BECKMAN COULTER
MADE IN France

ANNEXIN A5 FITC / 7-AAD KIT

REF IM3614 150 tests

For Research Use Only. Not for use in diagnostic procedures.

REAGENTS

	Annexin A5-FITC	7-AAD Viability Dye	10X concentrated Binding Buffer
Formulation	Liquid - ready-to-use	Liquid - ready-to-use	Liquid
Volume	1.7 mL	3 mL	1.7 mL
Number of vials	1 vial	1 vial	5 vials

Features of Annexin A5-FITC

Modified human recombinant annexin labelled with FITC. Displays no measurable anti-coagulant activity in vitro.

- Annexin A5: FITC; 1:1 stoichiometric complex.
- Purity: >99% pure according to Fast Protein Liquid Chromatography.
- Concentration: 2.5 µg/mL annexin A5-FITC.
- Maximum fluorescence absorption: 490 nm.
- Maximum fluorescence emission: 525 nm.

Features of 7-AAD

- Purity: >85% pure according to HPLC.
- Maximum fluorescence absorption for DNA/7-AAD complex: 543 nm.
- Maximum fluorescence emission for DNA/7-AAD complex: 655 nm.

REAGENT CONTENTS

- See Table

SPECIFICITY

The ANNEXIN A5-FITC / 7-AAD Kit is an apoptosis detection kit based on the binding properties of annexin A5 to phosphatidylserine (PS) and on the specificity of 7-amino-actinomycin D (7-AAD) for DNA guanine-cytosine base pair (1).

Apoptosis (or programmed cell death) was discovered in tissues on the basis of morphological changes of the cell (2). Gradually, the morphological criteria for apoptosis, like cell shrinkage, nuclear condensation and pyknosis, were complemented with biochemical criteria such as the cleavage of DNA between the nucleosomes, resulting in the ladder appearance of DNA bands on agarose gels (3). In the early studies this typical feature was considered the hallmark of apoptosis. However, not all cells in apoptosis appear to cleave their DNA strands between the nucleosomes (4) and those that do, do so only late in the apoptotic pathway.

Other parameters can be used to detect and measure apoptosis, one of which is the appearance on the surface of the cell of phosphatidylserine (PS), a negatively charged phospholipid usually located in the inner leaflet of the plasma membrane. In the early phase of apoptosis, the integrity of the cell membrane is maintained but the cells lose the asymmetry of their membrane phospholipids (5, 6, 7, 8). PS becomes exposed at the cell surface and forms one of the specific signals for recognition and removal of apoptotic cells by macrophages (6, 9).

As the apoptosis process progresses, the cell membrane integrity is lost. Using a DNA specific viability dye, like the 7-AAD makes possible the distinction between early apoptotic and late apoptotic or necrotic cells; Furthermore, 7-AAD emits in the far-red of the spectrum and therefore can be separated from phycoerythrin (PE) and PE-Texas Red tandem dyes emissions. Monoclonal antibodies directly conjugated to these fluorochromes may be used to identify different cell subsets simultaneously (10, 11).

Annexin A5, a Ca^{2+}-dependent and phospholipid-binding protein, binds specifically to PS, with high affinity. The conjugation of annexin A5 with FITC in a 1:1 stoichiometric complex does not change the native phospholipid-binding properties of annexin A5. Binding kinetics show a fast association of annexin A5-FITC with

the phospholipid membrane, if PS and Ca^{2+} are available. Apoptotic cell is stained by annexin A5 before the dying cell changes its morphology and hydrolizes its DNA (5, 11, 12, 13, 14).

Apoptosis-associated PS exposure is a phylogenetically conserved mechanism of mammalian and non-mammalian species, and its detection by annexin A5 has been demonstrated for human, mouse, rat, hamster, chick and drosophila cell types tested so far (6, 7, 8, 9, 11, 15, 16, 17, 18).

The early detection and the ubiquity of apoptosis-associated PS exposure makes the ANNEXIN A5-FITC/ 7-AAD Kit, in view of its simple and rapid protocol, a powerful tool for the study of apoptosis (11, 13, 15, 19).

WARNING AND PRECAUTIONS

1. 7-AAD Viability Dye contains 0.005% 7-AAD. Pure 7-AAD is a potential carcinogen. Although this compound is highly diluted, we recommend to avoid contact with skin and eyes, to wear suitable protective clothing, gloves, and appropriate eye/face protection.
2. 7-AAD Viability Dye contains 1% Dimethyl Sulfoxide (DMSO). Pure DMSO is an irritant. Although this compound is diluted, we recommend to avoid contact with skin and eyes, to wear suitable protective clothing, gloves, and appropriate eye/face protection.
3. As a chromophore, 7-AAD is sensitive to light. Store the 7-AAD Viability Dye within the kit box to avoid continuous exposure to light during storage.
4. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
5. Never pipet by mouth and avoid contact of samples with skin and mucous membranes
6. Avoid microbial contamination of reagents or erroneous results might occur.
7. Do not use reagent beyond the expiration date on the vial label.
8. Minimize exposure to light.
9. Use general good laboratory practices when handling this reagent.

GHS HAZARD CLASSIFICATION

7-AAD Viability Dye	WARNING	
	H316	Causes mild skin irritation.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
		Dimethyl Sulfoxide 1 - 2%

SDS	Safety Data Sheet is available at techdocs.beckmancoulter.com

STORAGE AND HANDLING CONDITIONS AND STABILITY

These reagents are stable up to the expiration date when stored at 2–8°C in the absence of light. Do not freeze.

REAGENT PREPARATION

Dilute the 10X concentrated Binding Buffer 10 fold with distilled water and place the diluted buffer on ice. Prepare a quantity sufficient for the expected number of assays.

Annexin A5-FITC and 7-AAD Viability Dye reagents are ready for use. Do not dilute.

After use, the reagents should be stored at 2–8°C in the dark.

PROCEDURE

Positive controls:

- Incubate cells with 3% formaldehyde-containing PBS for 30 minutes on ice. Centrifuge cells, discard the formaldehyde buffer, and resuspend cell pellets in cold 1X Binding Buffer to $5x10^6$–$10x10^6$ cells/mL. Proceed to staining from step 2 of the staining procedure.
- Induction of apoptosis of Fas/CD95-expressing cells such as human Jurkat cells or mouse thymocytes:

  Add 100 ng/mL of purified agonistic anti-Fas/CD95 antibody to the culture medium and incubate cells for 4–24 hours at 37°C (5% CO_2). Centrifuge cells, discard supernatant, and suspend cell pellets in cold 1X Binding Buffer to $5x10^6$–$10x10^6$ cells/mL. Proceed to staining from step 2 of the staining procedure.

Detection of apoptotic cells in a cell suspension:

1. Wash cell suspensions with ice-cold culture medium or PBS and centrifuge for 5 minutes at 500 x g at 4°C. Discard supernatant, and resuspend cell pellets in ice-cold 1X Binding Buffer to $5x10^6$–$10x10^6$ cells /mL. Keep tubes on ice.
2. Add 10 µL of Annexin A5-FITC solution and 20µL of 7-AAD Viability Dye to 100 µL of the cell suspensions prepared as given in step 1. Mix gently.
3. Keep tubes on ice and incubate for 15 minutes in the dark.
4. Add 400µL of ice-cold 1X Binding Buffer and mix gently.

Analyze cell preparations within 30 minutes by flow cytometry.

PRECAUTIONS

- The flow cytometer is preferably set such that the distribution of the Annexin A5-FITC-negative population is in the first decade of the FITC-parameter and the 7-AAD-negative population in the first decade of the 7-AAD-parameter.
- The incubation with Annexin A5-FITC and 7-AAD should be carried out on ice so as to arrest further progress of the cells through the stages of viability > apoptosis > secondary necrosis.
- For rat thymocytes, when kept on ice, the population distribution (viable, apoptotic, secondary necrotic) remains stable for at least 6 hours.

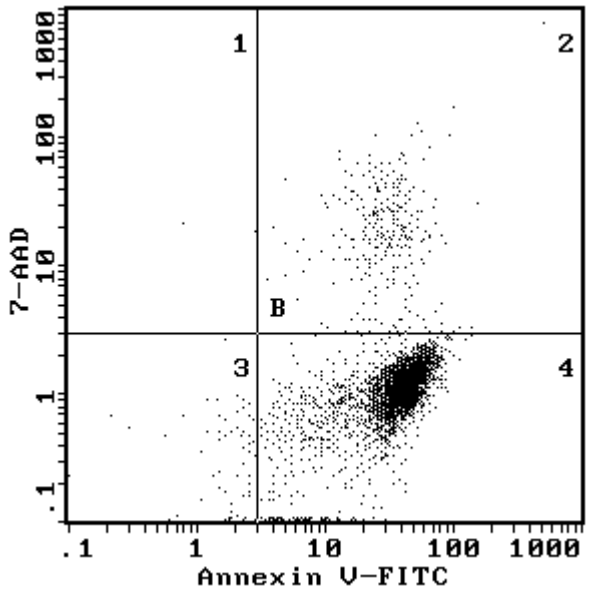
EXAMPLE DATA

Flow cytometric analysis of apoptotic Jurkat cells after staining with the reagents of the Annexin A5-FITC / 7-AAD Kit. Jurkat cells have been treated by 100ng/mL of agonistic anti-Fas/CD95 antibody for 5 hours. Acquisition and analysis are done on a COULTER EPICS XL equipped with the SYSTEM II software. The biparametric histogram LOG FL1 (525nm) vs LOG FL4 (675nm) shows three distinct populations, i) the viable cells which have low FITC and a low 7-AAD signal, ii) the apoptotic cells, which have high FITC and a low 7-AAD signal, and the secondary necrotic cells which have high FITC and a high 7-AAD signal (see figure). Depending on the cell type and on culture and centrifugation conditions, a fourth population corresponding to the damaged viable cells, with low FITC and a high 7-AAD signal, may be vizualized.

Quadrant 2: 14.0% (secondary necrotic cells)

Quadrant 3: 13.6% (viable cells)

Quadrant 4: 72.4% (early apoptotic cells)



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ADDITIONAL INFORMATION

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