invitrogen USER GUIDE

Dead Cell Apoptosis Kit with Annexin V FITC & Propidium Iodide 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 FITC & Propidium Iodide for flow cytometry provides a rapid and convenient assay for apoptosis. The kit contains recombinant Annexin V conjugated to fluorescein (FITC Annexin V), as well as 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 FITC 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.

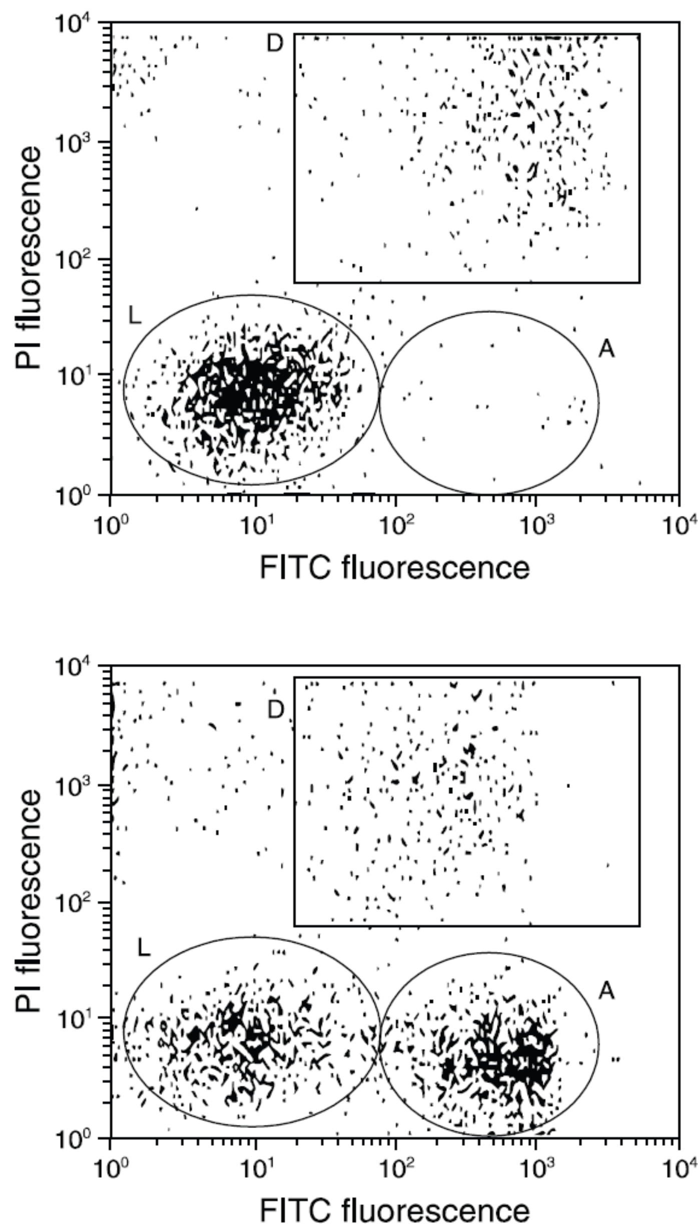

Figure 1 Jurkat cells (human T-cell leukemia) untreated (as control, top panel) or treated with 10 µM camptothecin for four hours (bottom panel).
Cells were then treated with the reagents in the kit, followed by flow cytometric analysis. Note that the camptothecin-treated cells (bottom panel) have a higher percentage of apoptotic cells (indicated by an "A") than the basal level of apoptosis seen in the control cells (top panel). L = live cells, D = dead cells.

Contents and storage

Component	Amount[1]	Composition	Storage[2, 3]
FITC 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.
Propidium iodide (PI, Component B)[5]	100 µL	1 mg/mL (1.5 mM) solution in deionized water	
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] The FITC 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 6 months.
[4] Approximate fluorescence excitation/emission maxima: 494/518 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 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, 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 of FITC Annexin V (Component A) and 1 µL of the 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 of 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 (e.g., FL1) and > 575 nm (e.g., FL3).

    Note: The population should separate into three groups: live cells will show only a low level of fluorescence, apoptotic cells show green fluorescence and dead cells show both red and 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) and rhodamine (TRITC) or Texas Red™ dye.

Label apoptotic cells for microscopy

Note: This protocol was developed using Jurkat cells treated with camptothecin to induce apoptosis and may be adapted for adherent cell lines.

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

2. Prepare 1X annexin-binding buffer. For example, to make 1 mL of the 1X buffer, add 200 µL 5X annexin-binding buffer (Component C) to 800 µL 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 for future experiments.

4. After the incubation period, wash the cells in cold 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 annexin-binding buffer to ~1 × 10^6 cells/mL, preparing a sufficient volume for deposition on a slide.

7. Add 5–25 µL of the Annexin V conjugate (Component A) and 1–2 µL of the 100 µg/mL PI working solution (prepared in step 3) to each 100 µL of cell suspension. Higher concentrations of the annexin V conjugate tend to produce better results; determine the optimal staining concentration empirically.

8. Incubate the cells at room temperature for 15 minutes.

9. Wash the cells with 1X annexin-binding buffer.

10. Deposit the cells onto slides, mount them using the desired method and observe the fluorescence using appropriate filters.

    Note: The cells should separate into three groups: live, apoptotic, and dead. Live cells show only weak annexin V staining of the cellular membrane, while apoptotic cells show a significantly higher degree of surface labeling. Dead cells show both membrane staining by annexin V and strong nuclear staining from the propidium iodide.

Related products

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

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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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