Dead Cell Apoptosis Kit with Annexin V Alexa Fluor™ 488 & Propidium Iodide

Catalog Numbers V13241, V13245

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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 Alexa Fluor™ 488 & Propidium Iodide 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 the red-fluorescent propidium iodide (PI) nucleic acid binding dye. PI is impermeant to live cells and apoptotic cells, but stains dead cells with red fluorescence, binding tightly to the nucleic acids in the cell. After staining a cell population with Alexa Fluor™ 488 Annexin V and PI in the provided binding buffer, apoptotic cells show green fluorescence, dead cells show red and 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.

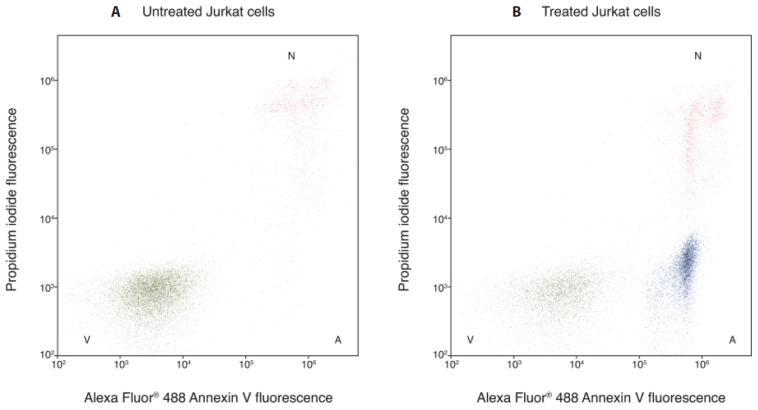

Figure 1 Jurkat cells (human T-cell leukemia) untreated control (panel A) or treated with 10 µM camptothecin for 4 hours (panel B). Cells were stained and analyzed by flow cytometry using 488 nm excitation on the Attune™ Acoustic Cytometer with 530/30 and 575/24 bandpass filters and collected by means of a standard 100 µL/minute collection rate. Note that the camptothecin-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.

Contents and storage

Component	Cat. No. V13241 (50 assays)[1]	Cat. No. V13245 (250 assays)[1]	Composition	Storage[2,3]
Alexa Fluor™ 488 Annexin V (Component A) [4]	250 µL	5 × 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 to 6°C. Protect from light. Do not freeze Component A.
Propidium iodide (PI, Component B)[5]	100 µL	100 µL	1 mg/mL (1.5 mM) solution in deionized water	
5X Annexin-binding buffer (Component C)	15 mL	50 mL	50 mM HEPES, 700 mM NaCl, 12.5 mM $CaCl_2$, pH 7.4	

[1] Based on 100 µL assay volume.
[2] The Alexa Fluor™ 488 annexin V and propidium iodide 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 1 year from the date of receipt.
[4] Approximate fluorescence excitation/emission maxima: 488/499 nm
[5] Approximate fluorescence excitation/emission maxima: 535/617 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
- Phosphate-buffered saline (PBS)
- Deionized water

Label apoptotic cells for flow cytometry

Note: 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 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 100 µg/mL working solution of PI by diluting 5 µL of the 1 mg/mL PI stock solution (Component B) in 45 µL 1X Annexin-binding buffer.

   Note: Store the unused portion of this working solution at 4°C for future experiments.
4. Harvest the cells after the incubation period and wash in cold phosphate-buffered saline (PBS).
5. Centrifuge the washed cells, 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 ~1×10^6 cells/mL, preparing a sufficient volume for a 100 µL per assay.
7. Add 5 µL Alexa Fluor™ 488 Annexin V (Component A) and 1 µL 100 µg/mL PI 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 and 575 nm (or equivalent) using 488-nm excitation.
11. Confirm the flow cytometry results by viewing the cells under a fluorescence microscope, using filters appropriate for fluorescein (FITC) and tetramethylrhodamine (TRITC) or Texas Red™ dye.

Related products

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

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Life Technologies Corporation | 29851 Willow Creek | Eugene, Oregon 97402 USA
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Revision	Date	Description
A.0	19 May 2022	The format and content were updated. This document supercedes Rev 3.0, revision date September 2010.

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