoes spectral shift upon association with DNA. 7-AAD/DNA complexes can be excited by the argon-ion laser and emit beyond 610 nm, making this nucleic acid stain useful for multicolor flow cytometry. After staining a cell population with Pacific Blue™ annexin V and 7-AAD in binding buffer (provided), apoptotic cells show violet fluorescence, dead cells show red fluorescence and live cells show little or



**Figure 1.** Jurkat cells (T-cell leukemia, human) treated with 10  $\mu$ M 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 #14 followed by flow cytometric analysis. 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.

no fluorescence (Figure 1). These populations are easily distinguished using a flow cytometer with the 405 nm (or 407 nm) and 488 nm lines for excitation. Pacific Blue™ annexin V and 7-AAD emit violet and red fluorescence, respectively and can be detected with a 440 nm and 660 nm bandpass filters. There is very little spectral overlap between the two dyes, therefore very little compensation is needed. We have optimized this assay using Jurkat cells, a human T-cell leukemia line, treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types.

Since 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](http://probes.invitrogen.com).

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## Materials

### Kit Components

- **Pacific Blue™ 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)
- **7-aminoactinomycin D (7-AAD)** (Component B), 200 µL of a 1mg/mL solution in DMSO
- **5X Annexin-Binding Buffer** (Component C), 15 mL of 50 mM HEPES, 700 mM NaCl, 12.5 mM CaCl<sub>2</sub>, 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  $2 \times 10^5$  to  $1 \times 10^6$  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 solutions containing the Pacific Blue™ annexin V conjugate. For long-term storage, store the 7-AAD solution at ≤–20°C. Pacific Blue™ annexin V and 7-AAD components are light sensitive. These compounds may be handled in normal room light, but avoid prolonged exposure to light.

**Caution:** No data are available addressing the mutagenicity or toxicity of 7-AAD dye. Because this reagent binds to nucleic acids, it should be treated as a potential mutagen and handled with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

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## 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.

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## 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);
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8. *J Immunol* 151, 4274 (1993);
9. *J Biol Chem* 265, 4923 (1990);
10. *Blood* 84, 1415 (1994).

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## Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
V35124	Vybrant® Apoptosis Assay Kit #14 *Pacific Blue™ annexin V/7-aminoactinomycin D* *for flow cytometry* *50 assays* .....	1 kit

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).

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<sub>2</sub>O).

4. Prepare a 0.1 mg/mL working solution of 7-AAD dye, for example, by diluting 5 µL of the 1 mg/mL 7-AAD stock solution (Component B) in 45 µL of 1X Annexin-Binding Buffer. The unused portion of this working solution may be stored at ≤–20°C for up to 1 month.

5. Recentrifuge the washed cells (from step 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  $\sim 1 \times 10^6$  cells/mL, preparing a sufficient volume to have 100 µL per assay.

6. Add 5 µL of Pacific Blue™ annexin V (Component A) and 1 µL of the 0.1 mg/mL 7-AAD working solution (prepared in step 4) to each 100 µL of cell suspension.

7. Incubate the cells at room temperature for 30 minutes.

8. After the incubation period, add 400 µL of 1X Annexin-Binding Buffer, mix gently and keep the samples on ice.

9. As soon as possible, analyze the stained cells by flow cytometry, measuring the fluorescence emission at 440 nm with violet excitation (Pacific Blue™ dye) and at 660 nm with 488 nm excitation (7-AAD). The population should separate into three groups: live cells with only a low level of violet and red fluorescence, apoptotic cells with high violet fluorescence and no red fluorescence and necrotic cells with high-intensity violet and red fluorescence (see Figure 1). Confirm the flow cytometry results by viewing the cells under a fluorescence microscope using the appropriate filters.

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