Annexin V-FITC Kit System for Detection of Apoptosis
PN IM2375 – 20 tests

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

SPECIFICITY
The ANNEXIN V-FITC Kit is an apoptosis detection kit based on the binding properties of annexin V to phosphatidylserine (PS) and the DNA-intercalating capabilities of propidium iodide (PI). Apoptosis (or programmed cell death) was discovered in tissues on the basis of morphological changes of the cell (1). 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 on agarose gels (2). Until recently, this typical feature was considered the hallmark of apoptosis. However, not all cells in apoptosis appear to cleave their DNA strands between the nucleosomes (3) and those that do, do so only late in the apoptotic pathway.

New insights in the apoptotic process led to new parameters, which can be used to detect and measure apoptosis. One of these parameters 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 (4 – 7). PS becomes exposed at the cell surface and forms one of the specific signals for recognition and removal of apoptotic cells by macrophages (5, 8, 9).

Annexin V, a Ca2+-dependent and phospholipid-binding protein, binds preferentially to PS, with high affinity. Apoptotic cell is stained by annexin V before the dying cell changes its morphology and stabilizes its DNA (4, 10 – 13). The early detection and the ubiquity of apoptosis-associated PS exposure makes the ANNEXIN V-FITC Kit, in view of its simple and rapid protocol, a powerful tool for the study of apoptosis (10, 12, 14, 15). In the use of the ANNEXIN V-FITC Kit, the affinity of annexin V-FITC for PS in the presence of Ca2+ is exploited. The conjugation of annexin V with FITC in a 1:1 stoichiometric complex does not change the native phospholipid-binding properties of Annexin V. Binding kinetics show a fast association of annexin V with FITC in a 1:1 stoichiometric complex. The ANNEXIN V-FITC protocol is designed for the convenient, rapid measure of apoptosis in a sample of suspended cells. Apoptosis-associated PS exposure is a phylogenetically conserved mechanism of mammalian and non-mammalian species, and its detection by annexin V has been demonstrated for human, mouse, rat, hamster, chick and drosophila cell types tested so far (5 – 7, 9, 10, 14, 16 – 18).

REAGENT
Annexin V-FITC Kit System for Detection of Apoptosis
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REAGENT CONTENTS
- 1 Vial annexin V-FITC ready-to-use, liquid, 100 µL, containing 1 mg / mL BSA.
- 1 vial 10X concentrated binding buffer, liquid, 1,7 mL.
- 1 vial Propidium iodide*, red powder, 250 µg.
*Warning: Propidium iodide is toxic.

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

Fluorescence
Annexin V-FITC
Absorbs 492 nm
Emits at 520 nm.

Propidium iodide
Absorbs at 370 – 550 nm
Emits at 560 – 680 nm

APPLICATION
Flow cytometry.

STATEMENTS OF WARNING
1. Propidium Iodide (PI) is toxic: ANNEXIN V Kits contain Propidium iodide which is a potential mutagen. We recommend to avoid contact with skin and eyes, to wear suitable protective clothing and gloves, and appropriate eye / face protection. Mechanical ventilation and respiratory protection are also recommended.
2. Specimens, samples and all material coming in contact with them should be considered potentially infectious and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.
7. Use good laboratory practices when handling this reagent.

STORAGE CONDITIONS AND STABILITY
This reagent is stable up to the expiration date when stored at 2 – 8°C in the dark. 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

Dilute the 250 µg Propidium Iodide in 1 mL of diluted binding buffer and place the solution on ice.

After use, the solutions should be stored at 2 – 8°C.

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 18 – 25°C prior to use.

PROCEDURE
Detection of apoptosis on cell suspensions:

1. Wash the cell samples with ice-cold culture medium or PBS after centrifugation for 5 minutes at 500 g x g at 4°C. Discard supernatant, and finally resuspend the cell pellet in ice-cold, diluted binding buffer to 5 x 10^5 – 5 x 10^6 cells / mL. Keep the tubes on ice.
2. Add 5 µL of Annexin V-FITC solution and 2,5 µL dissolved PI to 100 µL of the cell suspension prepared as given in step 1. Mix gently.
3. Keep the tube on ice and incubate for 10 minutes in the dark.
4. Add 400 µL of ice-cold 1X binding buffer to the preparations and mix gently.
5. Analyze the cell sample by flow cytometry (or fluorescence microscopy).

Positive Control
1. Incubate the cells with 3% formaldehyde in buffer for 30 minutes on ice. Centrifuge the cells, discard the formaldehyde buffer, and resuspend the cells in cold diluted binding buffer to 5 x 10^5 – 5 x 10^6 cells / mL. Proceed to staining.
2. 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 (clone 7C11; see catalog for PN for human cells, or clone RK-8; see catalog for PN for mouse cell) to the culture medium and incubate cells for 4-24 hours at 37°C (5% CO2). Centrifuge cells, discard supernatant, and suspend cell pellets in cold 1X binding buffer to 5 x 10^5 - 5 x 10^6 cells/mL. Proceed to staining from step 2 of the procedure.

GENERAL NOTES AND PRECAUTIONS
The flow cytometer is preferably set such that the Mean Fluorescence Intensity of the annexin V-negative population is in the first
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EXAMPLE DATA
Flow cytometric analysis of apoptotic Jurkat cells after staining by Annexin V-FITC / PI (performed on a Coulter EPICS XL flow cytometer). Jurkat cells have been treated by 100 ng/mL of agonistic anti-Fas / CD95 antibody (clone 7C11; See catalog for PN) for at least 6 hours. Analysis is done with the XL SYSTEM II™ software. The biparametric representation (FL1 vs FL4) shows three distinct populations, i) the viable cells which have low FITC and low PI signal, ii) the apoptotic cells, which have high FITC and low PI signal and iii) the secondary necrotic cells which have high FITC and high PI 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 high PI signal, may be visualized.

SELECTED RESEARCH REFERENCES

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