Pacific Blue™ Annexin V/SYTOX™ AADvanced™ Apoptosis Kit for Flow Cytometry

Catalog Number A35136

Pub. No. MAN0002443 Rev. A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

Annexins are a family of calcium-dependent phospholipid-binding proteins that preferentially bind phosphatidylserine (PS). Under normal physiologic conditions, PS is predominantly located in the inner leaflet of the plasma membrane. Upon initiation of apoptosis, PS loses its asymmetric distribution across the phospholipid bilayer and is translocated to the extracellular membrane leaflet marking cells as targets of phagocytosis. Once on the outer surface of the membrane, PS can be detected by fluorescently labeled Annexin V in a calcium-dependent manner.

The Pacific Blue™ Annexin V/SYTOX™ AADvanced™ Apoptosis Kit 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. Because Pacific Blue™ dye absorbs maximally at 415 nm with fluorescence emission 455 nm, it is a good choice for violet diode laser excitation in flow cytometry. The kit includes the red fluorescent dye, SYTOX™ AADvanced™ Dead Cell Stain, for identifying necrotic cells based on membrane integrity. After staining a cell population with Pacific Blue™ annexin V and SYTOX™ AADvanced™ stains in the supplied binding buffer, apoptotic cells show bright violet fluorescence, dead cells show red fluorescence, and live cells show dim violet fluorescence (Figure 1). Because there is very little spectral overlap between the two dyes, very little or no compensation is required. 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.

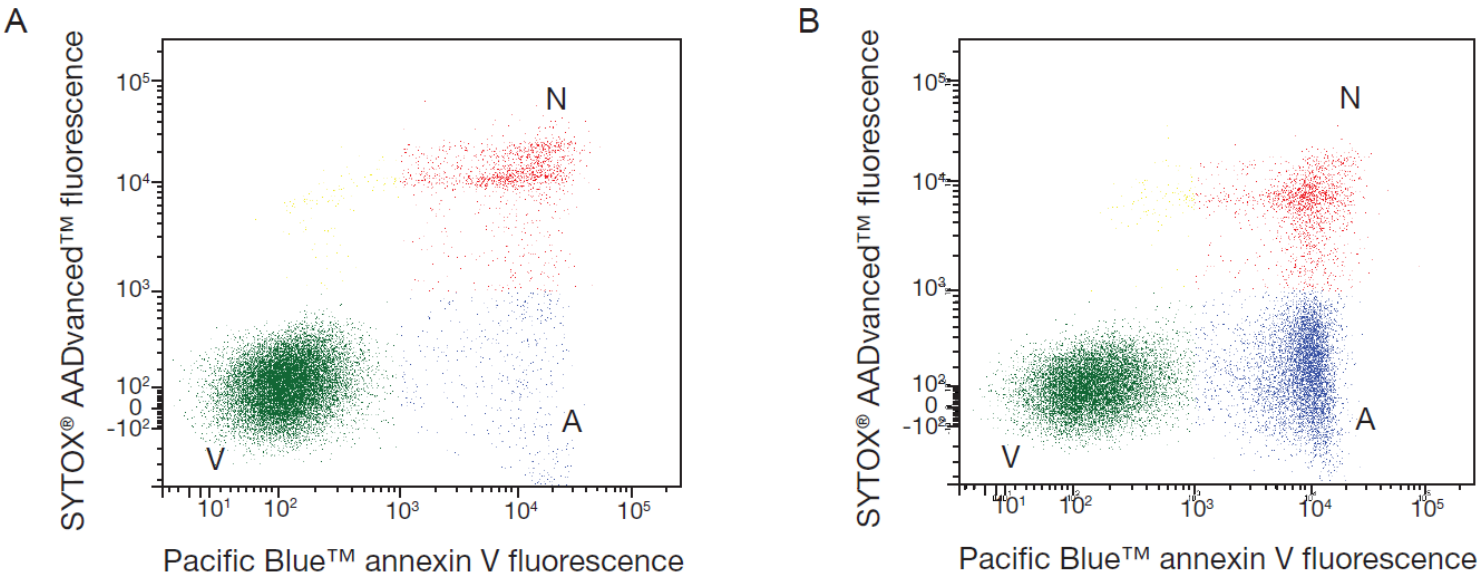

Figure 1 Jurkat cells (human T-cell leukemia) untreated control (panel A) or treated with 10 µM camptothecin for four hours (panel B). Cells were treated with the reagents in the kit and analyzed by flow cytometry using 405 nm and 488 nm excitation. Note that the camptothecin-treated cells have a higher percentage of apoptotic cells (panel B) than the basal level of apoptosis seen in the control cells (panel A). A = apoptotic cells, V = viable cells, N = necrotic cells.

Because no single parameter defines apoptosis in all systems, we strongly suggest using a combination of different measurements for reliable detection of apoptosis. Refer to thermofisher.com/apoptosis for a wide selection of products for apoptosis research.

Contents and storage

Component	Amount[1]	Storage[2]
Pacific Blue™ Annexin V (Component A)[3]	250 pL solution in 25 mM HEPES, 140 mM NaCl, 1 mM EDTA, pH 7.4, with 0.1% bovine serum albumin (BSA)	Store at 2–6°C. Protect from light. Do not freeze.
SYTOX™ AADvanced™ Dead Cell Stain (Component B)[4]	1 vial	Store at 2–6°C. Protect from light.
Dimethylsulfoxide (DMSO), anhydrous (Component C)	100 µL	Store at 2–6°C.
5X Annexin-binding buffer (Component D)	15 mL	

[1] Sufficient material is supplied for 50 assays, based on the protocol below.
[2] When stored as directed, this kit is stable for at least 6 months.
[3] Approximate fluorescence excitation/emission maxima: 415/455 nm
[4] Approximate fluorescence excitation/emission maxima: 546/647 nm, bound to DNA

Spectral characteristics

The fluorescence excitation and emission spectra of the Pacific Blue™ annexin V conjugate (Component A) has fluorescence excitation/emission maxima of 415 nm and 455 nm, respectively.

The fluorescence excitation and emission spectra of the SYTOX™ AADvanced™ Dead Cell Stain (Component B) were obtained from samples of the dye bound to DNA. 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.

Required materials not supplied

- Flow cytometry tubes
- Cells and culture medium
- Deionized water

Procedural guidelines

- 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.
- Make sure that all tubes contain the same number of cells for a given experiment. Tube-to-tube variation in cell number leads to significant differences in staining, and such variation can affect results.
- We recommend phosphate-buffered saline (PBS) for suspending cells during staining.
- Do **not** use glass containers or tubes.

Label apoptotic cells for flow cytometry

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

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

3. Prepare 500 µM SYTOX™ AADvanced™ Dead Cell Stain working solution by adding 200 µL DMSO (Component C) to one vial of SYTOX™ AADvanced™ Dead Cell Stain (Component B). Mix the solution well.

   Note: When stored at 2–6°C, the SYTOX™ AADvanced™ Dead Cell Stain working solution may be subjected to many freeze-thaw cycles without reagent degradation.

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

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

6. Centrifugre the washed cells, discard the supernatant, and resuspend the cells in 1X annexin-binding buffer (prepared in step 2) at ~1 × 10^6 cells/mL, preparing a sufficient volume to have 100 µL per assay.

7. Add 5 µL of Pacific Blue™ Annexin V (Component A) and 1 µL of 500 µM SYTOX™ AADvanced™ Dead Cell Stain working solution (prepared in step 3) to each 100 µL of cell suspension.

8. Incubate the cells at room temperature for 30 minutes, **protected from light**.

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

10. As soon as possible, analyze the stained cells by flow cytometry, measuring the fluorescence emission using a 450 nm bandpass or equivalent with 405 nm excitation (Pacific Blue™ dye) and a 670 bandpass or equivalent with 488 nm excitation (SYTOX™ AADvanced™).

    Note: The sample can contain three populations: live cells showing a low level of violet and red fluorescence, apoptotic cells showing a high level of violet fluorescence and no red fluorescence, and necrotic cells showing a high intensity red and violet fluorescence (see Figure 1).

11. Confirm the flow cytometry results by viewing the cells under a fluorescence microscope using the appropriate filters.

Related products

For more information on other products for apoptosis research, visit **thermofisher.com/apoptosis**.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 29851 Willow Creek Road | Eugene, Oregon 97402 USA
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0002443

Revision	Date	Description
A.0	19 May 2022	The format and content were updated. This document supercedes Rev. 2.01, revision date July 2010.