IK- 90314 Annexin V-FITC Kit 100 tests

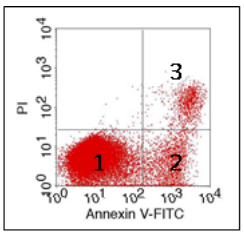
Annexin V (FITC)-conjugated is for the detection of phosphatidylserine exposed in the membrane of apoptotic cells. There is a 85 % homology of recombinant chicken Annexin V to the human Annexin V and a 100 % identity in the phosphatidylserine binding sites. Annexin V-FITC binding to PS is Ca 2+ dependent.

Introduction:
Apoptosis and necrosis are the two main forms of cell death. Apoptosis is mostly a physiological process and plays an essential role in the development and homeostasis of all multi-cellular organisms.
Apoptosis can be induced by several stimuli like UV- and gamma-irradiation or DNA damaging substances.
Apoptotic cells change the structure of their membrane, which leads to the exposure of phosphatidylserine (PS) on the membrane surface. Annexins are ubiquitous homologous proteins that bind phospholipids in the presence of calcium. Since the redistribution of phosphatidylserine from the internal to the external membrane surface represents an early indicator of apoptosis, Annexin V and its conjugates can be used for the detection of
apoptosis because they interact strongly and specifically with exposed phosphatidylserine. Detection of apoptotic cells with Annexin V can be achieved earlier than analysis of apoptotis by DNA-based assays.

Buffer/Additives/Preservative:
Each vial contains fluorescein conjugated Annexin with 0.1 % BSA in PBS.
Preservative: 0.09 % w/v sodium azide.

Applications:
An early event in apoptosis is the flipping of phosphatidyserine of the plasma membrane from the inside surface to the outside surface. Annexin V binds specifically to phosphatidylserine and FITC-conjugated

Annexin V can be used as a fluorescent probe to label apoptotic cells. Binding of Annexin V to the exposed charged head groups of PS is a Ca2+dependent process.
Propidium Iodide is used in conjunction with Annexin V-FITC. The cell membrane integrity excludes Propidium Iodide in viable and apoptotic cells, whereas necrotic cells are permeable to Propidium Iodide. Thus dual parameter FACS analysis allows for the discrimination between viable, apoptotic and necrotic cells.



Cells were treated with UV for 30 minutes. After an incubation of 4 hours they were subsequently stained according to the procedure described below.
1. Viable cells (double-negative)
2. Early apoptotic cells (Annexin-V-positive/PI-negative)
3. Late apoptotic/necrotic cells (double-positive)

IK-90314	KIT contents	Volume
1	**Annexin V-FITC – Use 5 ul/Test**	500 µl (100 tests)
2	**Propidium Iodide -Ready to Use- Use 5 ul/Test**	500 µl
3	**10X Annexin V Binding Buffer *** (Aqueous buffer solution) – Add 9 parts of sterile water before use	10 ml

PROCEDURE

*Preparation of 10X Annexin V Binding buffer to obtain 1X Annexin V Binding Buffer:
- To obtain 1X Annexin V Buffer dilute the 10X buffer at 1:10 in sterile deionized or distilled water 1:10 ratio (Mix 1ml of 10X buffer with 9ml water = Obtain 10 ml of 1X Annexin V Binding Buffer)

1. Dilute an appropriate amount of 10X Annexin-V binding buffer with deionized or distilled water in 1:10 ratio (= 1X buffer)
2. Wash cells with culture medium or PBS
3. Resuspend cells (typical 10^4 – 10^6 cells) in 90 µl of diluted (1X) Annexin-V binding buffer.
4. Add 5 µl of Annexin-V conjugate and 5 µl of propidium iodide solution.
5. Incubate 20 minutes in the dark.
6. Add 400 µl of Annexin-V binding buffer (1X).
7. Centrifuge at 400 x g for 5 minutes.
8. Resuspend cells in 1X Annexin-V binding buffer (typical 500µl) and analyze sample by flow cytometry

Storage: Upon arrival store at 2 - 8 °C in the dark. If stored properly reagent will be stable for 12 months.

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