Contact us
Email : info.intl@nacalai.co.jp
TEL : +81-75-251-1730
FAX : +81-75-251-1763

18146E_1908_1

Product No. 18146

Annexin V-633 Apoptosis Detection Kit

Apoptosis is one of the programmed cell death and plays an important role in maintaining the homeostasis and developmental processes in both plants and animals. As of today, various indicators have been established such as caspase activity variation, DNA fragmentation and phosphatidylserine transition by structural change of cell membrane.

Once apoptosis is initiated, the phosphatidylserine presents in the inner cell membrane migrates to cell surface through the cell membrane of the lipid bilayer.

Annexin V is a Ca^{2+} dependent phospholipid-binding protein with high affinity for phosphatidylserine. So, by using fluorescent-labeled Annexin V, cell membrane change that occur in the early stage of apoptosis can be detected and the apoptotic cells can be identified.

Features

- This kit contains Annexin V-red-fluorescent dye conjugate and propidium iodide(PI).
- This kit can identifies early and late apoptotic cells as well as necrotic cells.
- Easy handling
- Detect by flow cytometry or fluorescence microscopy
- Without fixation

Components

Reagents	Volume	Quantity	Cap
Annexin V-633 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 Binding 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 and remove supernatant.
2. Add PBS for wash cells and discard supernatant. Repeat this step one more time.
3. Add Annexin V Biding Solution(1x) to make final cell concentration of 1 x 10^6 cells/mL.
4. Transfer 100 µL of cell suspension prepared at step 3 to a new tube.
5. Add 5 µL of Annexin V-633 Solution and then add 5 µL of PI Solution to the cell suspension.
6. Incubate 15 minutes at room temperature with protection from light.
7. Add 400 µL of Annexin V Binding Solution (1x) and then slowly mix.
8. Apply the solution prepared in step 7 to flow cytometric assay or microscopic assay as soon as possible.

*Approximate fluorescence maximum excitation / emission

	excitation / emission
Annexin V-633	650 nm / 666 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 the cell suspension and remove supernatant.
6. Add PBS for wash cells and discard supernatant. Repeat this step one more time.
7. Add Annexin V Biding Solution(1x) to make final cell concentration of 1 x 10^6 cells/mL.

Manufactured by NACALAI TESQUE, INC.
498 Higashitamaya-cho, Nijo Karasuma,
Nakagyo-ku, Kyoto 604-0855, Japan

Contact us
Email : info.intl@nacalai.co.jp
TEL : +81-75-251-1730
FAX : +81-75-251-1763

8. Transfer 100 µL of cell suspension prepared at step 7 to a new tube.
9. Add 5 µL of Annexin V-633 Solution and then add 5 µL of PI Solution to the cell suspension.
10. Incubate 15 minutes at room temperature with protection from light.
11. Add 400 µL of Annexin V Binding Solution(1x) and then slowly mix.
12. Apply the solution to flow cytometric assay or microscopic assay as soon as possible.

*Although adherent cells are not frequently used for Annexin V conjugate to 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 conjugate for flow cytometry with adherent cell types.

Caution

- PI is a potential mutagen. Wear gloves and protective goggles when handle it. If it adhered to the skin, immediately wash with plenty of water.
- Both Annexin V-633 and PI Solution 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.18146-44)

*One test corresponds to the assay with cell concentration of 1 x 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)

Manufactured by NACALAI TESQUE, INC.
498 Higashitamaya-cho, Nijo Karasuma,
Nakagyo-ku, Kyoto 604-0855, Japan