invitrogen USER GUIDE

Mitochondrial Membrane Potential Apoptosis Kit, with MitoTracker™ Red & Annexin V Alexa Fluor™ 488 for Flow Cytometry

Catalog Number V35116

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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 Mitochondrial Membrane Potential Apoptosis Kit, with MitoTracker™ Red & Annexin V Alexa Fluor™ 488 for flow cytometry provides a rapid and convenient assay for apoptosis based on PS translocation and changes in mitochondrial membrane potential. The kit contains recombinant annexin V conjugated to one of our brightest fluorophores, the Alexa Fluor™ 488 dye, to provide 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 MitoTracker™ Red dye to indicate the status of the mitochondrial transmembrane potential.

After staining a cell population with Alexa Fluor™ 488 annexin V and MitoTracker™ Red dye in the provided binding buffer, apoptotic cells show green fluorescence with decreased red fluorescence, and live cells show very little green fluorescence and bright red fluorescence (Figure 1). These populations can easily be distinguished using a flow cytometer with the 488 nm line of an argon-ion laser to excite both dyes. Alexa Fluor™ 488 annexin emits a green fluorescence that can be detected in a 530 nm bandpass filter, and MitoTracker™ Red dye emits red fluorescence that can be detected using either orange (585 nm bandpass filter) or red (630 nm longpass filter) channels of a flow cytometer.

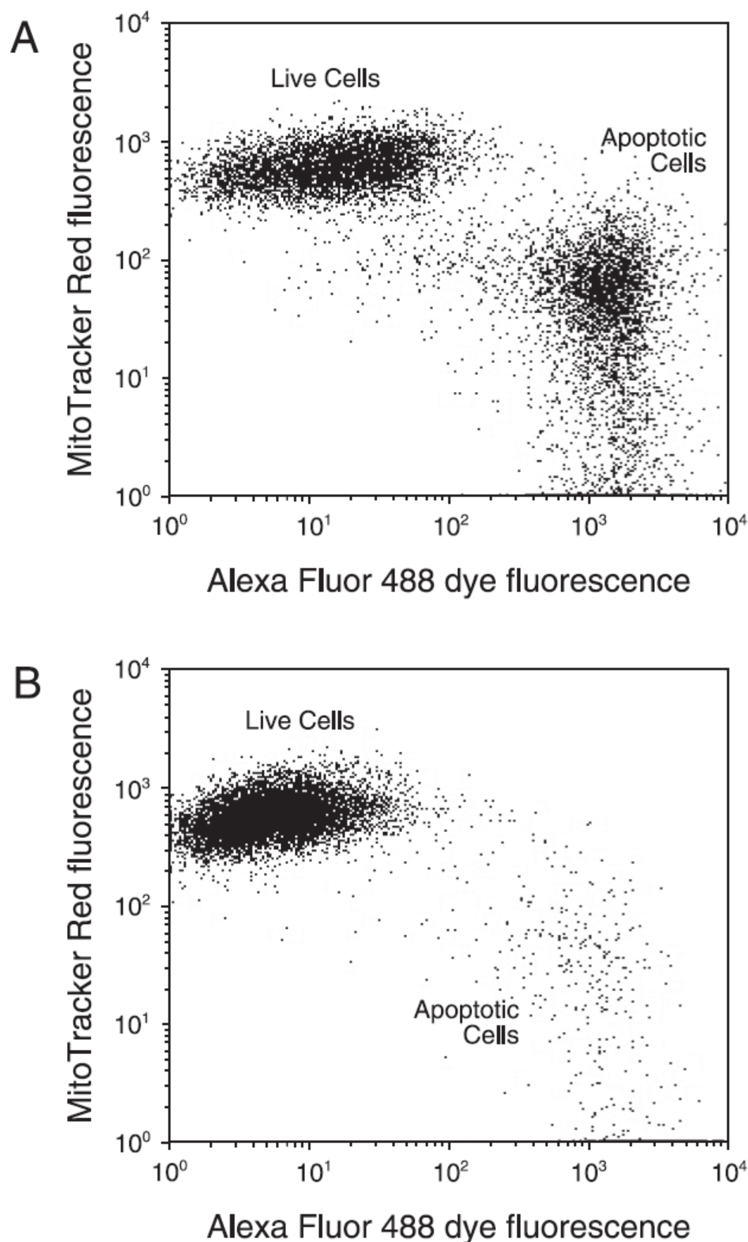

Figure 1 Jurkat cells (human T-cell leukemia) treated in complete medium with 10 pM camptothecin for four hours (A) or untreated (B).
Cells were reacted with the reagents in the kit followed by flow cytometric analysis. Note that the apoptotic cells show higher reactivity for annexin V and lower MitoTracker™ Red dye fluorescence than do live cells.

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. Since no single parameter defines apoptosis in all systems, we strongly suggest using a combination of different measurements for reliable detection of apoptosis. This kit could be easily combined with a cell impermeant dye, such as TO-PRO™-3 iodide (Cat. No. T3605), which indicates cell membrane integrity and would help distinguish dying or dead cells. TO-PRO™-3 iodide can be excited with a 633 nm laser and detected with a 660 nm bandpass filter. Refer to **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, except store Component B dessicated at ≤-20°C. Protect from light. Do not freeze Component A.
MitoTracker™ Red (CMXRos, Component B)[5]	3 × 50 µg	Not applicable	
5X Annexin-binding buffer (Component C)	15 mL	50 mM HEPES, 700 mM NaCl, 12.5 mM $CaCl_2$, pH 7.4	
Dimethylsulfoxide (DMSO, Component D)	100 µL	High-quality anhydrous DMSO	

[1] Sufficient material is supplied for 50 flow cytometry assays based on a 100 µL assay volume.
[2] The Alexa Fluor™ 488 annexin V conjugate and MitoTracker™ Red dye 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: 579/599 nm

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: This 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 a 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 1 mL 5X annexin-binding buffer (Component C) to 4 mL of deionized water.
3. Prepare a 10 mM stock solution of MitoTracker™ Red dye by adding 9.4 µL DMSO (Component D) to a vial of MitoTracker™ Red dye (Component B).

   Note: Store the unused portion of this stock solution at ≤–20°C for up to 1 month.
4. Prepare a 10 µM working solution of the MitoTracker™ Red dye. Pipet 1 µL of 10 mM MitoTracker™ Red stock solution into 1,000 µL of medium.
5. Harvest the cells and count them, adjusting their concentration to 5×10^6 cells/mL in culture medium.
6. To 1 mL of cells, add 4 µL of 10 µM MitoTracker™ Red working solution from the above step and stain for 30 minutes at 37°C in an atmosphere of 5% CO_2.
7. Wash the cells with PBS, resuspend the cells in 100 µL of 1X annexin-binding buffer, then add 5 µL of Alexa Fluor™ 488 Annexin V (Component A).

   Note: If also using TO-PRO™-3 iodide, add it to the cells at a concentration of 1 µM along with the Alexa Fluor™ 488 Annexin V (step 7).
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 place the samples on ice.

10. As soon as possible, analyze the stained cells by flow cytometry, measuring the fluorescence emission at 530 nm and 585 nm.

    Note: The cells should resolve into two principal populations: live cells with a low level of green fluorescence and high red fluorescence, and apoptotic cells with moderate level of green fluorescence and low red fluorescence (see Figure 1). If using TO-PRO™-3 iodide, cells with damaged membranes will show high red fluorescence when excited with a 633 nm laser line. Confirm the flow cytometry results by viewing the cells under a fluorescence microscope, using filters appropriate for fluorescein and Texas Red™.

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	27 May 2022	The content and format were updated. This document supercedes Rev. 2.0, revision date July 2010.

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