applied biosystems® by life technologies™

Apoptosis detection

Apoptosis assays for the Attune® Acoustic Focusing Cytometer

Apoptosis is a carefully regulated process of cell death that occurs as a normal part of development. Apoptosis is distinguished from necrosis, or accidental cell death, by characteristic morphological and biochemical changes, including compaction and fragmentation of nuclear chromatin, shrinkage of the cytoplasm, and loss of membrane asymmetry. Biochemically, apoptosis is distinguished by fragmentation of the genome and cleavage or degradation of several cellular proteins.

As with cell viability, no single parameter fully defines cell death in all systems; therefore, it is often advantageous to use several different approaches when studying apoptosis [1–4]. Life Technologies provides a broad offering of research reagents and kits for apoptosis testing for use with flow cytometry. Here, our most popular kits are demonstrated on the Attune® Acoustic Focusing Cytometer—three apoptotic plasma membrane assays and a mitochondrial membrane potential assay. Acoustic cytometry is compatible with our full range of apoptosis products.

Apoptotic plasma membrane assays for flow cytometry

Some of the earliest detectable apoptotic events involve the plasma membrane, including changes in membrane asymmetry and permeability. In addition to annexin V conjugates, Life Technologies provides unique assays to measure membrane changes under conditions where annexin V binding is problematic, such as in adherent cells, and without using special buffers.

Annexin V conjugates

In apoptotic cells, phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thus exposing PS to the external cellular environment. Annexin V labeled with a fluorophore can identify apoptotic cells by binding to PS exposed on the outer leaflet of the membrane. The Alexa Fluor® series of dyes, used in our Annexin V Dead Cell Apoptosis kits (Figure 1), has proven to make brighter and more photostable bioconjugates than other organic dyes with similar spectral characteristics.

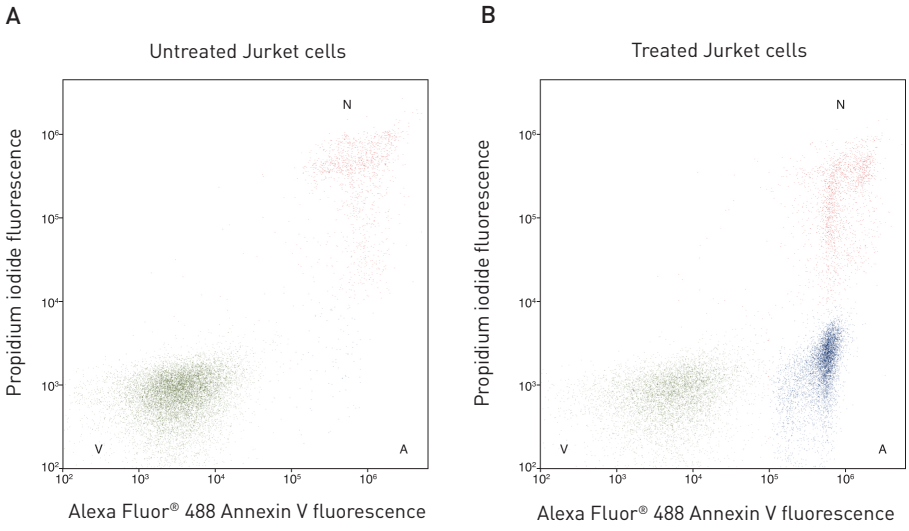


Figure 1. Apoptosis detection with the Annexin V Dead Cell Apoptosis Kit. Jurkat cells (T-cell leukemia, human) treated with 10 µM camptothecin for 4 hr (**B**) or untreated control (**A**). Cells were stained using the Annexin V Dead Cell Apoptosis Kit and analyzed by flow cytometry with 488 nm excitation on the Attune® Acoustic Focusing Cytometer with 530/30 nm and 575/24 nm bandpass filters. Data were acquired with a standard 100 µL/min collection rate. Note that the camptothecin-treated cells (**B**) have a higher percentage of apoptotic cells than the basal level of apoptosis seen in the control cells (**A**). A = apoptotic cells, V = viable cells, N = necrotic cells.

Monomeric cyanine dyes

There are some situations in which staining cells with annexin V is not the optimal method for detecting apoptosis. These include assays where cells are sensitive to the high calcium concentrations required for annexin V binding, assays where phosphatidylserine detection on adherent cells is adversely affected by trypsinization, and assays where washing of samples is prohibitive.

Three monomeric cyanine dyes (PO-PRO™-1, YO-PRO®-1, and TO-PRO®-3) (Figures 2 and 3) have been shown to penetrate apoptotic cells because of permeability changes associated with the loss in asymmetry of the plasma membrane [5]. These dyes enter apoptotic cells and bind to nucleic acids, while cell-impermeant dead cell stains are excluded. The three dyes have unique excitation wavelengths, providing enhanced flexibility in multiplexed assays.

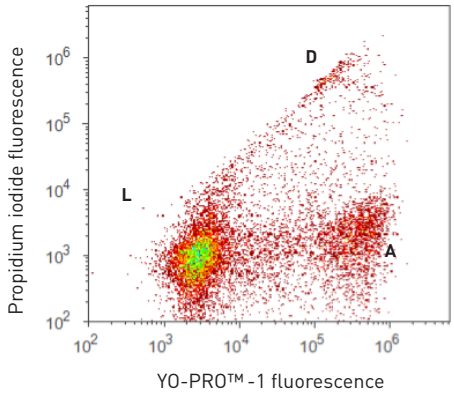


Figure 2. Apoptosis detection using YO-PRO®-1 stain. Jurkat cells (human T-cell leukemia) were treated for 4 hr with 10 µM camptothecin. Cells were then stained with 1.5 µM propidium iodide (PI) and 0.1 µM YO-PRO®-1 dye and analyzed on the Attune® Acoustic Focusing Cytometer using 488 nm excitation. YO-PRO®-1 dye fluorescence was collected with a 530/30 nm bandpass filter and PI fluorescence was collected with a 575/24 nm bandpass filter. Populations are labeled as L = live cells, A = apoptotic cells, and D = dead cells.

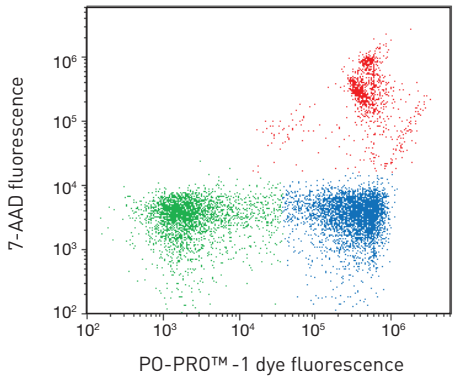


Figure 3. Apoptosis detection using PO-PRO™-1 stain. Jurkat cells (human T-cell leukemia) were treated for 4 hr with 10 µM camptothecin. Cells were then stained with 1.5 µM 7-AAD and 0.1 µM PO-PRO™-1 dye and analyzed on the Attune® Acoustic Focusing Cytometer using 405 nm and 488 nm excitation. PO-PRO™-1 dye fluorescence was collected with a 450/40 nm bandpass filter and 7-AAD fluorescence was collected with a 640 nm longpass filter. Populations are colored as green = live cells, blue = apoptotic cells, and red = dead cells.

Violet Ratiometric Membrane Asymmetry Probe/Dead Cell Apoptosis Kit

The Violet Ratiometric Membrane Asymmetry Probe/Dead Cell Apoptosis Kit (Figure 4) provides a simple and fast method for detecting apoptosis with dead-cell discrimination by flow cytometry. The violet ratiometric membrane asymmetry probe F2N12S (4´-N,N-diethylamino-6-(N-dodecyl-N-methyl-N-(3-sulfopropyl)) ammoniomethyl-3-hydroxyflavone) is a novel violet diode–excitable dye for the detection of membrane phospholipid asymmetry changes during apoptosis. This dye exhibits an excited-state intramolecular proton transfer (ESIPT) reaction, resulting in dual fluorescence with two emission bands corresponding to 530 nm and 585 nm and producing a two-color ratiometric response to variations in surface charge [6]. This ratiometric probe is therefore a self-calibrating absolute parameter of apoptotic transformation, independent of probe concentration, cell size, and instrument variations.

Mitochondrial JC-1 apoptosis assay for flow cytometry

A distinctive feature of the early stages of apoptosis is the disruption of the mitochondria, including changes in membrane and redox potential [7–9]. Life Technologies exclusively offers a number of fluorescent probes for analyzing mitochondrial activity in live cells by flow cytometry—the MitoProbe™ assays.

The JC-1 dye (Figure 5) exhibits potential-dependent accumulation in mitochondria, indicated by a fluorescence emission shift from green (~529 nm) to red (~590 nm). Consequently, mitochondrial depolarization is indicated by a decrease in the red/green fluorescence intensity ratio, which is dependent only on the membrane potential and not on other factors such as mitochondrial size, shape, and density, which may influence single-component fluorescence measurements.

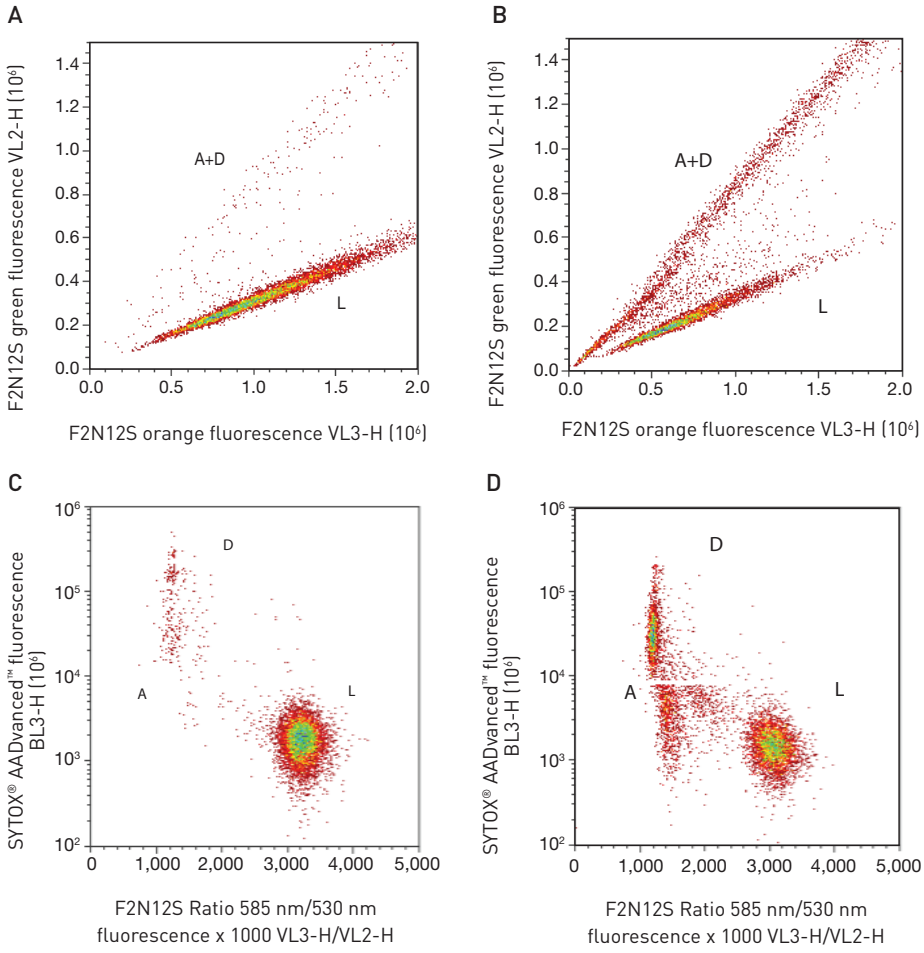
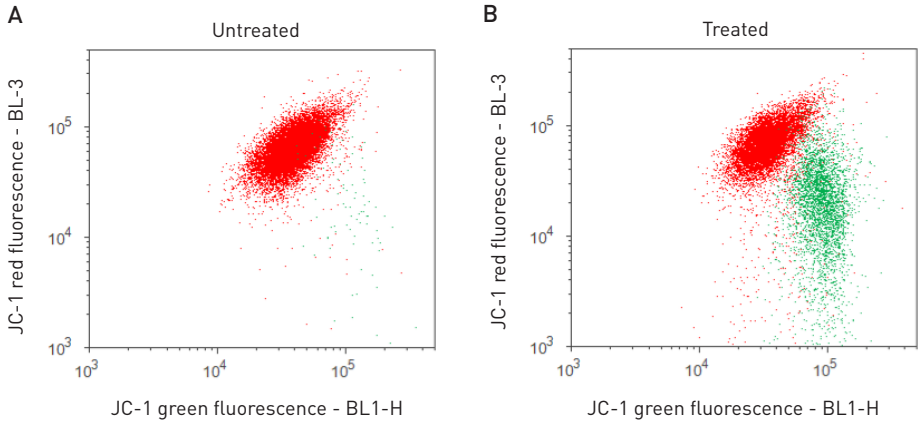


Figure 4. The Violet Ratiometric Membrane Asymmetry Probe/Dead Cell Apoptosis Kit. Jurkat cells (T-cell leukemia, human) were treated with 10 µM camptothecin for 4 hr (**B** and **D**) or left untreated (**A** and **C**). Cells were stained according to the protocol and analyzed on the Attune® Acoustic Focusing Cytometer. For F2N12S, 405 nm excitation and 522/31 nm and 603/48 nm bandpass filters were used; for SYTOX® AADvanced™ dead cell stain, 488 nm excitation and a 640 nm longpass filter was used. In panels **A** and **B**, live cells can be discriminated from apoptotic and dead cells by the relative intensities of the two emission bands from F2N12S. In panels **C** and **D**, SYTOX® AADvanced™ dead cell stain fluorescence is plotted against a derived ratio parameter from the two emission bands (585/530 nm) of F2N12S. A = apoptotic cells, L = live cells, D = dead cells.



5% CO_2, washed with PBS, and analyzed on the Attune® Acoustic Focusing Cytometer using 488 nm excitation with 530/30 nm bandpass and >640 longpass emission filters. Untreated cultured cells (**A**) are shown compared to treated cells (**B**), which were induced to undergo apoptosis with 10 µM camptothecin for 5 hr at 37°C.

Ordering Information—Current prices may be obtained from our website or by contacting Customer Service.

Product	Quantity	Cat. No.
Alexa Fluor® 488 Annexin V/Dead Cell Apoptosis Kit with Alexa Fluor® 488 Annexin V and PI (for flow cytometry)	1 kit (50 assays)	V13241
Alexa Fluor® 488 Annexin V/Dead Cell Apoptosis Kit with Alexa Fluor® 488 Annexin V and PI (for flow cytometry)	1 kit (250 assays)	V13245
Membrane Permeability/Dead Cell Apoptosis Kit with YO-PRO®-1 and PI (for flow cytometry)	1 kit (200 assays)	V13243
Membrane Permeability/Dead Cell Apoptosis Kit with PO-PRO™-1 and 7-aminoactinomycin D (for flow cytometry)	1 kit (200 assays)	V35123
Violet Ratiometric Membrane Asymmetry Probe/Dead Cell Apoptosis Kit (for flow cytometry)	1 kit (100 assays)	A35137
MitoProbe™ JC-1 Assay Kit	1 kit (100 assays)	M34152

Related Products—(these products are compatible with the Attune™ Acoustic Cytometer and the performance will be similar to the results shown on the previous pages)

Product	Quantity	Cat. No.
Single Channel Annexin V/ Dead Cell Apoptosis Kit (Alexa Fluor® 488 Annexin V/SYTOX® Green) (for flow cytometry)	1 kit (50 assays)	V13240
Annexin-binding buffer (5X concentrate) (for flow cytometry)	50 mL	V13246
PE Annexin V/ Dead Cell Apoptosis Kit (with SYTOX® Green) (for flow cytometry)	1 kit (50 assays)	V35112
Violet Annexin V/Dead Cell Apoptosis Kit (Pacific Blue™ Annexin V/SYTOX® AADvanced™) (for flow cytometry)	1 kit (50 assays)	V35136
Annexin V, Alexa Fluor® 350 conjugate	100 assays	A23202
Annexin V, Alexa Fluor® 488 conjugate	100 assays	A13201
Annexin V, Alexa Fluor® 555 conjugate	100 assays	A35108
Annexin V, Alexa Fluor® 568 conjugate	100 assays	A13202
Annexin V, Alexa Fluor® 594 conjugate	100 assays	A13203
Annexin V, Alexa Fluor® 647 conjugate	100 assays	A23204
Annexin V, Alexa Fluor® 680 conjugate	100 assays	A35109
Annexin V, allophycocyanin conjugate (APC Annexin V)	50 assays	A35110
Annexin V, biotin-X conjugate	100 assays	A13204
Annexin V, fluorescein conjugate (FITC Annexin V)	100 assays	A13199
Annexin V, Oregon Green® 488 conjugate	100 assays	A13200
Annexin V, R-phycoerythrin conjugate (R-PE Annexin V)	50 assays	A35111
Annexin V, Pacific Blue™ conjugate (for flow cytometry)	100 assays	A35122
PO-PRO™-1 iodide (435/455) (1 mM solution in DMSO)	1 mL	P3581
YO-PRO®-1 iodide (491/509) (1 mM solution in DMSO)	1 mL	Y3603
TO-PRO®-3 iodide (642/661) (1 mM solution in DMSO)	1 mL	T3605
MitoProbe™ $DiOC_2(3)$ (for flow cytometry)	100 assays	M34150
MitoProbe™ $DiIC_1(5)$ Assay Kit (for flow cytometry)	100 assays	M34151
MitoTracker® Orange CMTMRos (special packaging)	20 × 50 µg	M7510
MitoTracker® Red CMXRos (special packaging)	20 × 50 µg	M7512
MitoProbe™ Transition Pore Assay Kit (for flow cytometry)	100 assays	M34153
Vybrant® FAM Poly Caspases Assay Kit (for flow cytometry)	1 kit	V35117
Vybrant® FAM Caspase-3 and -7 Assay Kit (for flow cytometry)	1 kit	V35118
Vybrant® FAM Caspase-8 Assay Kit (for flow cytometry)	1 kit	V35119
Anti-PARP FITC Apoptosis Kit	1 kit	AHM2011
Hoechst 33342	100 mg	H1399
Vybrant® DyeCycle™ Violet stain (5 mM solution in DMSO)	200 assays	V35003
APO-BrdU™ TUNEL Assay Kit (with Alexa Fluor® 488 anti-BrdU)	60 assays	A23210

FITC Annexin V/Dead Cell Apoptosis Kit with FITC Annexin V and PI (flow cytometry)	50 assays	V13242
Chromatin Condensation/Dead Cell Apoptosis Kit with Hoechst 33342 and PI (for flow cytometry)	200 assays	V13244
Chromatin Condensation/Membrane Permeability/Dead Cell Apoptosis Kit with Hoechst 33342/YO-PRO®-1 and PI (flow cytometry)	200 assays	V23201
APC Annexin V/Dead Cell Apoptosis Kit with APC Annexin V and SYTOX® Green (for flow cytometry)	50 assays	V35113
Metabolic Activity/Annexin V/Dead Cell Apoptosis Kit with C_{12} Resazurin, APC Annexin V, and SYTOX® Green (flow cytometry)	50 assays	V35114
Mitochondrial Membrane Potential/Annexin V Apoptosis Kit with MitoTracker® Red and Alexa Fluor® 488 Annexin V (flow cytometry)	50 assays	V35116
Violet Annexin V/Dead Cell Apoptosis Kit with Pacific Blue™ Annexin V/SYTOX® AADvanced™ (for flow cytometry)	50 assays	A35136
Violet Chromatin Condensation/Dead Cell Apoptosis Kit with Vybrant® DyeCycle™ Violet and SYTOX® AADvanced™ (for flow cytometry)	200 assays	A35135

References

1. *Apoptosis* (2007) 12:803–813.
2. *Cell Death Differ* (2009) 16:3–11.
3. *Methods Mol Biol* (2009) 559:19–32.
4. *Methods* (2008) 44:222–228.
5. *J Immunol Meth* 185:249–258.
6. *J Am Chem Soc* (2007) 129:2187–2193.
7. *Trends Cell Biol* (2000) 10:369–377.
8. *Science* (2000) 289:1150–1151.
9. *Science* (2001) 292:624–626.