Single Channel Annexin V/Dead Cell Apoptosis Kit with Alexa Fluor® 488 annexin V and SYTOX® Green for Flow Cytometry

Catalog no. V13240

Table 1. Contents and storage information.

Material	Amount	Composition	Storage*	Stability
Alexa Fluor® 488 annexin V (Component A)	250 µL	Solution in 25 mM HEPES, 140 mM NaCl, 1 mM EDTA, pH 7.4, 0.1% bovine serum albumin (BSA)	• 2–6˚C • Protect from light • DO NOT FREEZE COMPONENT A	When stored as directed this kit is stable for 6 months.
SYTOX® Green dye (Component B)	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		
*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.				
Number of assays: Sufficient material is supplied for 50 flow cytometry assays based on a 100 µL assay volume.				
Approximate fluorescence excitation/emission maxima: Alexa Fluor® 488 annexin V**:** 499/521 in nm; SYTOX® Green: 503/524 in nm, bound to DNA.				

Introduction

Apoptosis is a carefully regulated process of cell death that occurs as a normal part of development. Inappropriately regulated apoptosis is implicated in disease states, such as Alzheimer's disease and cancer. Apoptosis is distinguished from necrosis, or accidental cell death, by characteristic morphological and biochemical changes, including compaction and fragmentation of the nuclear chromatin, shrinkage of the cytoplasm, and loss of membrane asymmetry.[1-5] In normal live cells, phosphatidylserine (PS) is located on the cytoplasmic surface of the cell membrane. However, in apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, thus exposing PS to the external cellular environment.[6] In leukocyte apoptosis, PS on the outer surface of the cell marks the cell for recognition and phagocytosis by macro phages.[7,8] The human vascular anticoagulant, annexin V, is a 35–36 kDa Ca^{2+}-dependent phospholipid-binding protein that has a high affinity for PS.[9] Annexin V labeled with a fluorophore or biotin can identify apoptotic cells by binding to PS exposed on the outer leaflet.[10]

The Single Channel Annexin V/Dead Cell Apoptosis Kit with Alexa Fluor® 488 annexin V and SYTOX® Green for flow cytometry provides a rapid and convenient assay for apoptosis. The kit contains recombinant annexin V conjugated to one of our best and brightest fluoro phores, the 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.

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 potentially can 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 probes.invitrogen.com for a wide selection of products for apoptosis research.

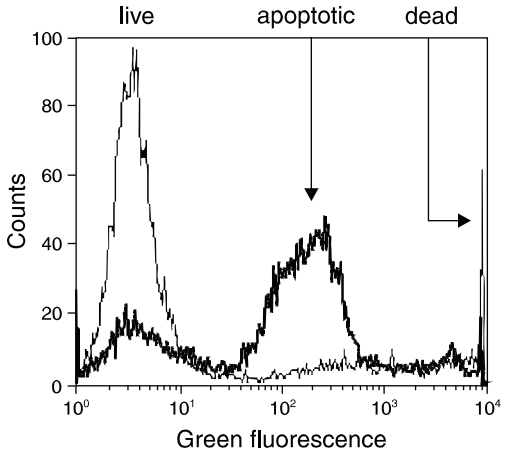

Figure 1A. Jurkat cells (T-cell lukemia, human) treated with 10 µM camptothecin for four hours (bold line) or untreated (as control, fine line). Cells were then treated with the reagents in the Kit followed by flow cytometric analysis. Note that the camptothecin-treated cells have a higher percentage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

Before Starting

Materials Required but Not Provided

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

Caution

No data are available addressing the mutagenicity or toxicity of SYTOX® Green dye. Because this reagent binds to nucleic acids, treated it as a potential mutagen and handle with appropriate care. Handle the DMSO stock solution with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues.

Experimental Protocols

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.

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

2. Harvest the cells after the incubation period and wash in cold phosphate-buffered saline (PBS).

3. Prepare 1X annexin-binding buffer.

   For example, for ~10 assays, add 1 mL 5X annexin-binding buffer (Component C) to 4 mL deionized water.

4. 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.

   Store the unused portion of this working solution at ≤–20°C for up to 1 month.

5. Re-centrifuge the washed cells (from step 2), discard the supernatant and resuspend the cells in 1X annexin-binding buffer.

   Determine the cell density and dilute in 1X annexin-binding buffer to ~1×10^6 cells/mL, preparing a sufficient volume to have 100 µL per assay.

6. Add 5 µL Alexa Fluor® 488 annexin V (Component A) and 1 µL 5 µM SYTOX® Green working solution (prepared in step 4) to each 100 µL of cell suspension.

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

8. After the incubation period, add 400 µL 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.

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

References

1. Immunol Cell Biol 76, 1 (1998); **2.** Cytometry 27, 1 (1997); **3.** J Pharmacol Toxicol Methods 37, 215 (1997); **4.** FASEB J 9, 1277 (1995); **5.** Am J Pathol 146, 3 (1995); **6.** Cytometry 31, 1 (1998); **7.** J Immunol 148, 2207 (1992); **8.** J Immunol 151, 4274 (1993); **9.** J Biol Chem 265, 4923 (1990); **10.** Blood 84, 1415 (1994).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
V13240	Single Channel Annexin V/Dead Cell Apoptosis Kit with Alexa Fluor® 488 annexin V and SYTOX® Green *50 assays* *for flow cytometry*	1 kit

Contact Information

Molecular Probes, Inc.
29851 Willow Creek Road
Eugene, OR 97402
Phone: (541) 465-8300
Fax: (541) 335-0504

Customer Service:
6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338
Fax: (541) 335-0305
probesorder@invitrogen.com

Toll-Free Ordering for USA:
Order Phone: (800) 438-2209
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Technical Service:
8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353
Toll-Free (800) 438-2209
Fax: (541) 335-0238
probestech@invitrogen.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100
Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

For country-specific contact information, visit www.invitrogen.com.

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