FITC-Annexin V/PI Apoptosis Kits(Green/Red)

Catalog No.: RA20046

Basic Information

Product name	FITC-Annexin V/PI Apoptosis Kits(Green/Red)
Sizes	20 T/50 T/100 T
Storage	2-8℃, keep away from light
Shipping	Shipped with ice pack
Validity	12 months
Ex/Em	FITC-Annexin V：494/518 nm；PI：535/617 nm

Product Introduction

The FITC -Annexin V/PI Apoptosis Detection Kit provides a rapid and simple method to detect apoptosis by labeling early apoptotic cells (green) and necrotic or late apoptotic cells (red). Annexin V is a Ca^{2+}-dependent phospholipid-binding protein with a molecular weight of 35–36 kDa that selectively binds to phosphatidylserine (PS). In normal cells, PS is located on the inner side of the plasma membrane. During early apoptosis, PS is translocated to the outer surface of the cell membrane and exposed to the extracellular environment. By binding FITC -labeled Annexin V to the exposed PS, this hallmark of apoptosis can be directly detected using flow cytometry or fluorescence microscopy. Propidium Iodide (PI) is a DNA-binding dye that stains the nuclei of necrotic or late apoptotic cells that have lost membrane integrity, emitting red fluorescence upon excitation.

Product Components

Components	10 T	50 T	100 T
A. 1 × Annexin V Binding Buffer	10 mL	50 mL	2 × 50 mL
B. FITC-Annexin V	50 µL	250 µL	500 µL
C. PI	100 µL	500 µL	1 mL

Note: Do not freeze Component B.

Materials Required but Not Provided

1. Consumables

6-, 12-, 24-, or 96-well plates or dishes

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2. Reagents

(1) Cell samples; (2) PBS; (3) Cell culture medium

3. Equipment

Flow cytometer, fluorescence microscope

Experimental Protocol

Operating Procedures

一、Experimental Group Design

Non-Autofluorescent Samples

Group	Annexin V	Nuclear Dye	Sample Type
Blank Control	-	-	Untreated, non-fluorescent cells
Negative Control	+	+	Untreated, autofluorescent cells
Single Stain 1	+	-	Non-fluorescent, apoptotic cells
Single Stain 2	-	+	Non-fluorescent, apoptotic cells
Single Stain 3	-	-	Autofluorescent cells
Experimental	+	+	Test sample cells

Autofluorescent Samples

Group	Annexin V	Nuclear Dye	Sample Type
Blank Control	-	-	Untreated cells
Negative Control	+	+	Untreated cells
Single Stain 1	+	-	Apoptotic cells
Single Stain 2	-	+	Apoptotic cells
Experimental	+	+	Test sample cells

Blank Control: Adjust threshold and instrument voltage.

Negative Control: Exclude interference from handling and subtract background fluorescence; also used for gating.

Single Stains: Used for compensation and voltage adjustment to prevent signal overflow.

Experimental Group: Acquire flow cytometry data after voltage and compensation adjustment.

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二、Cell Collection

(A) Suspension Cells

After apoptosis induction, centrifuge at 1000 rpm for 5 min, discard supernatant, collect cells, gently resuspend in PBS and count.

Note: PBS resuspension is essential for washing and ensuring Annexin V binding.

Take 50–1×10^6 cells, centrifuge at 1000 rpm for 5 min, discard supernatant, add 100 µL 1× Annexin V Binding Buffer and gently resuspend.

Add 5 µL FITC-Annexin V, mix gently.

Add 5 µL PI staining solution, mix gently.

Incubate at room temperature (20–25°C) for 10–15 min protected from light (e.g., wrapped in aluminum foil). Gently resuspend 2–3 times during incubation to improve staining.

(B) Adherent Cells

Collect culture medium into a tube. Wash cells with PBS, then add trypsin (without EDTA) to detach cells. Incubate at room temperature until cells detach with gentle pipetting. Avoid over-digestion.

Note: Over- or under-digestion may damage membranes and cause false positives or interfere with Annexin V binding.

Add the collected culture medium to neutralize trypsin, gently pipette cells into a tube, centrifuge at 1000 rpm for 5 min, discard supernatant, wash with PBS, and count.

Take 50–1×10^6 cells, centrifuge, resuspend in 100 µL 1× Binding Buffer.

Add 5 µL FITC-Annexin V, mix gently.

Add 5 µL PI, mix gently.

Incubate at room temperature for 10–15 min protected from light. Gently resuspend 2–3 times.

三、Data Analysis

(A) Flow Cytometry

After incubation, add 400 µL 1× Binding Buffer, mix, and analyze immediately.

FITC-Annexin V: excited at 494 nm, emission at 518 nm (FITC channel)

PI: emission at ~617 nm

On a dual-parameter plot:

Lower Left: Live cells (Annexin V–/PI–)

Lower Right: Early apoptosis (Annexin V+/PI–)

Upper Right: Late apoptosis/necrosis (Annexin V+/PI+)

Upper Left: Naked nuclei (Annexin V–/PI+)

(B) Fluorescence Microscopy

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Centrifuge cells, resuspend in 400 µL Binding Buffer, transfer to 96-well plate or slide, and observe under microscope.

Use FITC filter for FITC-Annexin V, and Cy3 or Texas Red filter for PI.

Notes

1. Briefly centrifuge reagents before use.
2. To slow apoptosis, incubate on ice, but extend time to ≥30 min.
3. Analyze samples within 1 h post-staining.
4. Use a large pipette tip with a small tip attached to avoid cell loss.
5. Protect dyes from light to prevent quenching.
6. For research use only. Not for diagnostic or therapeutic use.
7. Wear lab coat and gloves for safety.

This product is for research use only!