IMMUNOLOGICAL SCIENCES Product Data Sheet

IK-90316 Annexin V APC-conj. Kit (7-AAD)

Size: 500 ul (100 Tests)

Application: Flow Cytometry, Fluorescence Microscopy

Background:

APC-conjugated recombinant chicken Annexin V (AxV) 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-APC binding to PS is Ca^{2+} dependent.

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.

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 APC 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 Ca^{2+} dependent process. 7-AAD is used in conjunction with Annexin V-APC.
The cell membrane integrity excludes 7-AAD in viable and apoptotic cells, whereas necrotic cells are permeable to 7-AAD. Thus dual parameter FACS analysis allows for the discrimination between viable, apoptotic and necrotic cells.

IK-90316	KIT contents	Volume
1	**Annexin V-APC – Use 5 ul/Test**	500 µl (100 tests)
2	**7-AAD - Viability Staining solution 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)

Staining procedure:
Wash cells (up to 10^6) in 500 µl binding buffer (PBS with Ca^{2+} = add 0.33 g/l to PBS)
Spin at 250 xg for 5 minutes and discard supernatant
Resuspend the cell pellet in 70 µl binding buffer
Add 5 µl of AnnexinV-APC, incubate 15 minutes at room temperature in the dark.

Buffer: PBS containing 1% BSA and 0.1% sodium azide (pH 7.4)
Storage: Store at 4 ˚C. Do not freeze. Avoid prolonged exposure to light.

Warning: Sodium azide is harmful if swallowed (R22). Keep out of reach of children (S2). Keep away from food, drink and animal feeding stuff (S13). Wear suitable protective clothing (S36). If swallowed, seek medical advice immediately and show this container or label (S46). Contact with acids liberates very toxic gas (R32).

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