

Annexin V-FITC Apoptosis Detection Kit

Introduction

Apoptosis is a normal physiologic process which occurs during embryonic development as well as in maintenance of tissue homeostasis. Annexin V is a 35-36 kDa Ca²⁺ dependent phospholipid-binding protein that has a high affinity for PS (Phosphatidylserine), which is translocated from the inner to the outer leaflet of the plasma membrane during apoptosis process, and specifically binds to cells with exposed PS. Annexin V conjugated to FITC serves as a sensitive probe for flow cytometric analysis or fluorescence microscope analysis of cells that are undergoing apoptosis.

Propidium iodide (PI) is a vital dye which can turn the cell nucleus red. Viable cells with intact membranes exclude PI, whereas in the end stage apoptosis and death, the membranes of dead and damaged cells are permeable to PI. Using Annexin and PI can distinguish early apoptosis cells from end stage apoptosis cells and dead cells.

Reagents Provided (50/25 tests)

- Annexin V-FITC 25 uL/12.5 uL
- Binding Buffer 40 mL/20 mL
- Propidium Iodide (PI) 250 uL/125 uL

Storage and Expiry Date

All kit components must be stored at 2-8°C, stable for one year.

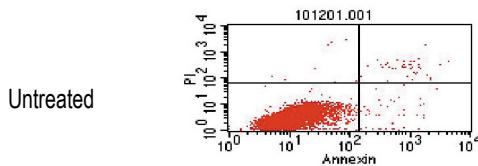
Precautions

1. To obtain optimal results, please read the datasheet in detail first before use.
2. Centrifuge before opening the cover to avoid liquids on inside wall being spilled out.
3. Annexin V-FITC needs to be detected and analyzed as soon as possible, FITC signal will be weakened after 1 hour.
4. PI and Annexin V-FITC are sensitive to light. Keep them away from light.
5. Be careful with Propidium iodide (PI). PI is absorbable through skin and is irritant to eyes.
6. This kit is only for research, not for diagnostic procedure.

Procedure

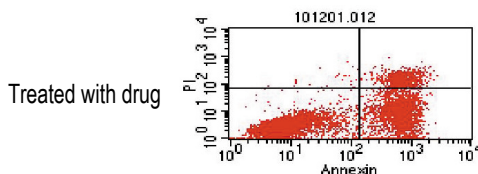
1. Adjust the cells concentration to 5×10⁵-1×10⁶/mL;
2. Take 1mL cells, 1000 rpm, 4 °C centrifuge for 10 minutes. Discard the supernate;
3. Add 1mL precooled PBS, then gently shake to keep cells in suspension. 1000 rpm, 4 °C centrifuge for 10 minutes. Discard the supernate;
4. Repeat procedure 3, 4 twice. (For adherent cells, first digest with trypsin, then wash with PBS);
5. Dilute Annexin V-FITC with Binding Buffer as a ratio of 1:400, then place on the ice out of light;
6. Resuspend cells with 200 uL Annexin V-FITC including Binding Buffer;
7. Incubate at room temperature for 10 minutes and protect from light. Determine within one hour, and add 5 uL PI before determination.
8. If determine with fluorescence microscope, centrifuge the mixed liquid then smear with cells deposit.

Below is the test result of Abgent's kit:



File: 101201.001
Gate: G1
Gated Events: 9330
Total Events: 10000

Quad	Events	%Gated	%Total	X Mean	X Geo Mean	Y Mean
UL	8	0.09	0.08	50.45	33.34	946.76
UR	42	0.45	0.42	1076.80	840.39	316.95
LL	9238	99.01	92.38	13.14	10.76	3.50
LR	42	0.45	0.42	703.86	496.76	7.87



File: 101201.012
Gate: G1
Gated Events: 7596
Total Events: 10000

Quad	Events	%Gated	%Total	X Mean	X Geo Mean	Y Mean
UL	25	0.33	0.25	69.02	49.78	177.27
UR	815	10.73	8.15	817.51	742.01	159.57
LL	4036	53.13	40.36	13.80	9.16	3.39
LR	2720	35.81	27.20	716.74	645.59	11.92

