

Vybrant® Apoptosis Assay Kit #12 (V35121)

with Vybrant® DyeCycle™ Violet stain/7-AAD

Quick Facts

Storage upon receipt:

- 2–6°C
- Protect from light
- Do not freeze

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 nuclear chromatin, shrinkage of the cytoplasm and loss of membrane asymmetry.¹⁻⁵

Vybrant® Apoptosis Assay Kit #12 provides a rapid and convenient assay for apoptosis based upon fluorescence analysis of the compacted state of the chromatin in apoptotic cells. The kit contains solutions of the cell-permeant Vybrant® DyeCycle™ Violet stain (excitation/emission maxima ~370/440 nm, after binding to dsDNA (Figure 1)), and red-fluorescent 7-aminoactinomycin D (7-AAD) (excitation/emission maxima ~546/650 nm, when bound to DNA (Figure 2)). 7-AAD is permeant only to dead cells. The condensed chromatin of apoptotic cells is stained more brightly by Vybrant® DyeCycle™ Violet stain than is the chromatin of normal cells. The staining pattern resulting from the simultaneous use of Vybrant® DyeCycle™ Violet stain and 7-AAD makes it possible to distinguish normal, apoptotic and dead cell populations by flow cytometry (Figure 3).^{2,6,7} The violet 405 nm laser is used for excitation of the Vybrant® DyeCycle™ Violet stain, whereas 7-AAD may be excited with the 488 nm line of an argon-ion laser. We have optimized this assay using Jurkat cells, a human T-cell leukemia cell line, treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types or buffers/media.

Materials

Kit Components

- **Vybrant® DyeCycle™ Violet stain** (Component A), 50 µL of a 5 mM solution in water

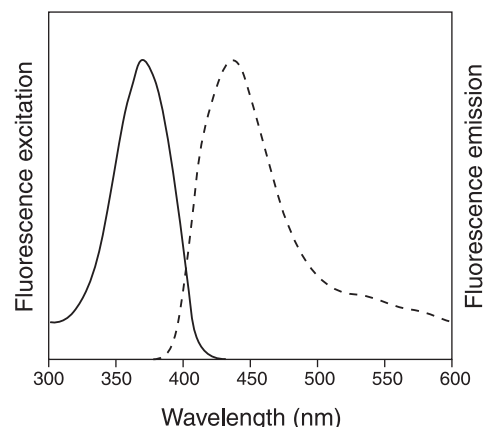


Figure 1. Fluorescence excitation and emission spectra of Vybrant® DyeCycle™ Violet stain bound to DNA in TBE pH 8.3.

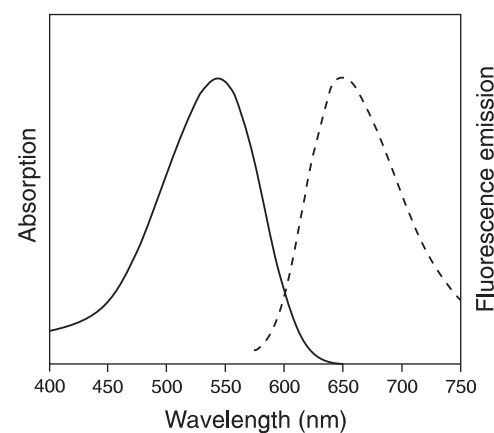


Figure 2. Fluorescence excitation and emission spectra of 7-aminoactinomycin D (7-AAD) bound to DNA.

- **7-aminoactinomycin D (7-AAD)** (Component B), 200 µL of a 1.0 mg/mL solution in DMSO

The kit provides sufficient reagents for 200 flow cytometry assays, each having 1×10^6 cells in a 1 mL volume.

Storage and Handling

Upon receipt, store the kit at 2–6°C, protected from light. The components of the kit should be stable in storage for at least 6 months. Vybrant® DyeCycle™ Violet stain and 7-AAD are light sensitive. They may be handled in normal room light, while avoiding prolonged exposure.

Caution: Vybrant® DyeCycle™ Violet stain and 7-AAD are known or suspected mutagens and should be used with 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. For a given experiment, it is important that all tubes contain the same number of cells. Tube-to-tube variation in cell number will lead to significant differences in staining and can affect results. We recommend Hanks' Balanced Salt Solution (HBSS) for suspending cells during staining.

Phosphate buffers are not recommended for staining with Vybrant® DyeCycle™ Violet stain. Do not use glass containers or tubes.

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, wash in HBSS and adjust the cell density to $\sim 1 \times 10^6$ cells/mL in HBSS. For each test a 1 mL volume will be used.
3. Make a working solution of Vybrant® DyeCycle™ Violet stain by diluting a sufficient amount of Component A 1:5 in deionized water. This working solution should be used within the same day.
4. Add 1 μ L of the Vybrant® DyeCycle™ Violet stain working solution and 1 μ L of the 7-AAD stock solution (Component B) to each 1 mL of cell suspension.
5. Incubate the cells on ice, protected from light, for 30 minutes.
6. As soon as possible after the incubation period, analyze the stained cells by flow cytometry, using 405/488 nm dual excitation while measuring the fluorescence emission at ~ 440 nm and ~ 660 nm. The population should separate into three groups: live cells will show only a low level of violet fluorescence, apoptotic cells will show a higher level of violet fluorescence and necrotic cells will show both violet and red fluorescence (see Figure 3, bottom panel).

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 17, 59 (1994);
7. Exp Cell Res 211, 322 (1994).

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

Cat #	Product Name	Unit Size
V35121	Vybrant® Apoptosis Assay Kit #12 *Vybrant® DyeCycle™ Violet/7-AAD*	1 kit

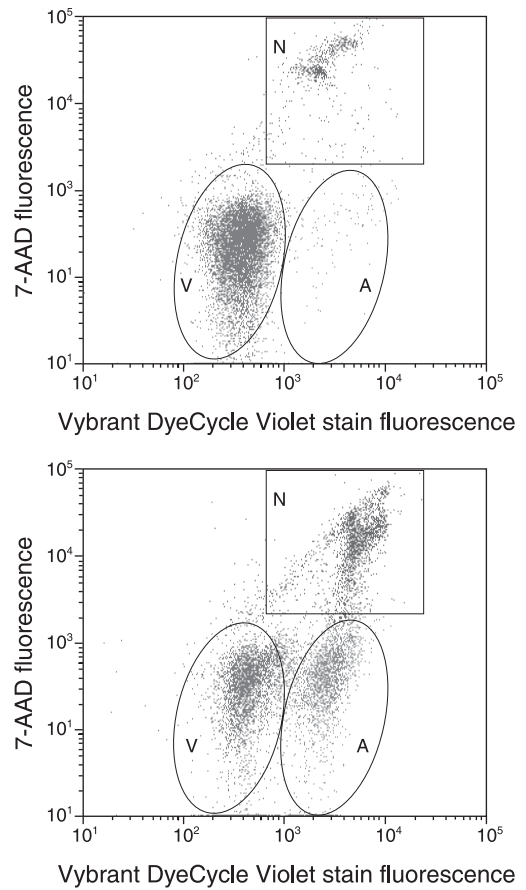


Figure 3. Jurkat cells (human T-cell leukemia) treated with 10 μ M camptothecin for 6 hours (bottom panel) or untreated (as control, top panel). Cells were then mixed with the reagents in the Vybrant® Apoptosis Assay Kit #12 and analyzed by flow cytometry using 405/488 nm dual excitation. Note that the 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). V = viable cells, N = necrotic cells. No instrument compensation was required in these examples.

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