

Product No. 15342

Annexin V-FITC Apoptosis Detection Kit

Apoptosis is one of the programmed cell death, that plays an important role in maintaining the homeostasis and developmental processes in both plants and animals. Any abnormal cells during the cytogenesis are eliminated by apoptosis. For instance, tumor growth from cancer cells occurred *in vivo* is inhibited by induction of apoptosis. However, apoptosis is not induced when the error occurs in tumor suppressor gene p53. Thus, the growth of cancer cells has been found to proceed. Apoptosis cells can be identified based on the alteration of cellular morphology as well as the alternation of biomedical changes.

As of today, various indicators have been established such as caspase activity variation, DNA fragmentation and phosphatidylserine transition on the cell surface-chromosomal. Annexin V stained cells are used to indicate cell membrane changes that occur in the early stage of apoptosis. Once apoptosis is initiated, the phosphatidylserine presents in the inner cell membrane migrates through the cell membrane of the lipid bilayer.

Annexin V specifically binds to phosphatidylserine in the presence of protein-dependent Ca ion. By using fluorescent-labeled Annexin V, the apoptotic cells can be identified by flow cytometry or fluorescence microscopy.

Features

- Annexin V-FITC Apoptosis Detection Kit contains ready-to-use solutions, Annexin V-FITC conjugate, propidium iodide(PI).
The kit can identify of apoptotic and necrotic cells.
- Detect by flow cytometry or fluorescence microscopy
- Without fixation

Components

Reagents	Volume	Quantity	Cap
Annexin V-FITC Solution	50 TESTS	2	Yellow
Propidium Iodide (PI) Solution	50 TESTS	2	Red
Annexin V Binding Buffer (10x)	50 TESTS	2	White

Preparation

Annexin V Biding Solution (1x)

Dilute Annexin V Binding Buffer (10x) by 10-fold with distilled water.

Protocol

General protocol for Suspension Cells

1. Centrifuge the cell suspension at 1,000 rpm for 3 minutes and remove supernatant.
2. Add PBS for wash cells and centrifuge at 1,000 rpm for 3 minutes, remove supernatant.
Repeat this step one more time.
3. Add Annexin V Biding Solution (1x) to make final cell concentration of 1×10^6 cells/mL.
4. Transfer 100 μ L of cell suspension prepared at step 3 to a new tube.
5. Add 5 μ L of Annexin V - FITC Conjugate, then 5 μ L of PI Solution to the cell suspension.
6. Incubate 15 minutes at room temperature with protect from light.
7. Add 400 μ L of Annexin V Biding Solution (1x).
8. Apply the solution prepared in step 7 to flow cytometric assay or microscopic assay.

*Approximate fluorescence maximum excitation / emission

	excitation / emission
Annexin V-FITC	494 nm / 518 nm
PI	535 nm / 617 nm

General protocol for Adherent Cells

1. Discard supernatant on the petri dish or plate.
2. Add PBS for wash cells and discard supernatant. Repeat this step one more time.
3. Detach the cells with Trypsin-EDTA.
4. Add appropriate volume of culture medium or PBS and transfer the cell suspension to a tube.
5. Centrifuge at 1,000 rpm for 3 minutes. Remove supernatant.
6. Add PBS for wash cells and centrifuge at 1,000 rpm for 3 minutes, remove supernatant.
Repeat this step one more time.

7. Add Annexin V Binding Solution (1x) to make final cell concentration of 1×10^6 cells/mL.
8. Transfer 100 μ L of cell suspension prepared at step 7 to a new tube.
9. Add 5 μ L of Annexin V - FITC Conjugate, then 5 μ L of PI Solution to the cell suspension.
10. Incubate 15 minutes at room temperature with protect from light.
11. Add 400 μ L of Annexin V Binding Solution (1x).
12. Apply this solution to flow cytometric assay or microscopic assay.

*Although adherent cells are not frequently used for Annexin V, FITC flow cytometric analyses because of avoiding the specific cell membrane damage from a cell detachment process, Casciola-Rosen *et al.* and van Engeland *et al.* have reported methods on utilizing Annexin V for flow cytometry with adherent cell types.

Caution

- PI is a potential mutagen. Wear gloves and protective goggles when handle it.
- Both FITC-labeled Annexin V and PI are light sensitive. All staining procedures must be performed without direct exposure to intense light.

Storage

Refrigerator, Protect from light (do NOT freeze)

Expiration

Expiration date is stated on the product label.

Packing

100 TESTS (Product No.15342-54)

*One assay corresponds to the assay with cell concentration of 1×10^6 cells/mL.

References

- 1) Casciola-Rosen L, Rosen A, Petri M, Schlissel M, *Proc Natl Acad Sci USA*, **93**(4), 1624 (1996)
- 2) van Engeland M, Remaekers FC, Schutte B, Reutelingsperger CP, *Cytometry*, **24**(2), 131 (1996)