Violet Chromatin Condensation/Dead Cell Apoptosis Kit with Vybrant® DyeCycle™ Violet and SYTOX® AADvanced™ for Flow Cytometry

Catalog no. A35135

Table 1. Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Vybrant® DyeCycle™ Violet stain (Component A)	50 µL	5 mM solution in water	• 2–6°C • Protect from light • Do not freeze	When stored as directed, the product is stable for at least 6 months.
SYTOX® AADvanced™ Dead Cell Stain (Component B)	100 µg	NA	• 2–6°C • Protect from light	
Dimethylsulfoxide (DMSO) (Component C)	200 µL	NA		
Number of assays: Sufficient material is supplied for 200 flow cytometry assays each having 1×10^6 cells/mL based on the protocol below.				
Approximate fluorescence excitation/emission maxima: Vybrant® DyeCycle™ Violet stain: 369/437 in nm, bound to DNA; SYTOX® AADvanced™ dead cell stain: 546/647 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 nuclear chromatin, shrinkage of the cytoplasm, and loss of membrane asymmetry.[1–5]

The Violet Chromatin Condensation/Dead Cell Apoptosis Kit with Vybrant® DyeCycle™ Violet and SYTOX® AADvanced™ 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 the cell-permeant Vybrant® DyeCycle™ Violet stain and the impermeant red-fluorescent SYTOX® AADvanced™ dead cell stain. The condensed chromatin of apoptotic cells are stained more brightly by Vybrant® DyeCycle™ Violet stain than the chromatin of normal cells. The SYTOX® AADvanced ™ stain labels only necrotic cells, based on membrane integrity. The staining pattern resulting from the simultaneous use of Vybrant® DyeCycle™ Violet and SYTOX® AADvanced ™ stains makes it possible to distinguish normal, apoptotic, and necrotic cell populations by flow cytometry (Figure 3).[2,6,7] The Vybrant® DyeCycle™ Violet and SYTOX® AADvanced™ stains are excited with the violet 405 nm and blue 488 nm lasers, respectively. 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 to use this assay with other cell types or buffers/media.

Spectral Characteristics

The fluorescence excitation and emission spectra of the Vybrant® DyeCycle™ Violet stain (Component A), obtained from samples of the dye bound to DNA, are shown in Figure 1. The Vybrant® DyeCycle™ stain/DNA complex has fluorescence excitation/emission maxima of 370 nm and 440 nm, respectively.

The fluorescence excitation and emission spectra of the SYTOX® AADvanced™ dead cell stain (Component B), obtained from samples of the dye bound to DNA, are shown in Figure 2. The SYTOX® AADvanced™ dead cell stain exhibits a fluorescence enhancement of greater than 500-fold. The SYTOX® AADvanced™ dead cell stain/DNA complex has fluorescence excitation and emission maxima of 546 nm and 647 nm, respectively.

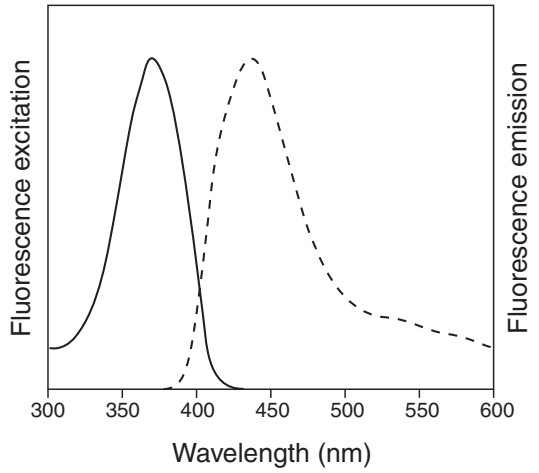

Figure 1. Fluorescence excitation and emission spectra of the Vybrant® DyeCycle™ Violet stain bound to DNA in TBE, pH 8.3.

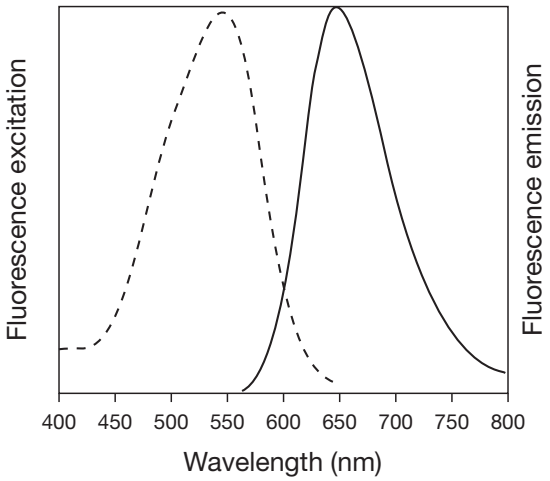

Figure 2. Fluorescence excitation and emission spectra of the SYTOX® AADvanced™ dead cell stain bound to DNA.

Before You Begin

Materials Required but Not Provided

- Hank's Balanced Salt Solution (HBSS) or other buffer
- Flow cytometry tubes
- Cells and culture medium
- Deionized water

Caution

The hazards posed by these stains have not been fully investigated. Since Vybrant® DyeCycle™ Violet and SYTOX® AADvanced™ stains are known to bind to nucleic acids, treat each stain as a potential mutagen and use with appropriate care. Handle the DMSO dye solution with particular caution, as DMSO is known to facilitate the entry of organic molecules into tissues. Always wear suitable protective clothing, gloves, and eye/face protection when handling this reagent. Dispose of the reagents in compliance with all pertaining local regulations.

Preparing Reagents

1.1 Remove kit from refrigerator, and allow the contents to equilibrate to room temperature.

1.2 To prepare 500 µM working solution of SYTOX® AADvanced™ stain, add 200 µL DMSO (Component C) to one vial of SYTOX® AADvanced™ dead cell stain (Component B) and mix well. This will give a concentration of 500 µM SYTOX® AADvanced™ stain in DMSO.

Note: The SYTOX® AADvanced™ dead cell stain solution in DMSO may be subjected to many freeze-thaw cycles without reagent degradation when stored at 2–6°C.

General Guidelines

- We have optimized this assay using Jurkat cells treated with camptothecin to induce apoptosis. The assay may require some modifications 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's Balanced Salt Solution (HBSS) for suspending cells during staining. We do **not** recommend phosphate buffers for staining with Vybrant® DyeCycle™ Violet stain.
- Do not use glass containers or tubes.

Experimental Protocols

2.1 Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.

2.2 Harvest the cells after the incubation period, wash cells in HBSS, and adjust the cell density to ~1×10^6 cells/mL in HBSS. For each test, use 1 mL of cell suspension.

2.3 Prepare a 1 µM working solution of Vybrant® DyeCycle™ Violet stain (Component A) by diluting a sufficient amount of Component A 1:5 in deionized water. Use this working solution within the same day.

2.4 Add 1 µL of the 1 µM Vybrant® DyeCycle™ Violet stain working solution (prepared in step 2.3) and 1 µL of the 500 µM SYTOX® AADvanced™ DMSO solution to each tube containing 1 mL of cell suspension. Mix well.

2.5 Incubate the cells on ice, **protected from light**, for 30 minutes.

2.6 Immediately after the incubation period, analyze the stained cells without washing by flow cytometry, using 405/488 nm dual excitation while measuring the fluorescence emission at ~440 nm and ~660 nm.

The sample can contain three populations: live cells showing a low level of violet fluorescence, apoptotic cells showing a higher level of violet fluorescence, and necrotic cells showing violet and red fluorescence (see Figure 3).

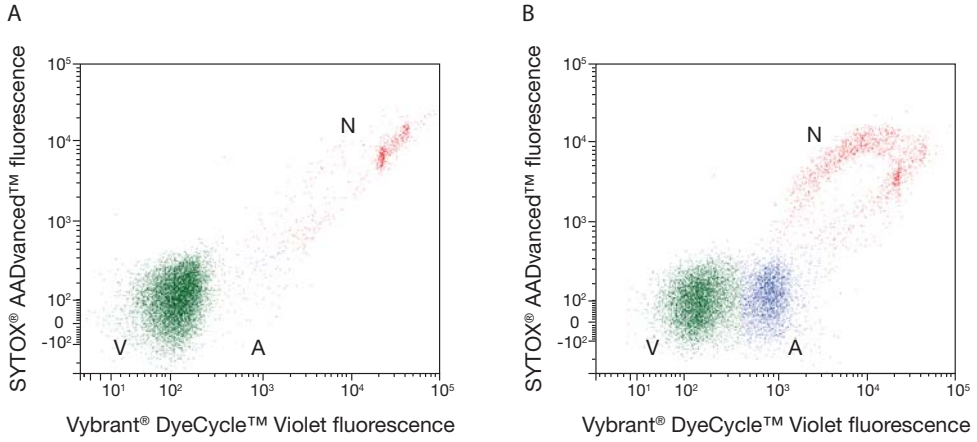

Figure 3. Jurkat cells (human T-cell leukemia) treated with 10 µM camptothecin for 6 hours (panel B) or untreated (as control, panel A). Cells were then mixed with the reagents in the kit and analyzed by flow cytometry using 405/488 nm dual excitation. Note that the campthothecin-treated cells (panel B) have a higher percentage of apoptotic cells than the basal level of apoptosis seen in the control cells (panel A). A=apoptotic cells, V = viable cells, N = necrotic cells.

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 143, 3 (1995); **6.** Cytometry 17, 59 (1994); **7.** Exp Cell Res 211, 322 (1994).

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

Cat. no.	Product Name	Unit Size
A35135	Violet Chromatin Condensation/Dead Cell Apoptosis Kit with Vybrant® DyeCycle™ Violet and SYTOX® AADvanced™ *for flow cytometry* *200 assays*	1 kit
Related products		
S10274	SYTOX® AADvanced™ dead cell stain *for 488 excitation* *for flow cytometry* *500 tests*	1 kit
S10349	SYTOX® AADvanced™ dead cell stain *for 488 excitation* *for flow cytometry* *100 tests*	1 kit
V13244	Chromatin Condensation/Dead Cell Apoptosis Kit with Hoechst 33342 and PI *for flow cytometry* *200 assays*	1 kit
V23201	Chromatin Condensation/Membrane Permeability/Dead Cell Apoptosis Kit with Hoechst 33342/YO-PRO®-1 and PI *for flow cytometry* *200 assays*	1 kit
V35003	Vybrant® DyeCycle™ Violet stain *5 mM solution in DMSO* *200 assays*	200 µL
14025-092	Hanks' Balanced Salt Solution (HBSS) (1X), liquid, contains calcium and magnesium, but no phenol red	500 mL
14170-112	Hanks' Balanced Salt Solution (HBSS) (1X), liquid, contains no calcium chloride, magnesium chloride, or magnesium sulfate	500 mL
14175-095	Hanks' Balanced Salt Solution (HBSS) (1X), liquid, contains no calcium chloride, magnesium chloride, magnesium sulfate, or phenol red	500 mL
24020-117	Hanks' Balanced Salt Solution (HBSS) (1X), liquid, contains calcium and magnesium	500 mL

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