Dead Cell Apoptosis Kit with Annexin V APC and SYTOX™ Green for Flow Cytometry

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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 Dead Cell Apoptosis Kit with Annexin V APC and SYTOX™ Green for flow cytometry provides a rapid and convenient assay for apoptosis. The kit contains recombinant Annexin V conjugated to allophycocyanin (APC). APC is an extremely fluorescent phycobiliprotein, which can be easily excited with a helium-neon (HeNe) laser at 633 nm on a standard flow cytometer and has an emission maximum at approximately 660 nm. In addition to APC Annexin V, the kit includes a solution of SYTOX™ Green nucleic acid stain. The SYTOX™ Green dye is impermeant to live cells and apoptotic cells, but stains dead cells with intense green fluorescence by binding to cellular nucleic acids. After staining a cell population with APC Annexin V and SYTOX™ Green stain, apoptotic cells show red fluorescence and very little green fluorescence, dead cells show a higher level of green and red fluorescence, and live cells show little or no fluorescence (Figure 1). These populations can easily be distinguished using a flow cytometer with both the 488 nm line of an argon-ion laser and the 633 nm line of a HeNe laser for excitation.

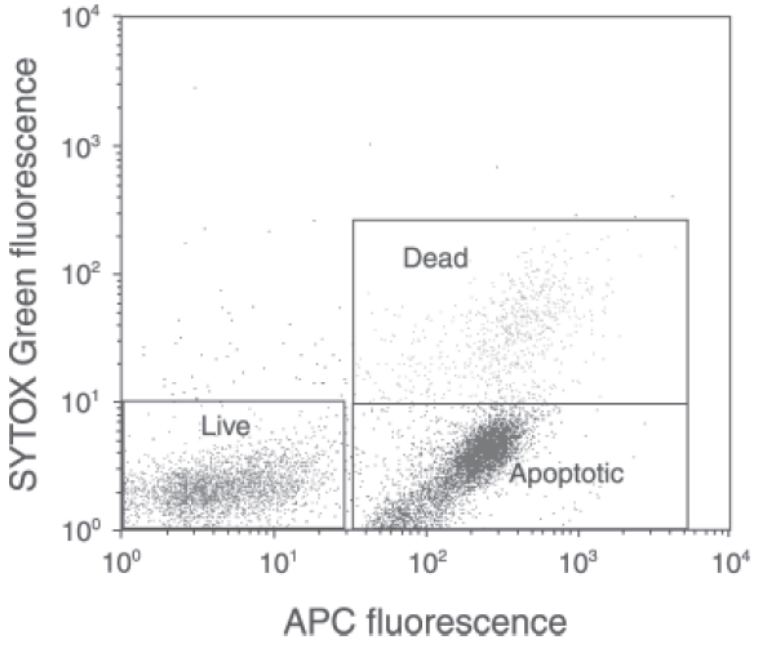

Figure 1 Jurkat cells (human T-cell leukemia) were treated with 10 pM camptothecin for 4 hours at 37°C, 5% CO_2. The cells were incubated with the reagents in the kit, and then analyzed by flow cytometry. The SYTOX™ Green fluorescence versus APC (allophycocyanin) fluorescence dot plot shows resolution of live, apoptotic, and dead cell populations.

The assay has been optimized using Jurkat cells, a human T-cell leukemia clone, 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 recommend

to use 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]	Composition	Storage[2,3]
Allophycocyanin (APC) Annexin V (Component A)[4]	250 µL	Solution in 25 mM HEPES, 140 mM NaCl, 1 mM EDTA, pH 7.4, 0.1% bovine serum albumin (BSA)	Store at 2–6°C. Protect from light. Do not freeze Component A.
SYTOX™ Green Stain (Component B)[5]	100 µL	10 µM solution in DMSO	
5X Annexin-binding buffer (Component C)	15 mL	50 mM HEPES, 700 mM NaCl, 12.5 mM $CaCl_2$, pH 7.4	

[1] Sufficient material is supplied for 50 flow cytometry assays based on a 100 µL assay volume.

[2] For long-term storage, store the vial of SYTOX™ Green stain at ≤–20°C. The SYTOX™ Green stain and APC annexin V are light sensitive and may be handled in normal room light, but avoid prolonged exposure to light.

[3] When stored as directed this kit is stable for 6 months.

[4] Approximate fluorescence excitation/emission maxima: 650/660 nm

[5] Approximate fluorescence excitation/emission maxima: 503/554 nm, bound to DNA

Required materials not supplied

- Samples (appropriate sample concentrations range from 2×10^5 to 1×10^6 cells/mL)
- Inducing agent
- 2 mM hydrogen peroxide
- Phosphate buffered saline (PBS)
- Deionized water

Label apoptotic cells for flow cytometry

Note: The assay has been optimized using Jurkat cells treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types. Because no single parameter defines apoptosis in all systems, we strongly suggest using a combination of different measurements for reliable detection of apoptosis. A wide selection of products for apoptosis research can be found at **thermofisher.com/apoptosis**.

1. Induce apoptosis in cells using the desired method. Prepare negative control by incubating cells in the absence of inducing agent. Prepare a positive control for necrosis by incubating cells with 2 mM hydrogen peroxide for 4 hours at 37°C.

2. Prepare 1X annexin-binding buffer. For example, for ~10 assays, add 2 mL 5X annexin-binding buffer (Component C) to 8 mL deionized water.

3. Prior to opening, warm the vial of SYTOX™ Green stain to room temperature. Prepare a 1 M working solution of SYTOX™ Green stain. For example, dilute 5 µL of the 10 µM SYTOX™ Green stain stock solution (Component B) in 45 µL of 1X annexin-binding buffer. The SYTOX™ Green stain concentration may have to be adjusted for individual cell types.

   Note: Store the unused portion of this working solution at ≤–20°C for up to 1 month. Reseal this vial tightly before returning to storage.

4. Harvest the cells following apoptosis induction and wash in 1X annexin-binding buffer.

5. Centrifuge the washed cells, discard the supernatant, and resuspend the cells at a concentration of ~1×10^6 cells/mL in 1X annexin-binding buffer.

6. Add 5 µL APC Annexin V (Component A) and 1 µL of the 1 µM SYTOX™ Green stain working solution (prepared in step 3) to each 100 µL cell suspension.

7. Incubate the cells at 37°C in an atmosphere of 5% CO_2 for 15 minutes.

8. After the incubation period, add 400 µL of the 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 530 nm and 660 nm.

   Note: The population should separate into three groups: live cells with only a low level of green and red fluorescence, apoptotic cells with a high level of red fluorescence and little green fluorescence, and dead cells with a high level of green and red fluorescence. Confirm the flow cytometry results by viewing the cells with a fluorescence microscope, using filters appropriate for fluorescein (FITC) and allophycocyanin (APC).

Related products

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

Limited product warranty

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Revision history: Pub. No.MAN0002430

Revision	Date	Description
A.0	19 May 2022	The content and format were updated. This document supercedes Rev 2.0, revision date July 2010.