Single-Channel Dead Cell Apoptosis Kit with Annexin V Alexa Fluor™ 488 & 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 Single-Channel Dead Cell Apoptosis Kit with Annexin V Alexa Fluor™ 488 & SYTOX™ Green for flow cytometry provides a rapid and convenient assay for apoptosis. The kit contains recombinant annexin V conjugated to Alexa Fluor™ 488 dye to provide the maximum sensitivity. Alexa Fluor™ 488 dye is an almost perfect spectral match to fluorescein (FITC), but it creates brighter and more photostable conjugates. In addition, the kit includes a ready-to-use solution of SYTOX™ Green nucleic acid binding dye. 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 Alexa Fluor™ 488 Annexin V and SYTOX™ Green dye in the supplied binding buffer, apoptotic cells show green fluorescence, dead cells show a higher level of green fluorescence and live cells show little or no fluorescence (Figure 1). These populations can easily be distinguished using a flow cytometer with the 488 nm line of an argon-ion laser for excitation. Both Alexa Fluor™ 488 Annexin and SYTOX™ Green dye emit a green fluorescence that can be detected in the FL1 channel, freeing the other channels for the addition of other probes in multi-color labeling experiments.

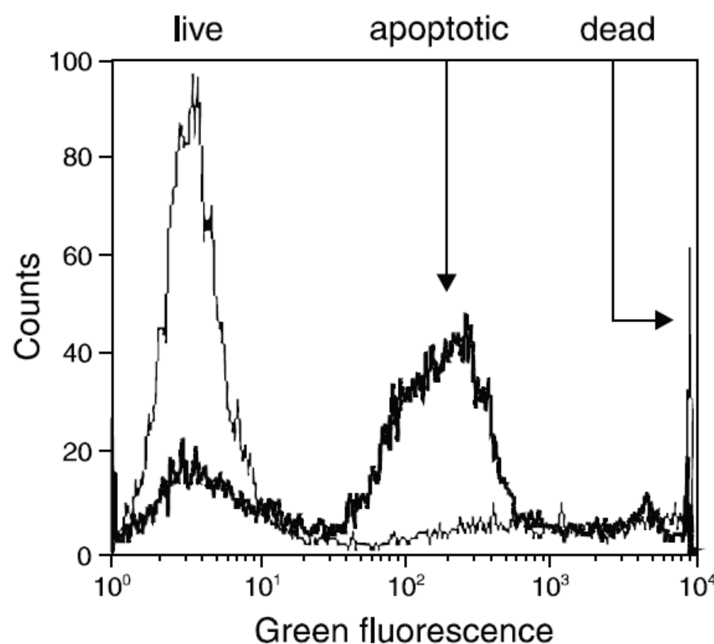

Figure 1 Jurkat cells (human T-cell lukemia) treated with 10 µM camptothecin for four hours (bold line) or untreated (as control, fine line).
Cells were then treated with the reagents in the Kit followed by flow cytometric analysis. Note that the camptothecin-treated cells have a higher percentage of apoptotic cells (intermediate green fluorescence) than the basal level of apoptosis seen in the control cells.

For Research Use Only. Not for use in diagnostic procedures.

We have optimized this assay 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. Because this assay requires only the FL1 channel for detection, other parameters might be measured simultaneously using fluorescent probes with different emission spectra. Indeed, since no single parameter defines apoptosis in all systems, we strongly suggest using a combination of different measurements for reliable detection of apoptosis. Refer to our website at **thermofisher.com/apoptosis** for a wide selection of products for apoptosis research.

Contents and storage

Component	Amount[1]	Composition	Storage[2,3]
Alexa Fluor™ 488 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 dye (Component B)[5]	100 µL	50 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 SYTOX™ Green solution at ≤–20°C. The Alexa Fluor™ 488 Annexin V and SYTOX™ Green components 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: 499/521 nm
[5] Approximate fluorescence excitation/emission maxima: 503/524 nm

Required materials not supplied

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

Label apoptotic cells for flow cytometry

Note: 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. 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 a negative control by incubating cells in the absence of inducing agent.
2. Prepare 1X annexin-binding buffer. For example, for ~10 assays, add 1 mL 5X annexin-binding buffer (Component C) to 4 mL deionized water.
3. Prepare a 5 µM working solution of SYTOX™ Green dye. For example, dilute 5 µL 50 µM SYTOX™ Green stock solution (Component B) in 45 µL 1X annexin-binding buffer.

   Note: Store the unused portion of this working solution at ≤–20°C for up to 1 month.
4. Harvest the cells after the incubation period and wash in cold phosphate-buffered saline (PBS).
5. Centrifuge the washed cells, then discard the supernatant, and resuspend the cells in 1X annexin-binding buffer.
6. 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.
7. Add 5 µL Alexa Fluor™ 488 Annexin V (Component A) and 1 µL 5 µM SYTOX™ Green working solution (prepared in step 3) to each 100 µL of cell suspension.
8. Incubate the cells at room temperature for 15 minutes.
9. After the incubation period, add 400 µL 1X annexin-binding buffer, mix gently, and keep the samples on ice.

10. As soon as possible, analyze the stained cells by flow cytometry, measuring the fluorescence emission at 530 nm.

    Note: The population should separate into three groups: live cells with only a low level of fluorescence, apoptotic cells with moderate green fluorescence, and dead cells with high-intensity green fluorescence (see Figure 1).

11. Confirm the flow cytometry results by viewing the cells under a fluorescence microscope, using filters appropriate for fluorescein (FITC).

Related products

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

Limited product warranty

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

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