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17-1002: Annexin V-FITC Apoptosis Detection Kit

Application : FACS
Alternative Name : ,

Description

Apoptosis or programmed cell death is an evolutionary conserved, intrinsic program of cell death that occurs in various physiological and pathological situations. It is a key regulator of physiological growth control and regulation of tissue homeostasis. Apoptosis is considered a vital component of various processes including normal cell turnover, proper development and functioning of the immune system, hormone-dependent atrophy, embryonic development and chemical-induced cell death. The term apoptosis (a-po-toe-sis) was first used in a now-classic paper by Kerr, Wyllie, and Currie in 1972 to describe a morphologically distinct form of cell death, although certain components of the apoptosis concept had been explicitly described many years previously. Apoptosis is characterized by specific morphological changes of the dying cells; namely, loss of membrane asymmetry, cytoskeleton remodeling, plasma membrane blebbing, loss of the mitochondrial membrane potential, caspase activation, chromatin condensation, and DNA fragmentation.

During apoptosis, the asymmetric distribution of phospholipids of the plasma membrane gets lost and phosphatidylserine (PS) is translocated to the outer leaflet of the plasma membrane. There, PS acts as one major “eat me” signal that ensures efficient recognition and uptake of apoptotic cells by phagocytes. Once on the cell surface, PS can be easily detected by staining with a fluorescent conjugate of Annexin V, a 35-36 kDa Ca2+ dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS. The one-step staining procedure takes only 10 minutes. Detection can be analyzed by flow cytometry or by fluorescence microscopy. Annexin V may be conjugated to fluorochromes including FITC. This format retains its high affinity for PS and thus serves as a sensitive probe for flow cytometric analysis of cells that are undergoing apoptosis.

Si. No.	Components	Contents
1	Annexin V-FITC	0.5 ml
2	Propidium Iodide (PI)	0.5 ml
3	10X PBS	20 ml
4	10X Binding Buffer	5 ml
5	Control Cells: 1 x 10^6 cells/ml	3 ml

Product Info

Amount : 100 Tests
Storage condition : Store all kit components at 4°C.

Application Note

STAINING PROTOCOL

1. Before beginning of the experiment, please dilute 10X PBS and 10X Binding Buffer to respective 1X by using distilled

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water.Please label the vials as follows (Table 1).

Vial	Cells	Stain
1	Control Cells	none
2	Control Cells	5 µl of Annexin V–FITC
3	Control Cells	5 µl Propidium Iodide (PI)
4	Un-induced Experimental Cells	5 µl of Annexin V–FITC + 5 µl PI
5	Apoptosis Induced Experimental Cells	5 µl of Annexin V–FITC + 5 µl PI

2. Control cells should be re-suspended properly by gently tapping the tube before initiation of staining. Aliquot 100ul of control cells to 3 individual vials as indicated in the Table 1 (Tube no 1, 2 and 3).

3. Resuspend the cells with 1 ml of pre-cooled PBS. Centrifuge the cells at 300 x g for 5 min.

4. Decant the supernatant carefully and repeat the washing step.

6. After decanting the supernatant, resuspend the pellet with 100 µl 1X Binding Buffer by gently tapping the tube. DO NOT VORTEX.

7. Wash the experimental cells twice with 1 ml of pre-cooled 1X PBS by centrifuge the cells at 300 x g for 5 min. Resuspend the pellet in (~1 x 10^6 cells/ml) 1X Binding Buffer.

8. Aliquot 100 µl of Experimental Cells to tubes no 4 and 5 as indicated in Table 1.

9. Add 5 µl of the Staining Reagents to each tube as indicated in Table 1 and mix the cells suspension thoroughly.

10. Incubate for 20 min at RT (20-25°C) in the dark. After incubation add 400 µl of 1X Binding Buffer to each tube.

11. Samples should be analyzed within 1 hr of the staining. Data acquisition should be done in FL1 channel for detecting Annexin V- FITC staining and the FL2 channel for detecting PI staining.

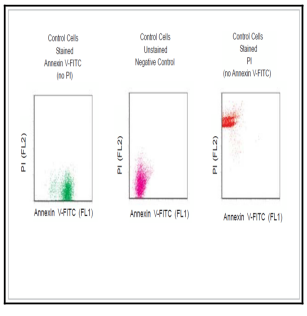

Fig.1: Control Cells were stained or unstained as indicated using the Annexin V-FITC Apoptosis Detection Kit.

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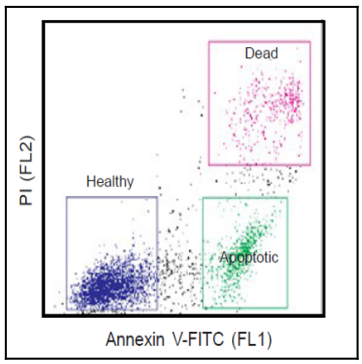

Fig.2: Adherent RAW cells were treated with 0.05 Î¼g/ml actinomycin-D for 17 hours to induce apoptosis. Cells were detached with Celltase and stained with the Annexin VFITC Apoptosis Detection Kit.