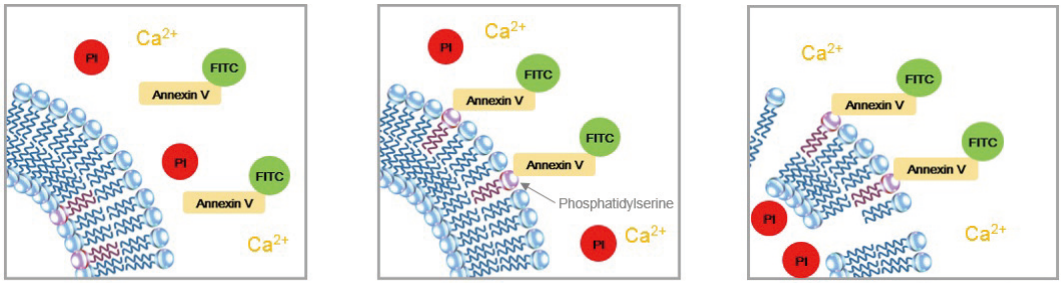
nacalai tesque
The quality for certainty.

Annexin V-FITC Apoptosis Detection Kit

Features

- Annexin V-FITC Apoptosis Detection Kit contains ready-to-use solutions, Annexin V-FITC conjugate, propidium iodide (PI).
- The kit can identify apoptotic and necrotic cells
- Detect by flow cytometry or fluorescence microscopy
- No need to fix cells

Mechanism

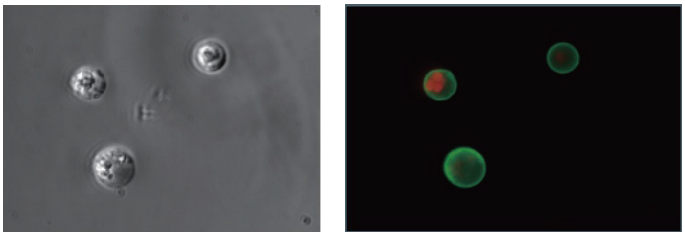


Normal Cell | Early stages of apoptosis | Late stage of apoptosis

In normal cells, phosphatidylserines (PS, membrane phospholipids) are held on the inner layer of the cell membrane, so Annexin V does not attach to the cells. During early apoptosis, the PS are exposed on the outer layer, where they attach to the FITC-labeled Annexin V and stain the cell surface green. During late apoptosis, propidium iodide (PI) enters the cell and stains the contents red.

Application 1

Fluorescent imaging of apoptosis induced cells



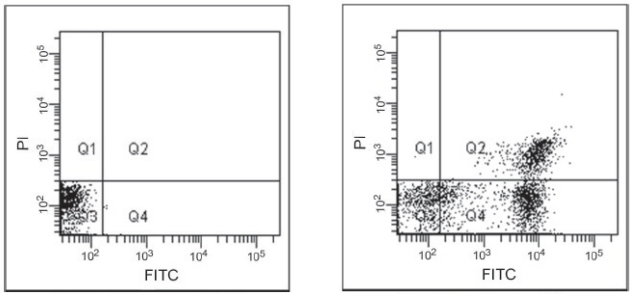
Bright Field | Fluorescent Image

Jurkat cells were apoptosis induced with staurosporine (1 µg/ml) at 37 °C for 3.5 hours and then observed under a fluorescent microscope.

FITC-labeled Annexin V (Green)
PI (Red)

Application 2

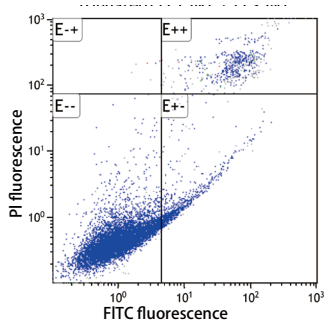
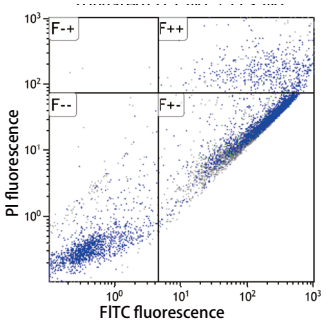
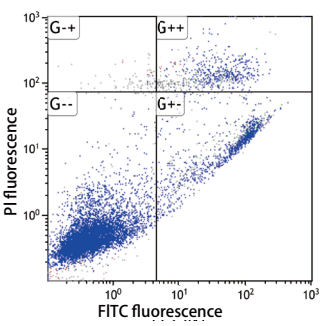
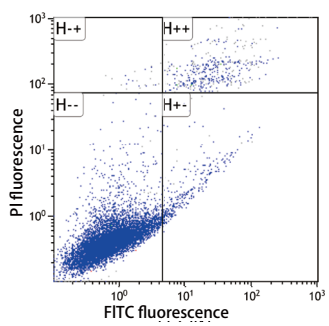
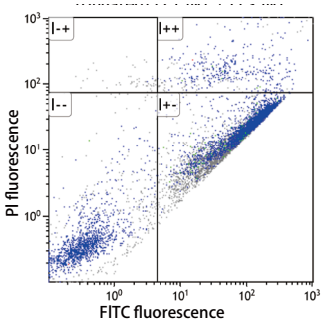
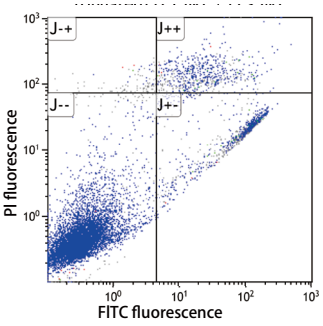
Flow cytometric analysis of apoptosis induced cells



A : Control (non-treated cells) | B : Apoptosis induced cells

Jurkat cells were apoptosis induced with staurosporine with its concentration of 1 µg/ml at 37 °C for 3.5 hours and then analyzed with a flow cytometer.

Apoptosis Detection Kit Performance Comparison

	Control (untreated)	Cycloheximide treatment	UV treatment
Company A			
Annexin V			

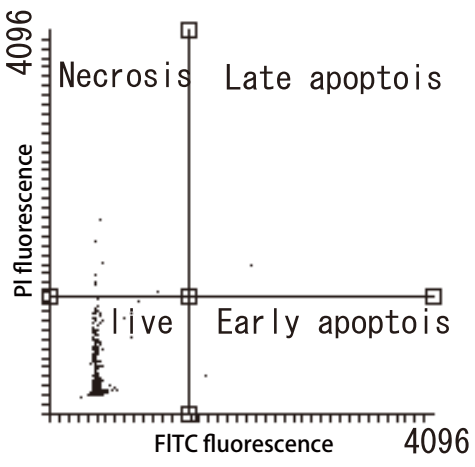
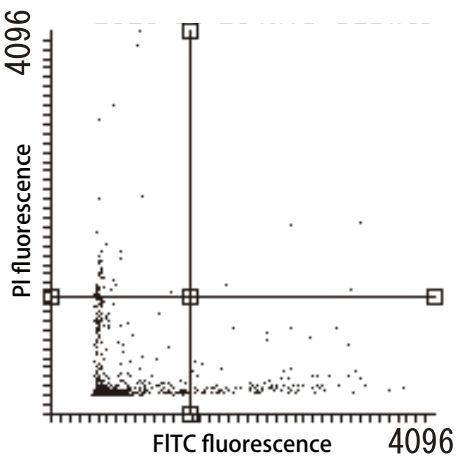
Cell type: Mouse B16 melanoma cells
Number of cells: 10,000
Instrument: Gallios (Beckman Coulter Inc.)

Apoptosis was induced by cycloheximide (1 µg/ml) or UV irradiation at 37°C for 3.5 hours. Cells were analyzed by flow cytometry. Our product showed about the same performance as the product from company A.
The control cells did not display any signs of apoptosis. Treatment with cycloheximide resulted in many cells in early apoptosis. UV irradiation also induced apoptosis in the tested cells.

--: FITC-labeled Annexin V and PI both had low fluorescence values. Live cells.
+-: FITC-labeled Annexin V had high fluorescence value, PI was low. Early apoptosis.
++: FITC-labeled Annexin V and PI both had high fluorescence values. Late apoptosis.

Data Courtesy of Assistant professor Yuuki Takahashi , Graduate School and Faculty of Pharmaceutical Sciences, Kyoto University

Apoptosis Detection Kit Performance Evaluation

Control (untreated)	Starvation (48 hours)
	

Cell type: U-87 MG cells
Instrument: Tali® Image-Based Cytometer (Thermo Fisher Scientific Inc.)

Apoptotsis was induced by removing glucose and starving the cells for 48 hours. Upon analysis using an imaging cytometer, some cells were observed in early apoptosis.

Data Courtesy of Assistant Professor Hitoshi Gotoh Department of Biology / Developmental Neurobiology, Liberal Arts and Sciences, Department of Biology, Kyoto Prefectural University of Medicine

Components

Reagents	for 100 assays	
	Volume	Quantity
Annexin V-FITC Conjugate	250 µl	2
PI Solution	250 µl	2
Annexin V Binding Buffer (10x)	10 ml	2

Preparation

Annexin V Binding Solution

Dilute Annexin V Binding Buffer (10x) 10-fold with distilled water.

Protocols

General Protocol for Suspension Cells

1. Centrifuge the cell suspension at 1,000 rpm for 3 minutes and remove supernatant.
2. Add PBS to wash cells and centrifuge at 1,000 rpm for 3 minutes, remove supernatant. (Do this step twice.)
3. Add 10-fold diluted Annexin V Binding Solution to make final cell concentration of 1 x 10^6 cells/ml.
4. Transfer 100 µl of cell suspension prepared in step 3 to a new tube.
5. Add 5 µl of Annexin V - FITC Conjugate, then 5 µl of PI Solution to the cell suspension.
6. Incubate 15 minutes at room temperature with protect from light.
7. Add 400 µl of 10-fold diluted Annexin V Binding Solution.
8. Apply the solution prepared in step 7 to flow cytometric assay or microscopic assay.

General Protocol for Adherent Cells

1. Discard supernatant on the petri dish or plate.
2. Add PBS for wash cells and discard supernatant. (Do this step twice.)
3. Detach the cells with Trypsin-EDTA.
4. Add appropriate volume of culture medium or PBS and transfer the cell suspension to a tube.
5. Centrifuge at 1,000 rpm for 3 minutes. Remove supernatant.
6. Add PBS to wash cells and centrifuge at 1,000 rpm for 3 minutes, remove supernatant. (Do this step twice.)
7. Add 10-fold diluted Annexin V Binding Solution to make final cell concentration of 1 x 10^6 cells/ml.
8. Transfer 100 µl of cell suspension prepared at step 7 to a new tube.
9. Add 5 µl of Annexin V - FITC Conjugate, then 5 µl of PI Solution to the cell suspension.
10. Incubate 15 minutes at room temperature with protection from light.
11. Add 400 µl of 10-fold diluted Annexin V Binding Solution.
12. Apply this solution to flow cytometric assay or microscopic assay.

*Although adherent cells are not frequently used for Annexin V, FITC flow cytometric analyses to avoid cell membrane damage from the cell detachment process, Casiola-Rosen et al. and van Engelend et al. have reported methods on utilizing Annexin V for flow cytometry with adherent cell types.

	excitation / emission
Annexin V-FITC	494 nm / 518 nm
PI	535 nm / 617 nm

References

1. Casciola-Rosen L, Rosen A, Petri M, Schlissel M, *Proc Natl Acad Sci USA*, **93**(4), 1624 (1996)
2. van Engeland M, Remaekers FC, Schutte B, *Reutelingsperger CP, Cytometry*, **24**(2), 131 (1996)

Ordering Information

Product Name	Storage	Product No.	PKG Size
Annexin V-FITC Apoptosis Detection Kit	4°C	15342-54	100 tests

*1 test = 1 assay using $1x10^6$ cells/ml solution

Related Products

Product Name	0.5%-Trypan Blue Stain Solution	MTT Cell Count Kit	Cell Count Reagent SF	
Product Image				
Product No.	29853-34	23506-80	07553-15	07553-44
PKG Size	100 ml	1 kit (for 1,000 tests)	500 tests	2,500 tests
Features	• For determining whether cells are alive and counting them. • Simple and inexpensive. • Cannot screen for cells in apoptosis or dysgonic cells. • High margin of error. • Not suitable for processing large volumes of samples.	• For assays of the metabolic activity of viable cells. • An increase in number of living cells results in an increase in the amount of formazan formed.	• For counting cells. • More sensitive than other water-soluble tetrazolium salts (XTT, MTS).	

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