Product datasheet

FITC Annexin V Apoptosis Detection Kit with PI

Product Information

Catalog# / Size K-01 / 100 tests

Storage Temperature 2–8 °C. Do not freeze.

Kit Components

10X Annexin V Binding Buffer (10 mL)

- Contains aqueous buffered solution containing 0.1 M Hepes/NaOH (pH 7.4), 1.4 M NaCl, 25 mM CaCl2 with no preservative.
- Prepare Freshly. For a 1X working solution, dilute 1 part of the 10X Annexin V Binding Buffer to 9 parts of distilled water.

Annexin V FITC (100 µL)

- Volume recommends per test; 1 µL
- Contains aqueous buffered solution containing BSA and ≤0.09% sodium azide.

Propidium Iodide (PI) Staining Solution (100 µL)

- Volume recommends per test; 1 µL
- Contains aqueous buffered solution containing no preservative.

The components supplied in this kit are 0.2 µm filtered and aseptically filled.

Storage Note Store undiluted at 4°C and protect from prolonged exposure to light. Do not freeze.

Reactivity All mammalian species

Application Flow Cytometry - *Quality Tested*

Kit Properties FITC Annexin V Apoptosis Detection Kit with PI has been specifically designed for the identification of apoptotic and necrotic cells. Annexin V FITC serves as a fluorescent probe for apoptotic cells. They will not bind normal and intact cells. However, necrotic cells are leaky enough to pass Annexin V FITC into cytoplasm and provide accessibility for binding to inner membrane phosphatidylserine (false positive apoptosis). Indeed, apoptotic cells have to be differentiated from necrotic cells by PI-stained apoptotic nuclei in comparison to PI-stained necrotic nuclei. But this assay due to its distinctive design is probably able to distinguish between cells that have undergone apoptotic death versus those that have died as a result of a necrotic pathway. The dead cells will probably stain only with both PI. However, when apoptosis is measured over time, cells can be often tracked from FITC Annexin V and PI negative (viable, or no measurable apoptosis), to FITC Annexin V positive and PI negative (early apoptosis, membrane integrity is yet present) and finally to FITC Annexin V and PI positive (end stage apoptosis). In fact, the movement of cells through these three stages suggests apoptosis (Figs. 1-3)

Description

Apoptosis is a normal physiologic process which occurs during embryonic development as well as in maintenance of tissue homeostasis. However, the program cell death is triggered in non-physiological conditions such as, drug induced apoptosis. The apoptosis program is characterized by certain morphological features, including loss of plasma membrane asymmetry at earlier stage and at later stage distinguish with subsequent loss of membrane integrity, condensation of the cytoplasm and nucleus, and internucleosomal cleavage of DNA. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby PS exposes to the extracellular environment. Annexin V is a 35-36 kDa Ca2+ dependent phospholipid-binding protein that binds phosphatidylserine with high affinity.

The conjugation of Annexin V to fluorochromes including FITC, while retains its high affinity for PS, provide this conjugate as a sensitive probe for flow cytometric analysis of cells that are undergoing apoptosis and microscopic imaging of this stained population. Since externalization of PS occurs in the earlier stages of apoptosis, FITC Annexin V staining can identify apoptosis at an earlier stage, and then quantitative evaluation of apoptotic nuclei based on nuclear changes such as DNA fragmentation can be distinguished by fluorophore staining nuclei, such as propidium iodide (PI) or 7-Amino-Actinomycin (7-AAD) to allow the investigator to identify early apoptotic cells (PI or 7-AAD negative, FITC Annexin V positive).

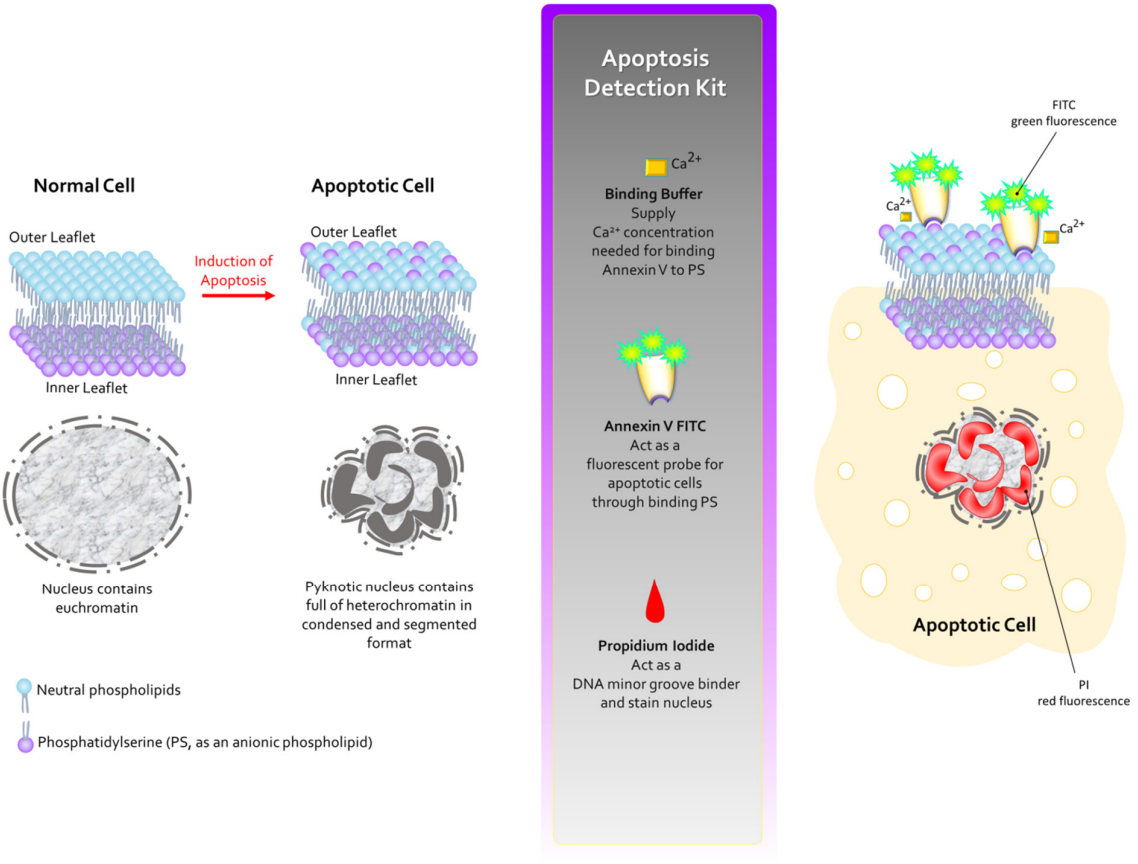

Figure 1: Test principle.

Viable and dead cells Viable cells with intact membranes represent preserved plasma membrane integrity so are not permeable to PI, whereas the membranes of dead and damaged cells are permeable to PI. For example, cells that are considered viable are FITC Annexin V and PI negative

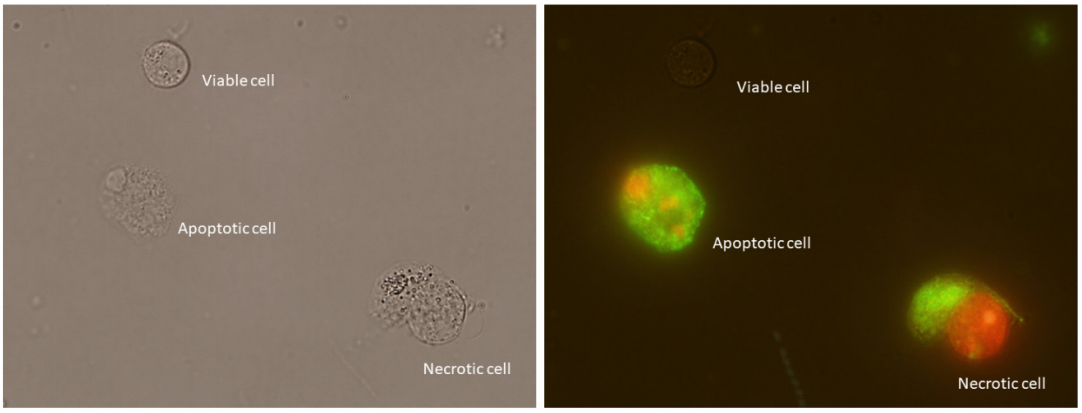

Figure 2: The illustration of viable, necrotic and apoptotic cells.

Early apoptotic cells The cells that are in early apoptosis are FITC Annexin V positive and PI negative

Late apoptotic cells The cells that are in late apoptosis or already dead are both FITC Annexin V and PI positive.

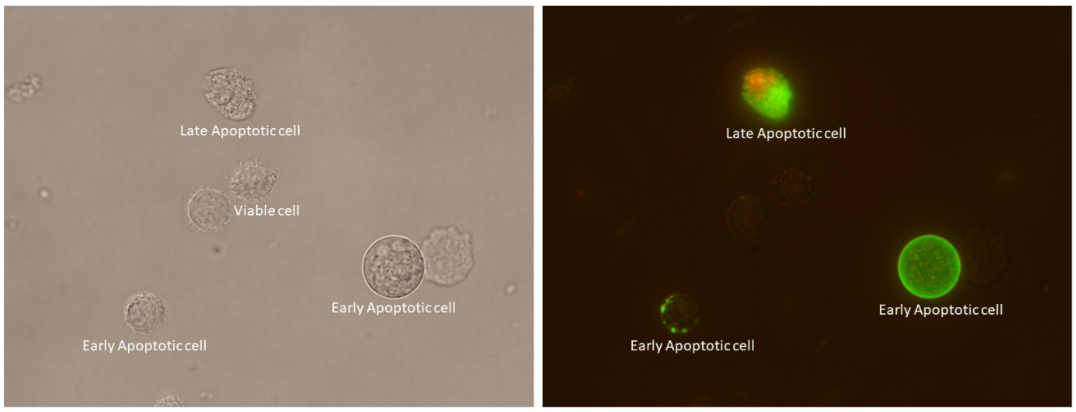

Figure 3: The illustration of viable, early and late apoptotic cells.

Flow Cytometric Analysis of FITC Annexin V/PI staining

Jurkat cells (Human T-cell leukemia; ATCC TIB-152) were untreated on left panels or treated on right panels during three time points, 3, 6 and 18 hours with 1 µM staurosporine (right panels). Cells were incubated with FITC Annexin V in binding buffer and then incubated with propidium iodide (PI) and analyzed by flow cytometry.

At left panels, untreated cells at three time points were FITC Annexin V and PI negative, indicating that they were viable and not undergoing apoptosis. At right panels, after 3 hours treatment (top panel), after 6 hours treatment (middle panel) and after 18 hour treatment (bottom panel) there were two populations of cells: Cells that were viable and not undergoing apoptosis (FITC Annexin V and PI negative; Q4 area in each panel) and cells undergoing early apoptosis (FITC Annexin V positive and PI negative; Q3 area) and late apoptosis (FITC Annexin V positive and PI positive; Q2 area) (Fig. 4).

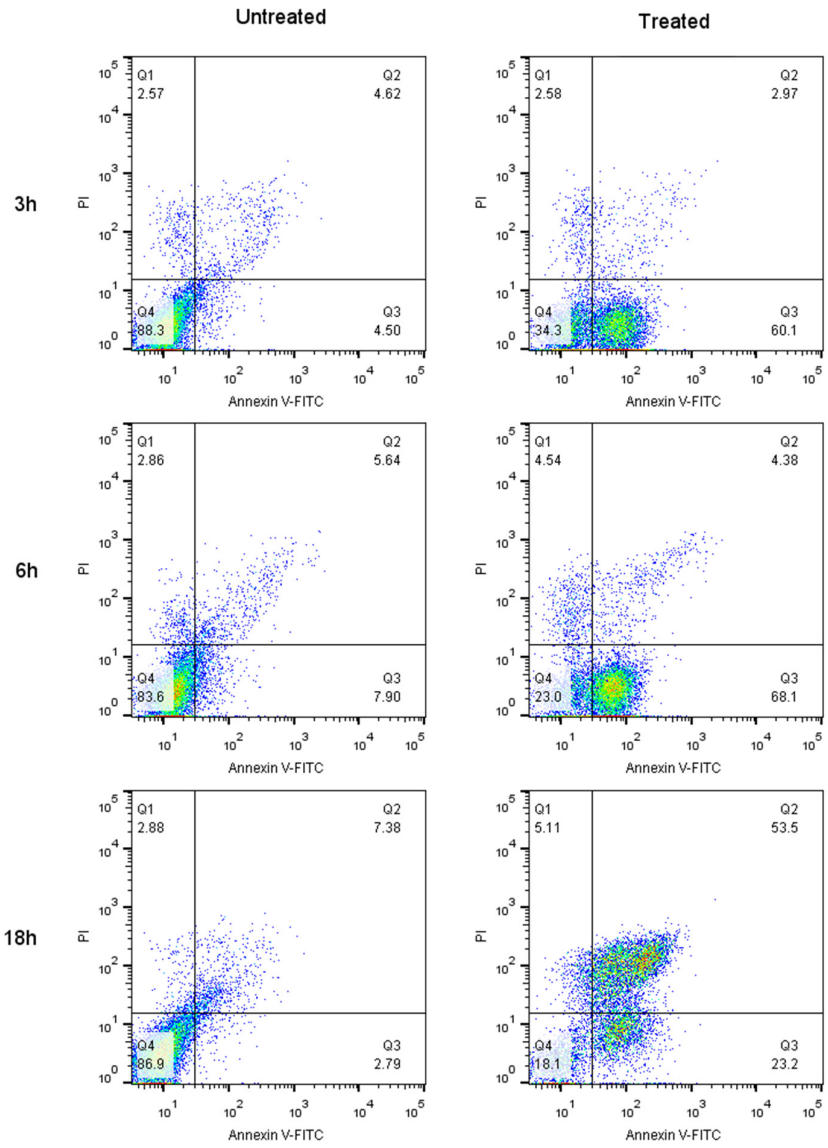

Figure 4. Flow Cytometric Analysis of FITC Annexin V staining.

Materials	- Jurkat T cells (ATCC TIB-152). - Prepare staurosporine (abcam; ab120056): final concentration 1µM of staurosporine in cell culture media contains 1 x 10^5 – 1 x 10^6 cells/mL. - 10X Annexin V Binding Buffer (component no. PZ03071): Prepare 1X working Solution through dilution 1 part of the 10X Annexin V Binding Buffer to 9 parts of distilled water. - FITC Annexin V (component no. PDZ233): Use 1 µl per test. - Propidium Iodide (PI) (component no. PZ03072) is ready-to-use as a nucleic acid dye. Use 1 µl per test.
Staining Procedure	1- Wash cells twice with cold PBS and then resuspend cells in 1X Binding Buffer at a concentration of 1 x 10^5 to 1 x 10^6 cells/100µL. **2**- Add 1 µl of Annexin V FITC to the test tube. **3**- Gently vortex the cells and incubate for 15 min at RT (25°C) in the dark. **4**- Add 1 µl of PI to the test tube and incubate for 2 minutes. **5**- Add 400 µl of 1X Binding Buffer to each tube. Analyze by flow cytometry within 1 hr, while samples keep on ice.
Required Controls for Setting up flow cytometry	- Unstained cells. - Cells stained with Annexin V FITC (no PI), as a single FITC color while, has to contain negative cells to set up compensation and quadrants. - Cells stained with PI (no FITC Annexin V), as a single PI color while, has to contain negative cells to set up compensation and quadrants.
References	-