Annexin V-FITC Apoptosis Kit

Cat. Number: UAFP050
For Research Only

Components:

Material name:	Annexin V-FITC Apoptosis Kit
10X Annexin V Binding Buffer:	15ml
FITC Annexin V:	250ul (5ul/test)
Propidium Iodide Staining Solution:	250ul (5ul/test)

Formulation and Storage:

Formulation:	Liquid
Storage:	4℃

Background:

Apoptosis is a normal physiologic process which occurs during embryonic development as well as in maintenance of tissue homeostasis. The apoptotic program is characterized by certain morphologic features, including loss of plasma membrane asymmetry and attachment, condensation of the cytoplasm and nucleus, and internucleosomal cleavage of DNA. Loss of plasma membrane is one of the earliest features. In apoptotic cells, the membrane phospholipid phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V is a 35-36 kDa Ca2+ dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS.

Suggested staining Protocol:

SUGGESTED CONTROLS FOR SETTING UP FLOW CYTOMETRY

1. Unstained cells.
2. Cells stained with FITC Annexin V (no PI).
3. Cells stained with PI (no FITC Annexin V).

SUGGESTED EXPERIMENT POSITIVE CONTROL

Cells directly exposed in UV for 5min. Then incubate them overnight.

SUGGESTED EXPERIMENT NEGATIVE CONTROL

The untreated population is used to define the basal level of apoptotic and dead cells.

OTHER PROCEDURE NOTICE

Please wash the cells very gently, and centrifuge the tube under 200g.

PROTOCOL

1. Dilute 3 mL 10× binding buffer with 27 mL distilled water for 10 tests.
2. Harvest cell（about 1×10^5 cells per test）then wash with cold PBS.
3. Suspend cells in 1 mL 1× Binding Buffer, 200×g centrifugation for 10 minutes, and then remove the Binding Buffer from the cell pellet.
4. Resuspend cells in 1 mL 1× Binding Buffer; adjust cell concentration to 1×10^6cells/mL.
5. Add 100 µL of cells (1×10^5 cells) to each labeled tube.
6. Add 5 µL of Annexin V-FITC to appropriate tubes.
7. Gently vortex each tube and incubate for 10 minutes in room temperature, protected from light.
8. Add 5 µL PI solution incubation for 5min in room temperature, protected from light.
9. Add PBS to 500µL and vortex gently.
10. Analyze by flow cytometry in 1 hour.