Annexin V-FITC/PI apoptosis kit

(Specific for BD Accuri C6)

Product Information

Catalog#	AT101C
Size	30 Tests/100 Tests
expiration date	one year
Storage & Handling	Store between 2°C and 8°C. Do not freeze

Product Description

Annexins are a family of calcium-dependent phospholipid-binding proteins that preferentially bind phosphatidylserine (PS). Under normal physiologic conditions, PS is predominantly located in the inner leaflet of the plasma membrane. Upon initiation of apoptosis, PS loses its asymmetric distribution across the phospholipid bilayer and is translocated to the extracellular membrane leaflet marking cells as targets of phagocytosis. Once on the outer surface of the membrane, PS can be detected by fluorescently labeled Annexin V in a calcium-dependent manner.

Note:that this product is not suitable for automatic voltage-adjusting flow cytometers such as the BD C6; if used with such devices, we recommend using the Annexin V-FITC/PI apoptosis kit (C6-specific).

Kit Contents

Components Size	30 T	100 T
Annexin V-FITC	150 uL	500 uL
PI	300 uL	1000 uL
5 × Binding buffer	5 mL	15 mL
Apoptosis Positive Control Solution	5 mL	5 mL
Accutase	100 mL	100 mL

Assay Procedure

A. Cell Digestion (Optimization required for different cell types)

1) Thaw Accutase at 4°C or room temperature. Do not thaw at 37°C.

2) Carefully aspirate the cell culture medium. (If apoptotic cells are present in the supernatant, collect it.)

3) (Optional) Wash the cells with sterile PBS.

4) Add an appropriate amount of thawed Accutase (no need to warm to 37°C) to cover the cells. Depending on cell confluency and density, typically add 2.5 - 5 ml of Accutase to a T25 flask.

5) Incubate at room temperature for 5 - 10 minutes (can be extended up to 1 hour if necessary). No termination step is required.

6) When the cells begin to round up, gently tap the culture flask to detach the cells.

7) Gently pipette to dissociate the cells and collect them.

B. Adjustment of instrument parameters

1) Collect 1×10^6-3×10^6cells were centrifuged and washed twice with precooled PBS, and the supernatant was discarded.

2) Add 500 uL Apoptosis Positive Control Solution heavy suspension and incubate on ice for 30 minutes.

3) Wash centrifugally with pre-cooled PBS and discard the supernatant.

4) Add appropriate amount of precooled 1 × Binding Buffer resuspension, and add the same number of untreated living cells to mix with it. Adding precooled 1 × Binding Buffer to 1.5 mL, it was equally divided into three tubes, of which one tube was blank control tube and two tubes were single dye tube.

5) The single dye tube was incubated with 5 uL Annexin V-FITC or 10 uL PI at room temperature and away from light for 5 minutes.

6) On the flow cytometry, the voltage of FSC, SSC and fluorescence channel is reguLated by blank tube, and under this voltage condition, the voltage is reguLated by single dye tube.Compensation of fluorescence channels.

Note: some treatment of adherent cells into single cells will cause damage to the cell membrane, resuLting in false positive of Annexin V. therefore.

Optimization is needed. Enzymes that are milder to cells such as Accutase can be used

C. Sample testing

1) Apoptosis was induced according to the experimental scheme.

2) Wash centrifugally with precooled PBS and collect 1-10×10^5 cells (including cells in the cuLture supernatant). Dilute 5 × Binding with double distilled water.Buffer is 1× working solution, and 500 uL 1 × Binding Buffer resuspension cells are taken.

3) 5 uL Annexin V-FITC and 10 uL PI were added to each tube.

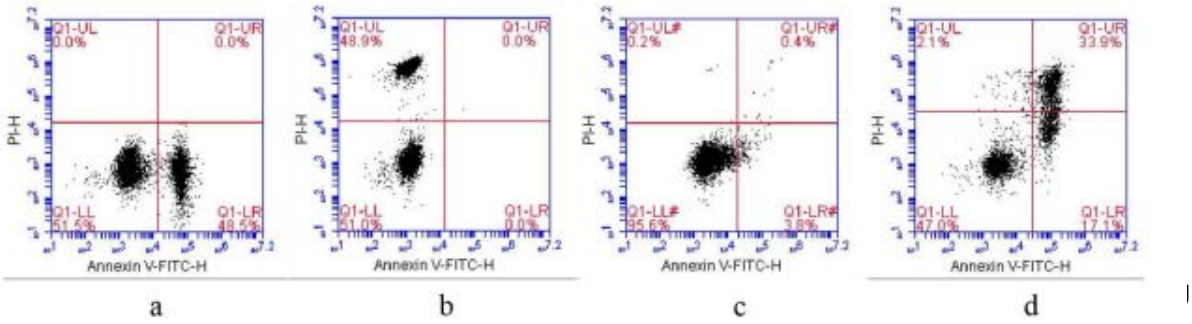
4) After mixing gently and swirling, incubate at room temperature away from light for 5 minutes.

5) According to the experimental method, flow analysis is carried out.

6) Flow analysis.

Annexin V-FITC was detected by FITC detection channel (Ex = 488 nm; Em = 530 nm) and by PI on flow cytometry.PI was detected by Ex (535 nm; Em = 615 nm).

Product Data



the unstimuLated HL-60 cells(c).Staining the Camptothecin-stimuLated HL-60 cells with both Annexin V-FITC and PI(d). Total viable cells were used for analysis.

Application Notes

1. Please read the instructions carefully before using this product.This product is only used for scientific research, not for diagnosis.
2. For your safety and health, please wear experimental protective clothing,gloves, masks and other necessary protective equipment.
3. For more apoptosis-related products, please follow the UNOCI biology website or call for advice.

Related Products

Catalog	Product Name	Size
AP101C	AnnexinV-FITC/PI Apoptosis Kit(Exclusively for BD Accuri C6)	30T/100T
AP104	AnnexinV-PE/7-AAD Apoptosi Kit	30T/100T
AP105	AnnexinV-APC/7-AAD Apoptosis Kit	30T/100T
AP107	Annexin V-APC/PI Apoptosis Kit	30T/100T
CCS012	Cell Cycle Staining Kit	50 T
MJ101	Mitochondria Staining Kit	125 T