Annexin V, FITC Labeled

Catalog number: 20030, 20032
Unit size: 100 tests, 300 tests

Component	Storage	Amount (Cat No. 20030)	Amount (Cat No. 20032)
Annexin V, FITC Labeled	Freeze (< -15 °C), Minimize light exposure	100 tests	300 tests

OVERVIEW

FITC Annexin V is a highly fluorescent conjugate used to identify and quantitate apoptotic cells by flow cytometry.

Product Description

Annexin V is a 35-36 kDa phospholipid-binding protein that has a high affinity for phosphatidylserine (PS) residues. In apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, making it accessible to Annexin V conjugated to fluorescein isothiocyanate (FITC). By binding to PS, Annexin V FITC can be used to detect and quantify apoptotic cells via flow cytometry or fluorescence microscopy.

Key Features

- **High Sensitivity and Specificity** to efficiently detect early apoptotic cells by binding to exposed PS.
- **Dual Staining Capability** can be used in conjunction with propidium iodide (PI) to distinguish between apoptotic and necrotic cells.
- **Non-Radioactive Assay** provides a safe and easy method for apoptosis detection without the need for radioactive materials.
- **Rapid and Simple Protocol** enables minimal hands-on time with straightforward staining and analysis procedures.
- **Versatile Applications** suitable for use in various cell types and experimental conditions.

Mechanism of Action

During apoptosis, the integrity of the plasma membrane changes, leading to the translocation of phosphatidylserine (PS) from the inner to the outer leaflet. Annexin V FITC, a fluorophore-conjugated protein with a high affinity for PS, selectively binds to the externalized PS residues. This binding event is detectable and quantifiable via flow cytometry or fluorescence microscopy. The intensity of the FITC signal enables precise identification and quantification of apoptotic cells, as it directly correlates with Annexin V binding to PS on the cell surface.

AT A GLANCE

Protocol Summary

1. Prepare cells with test compounds (200 µL/sample).
2. Add Annexin V conjugate assay solution.
3. Incubate at room temperature for 30-60 minutes.
4. Analyze with a flow cytometer or a fluorescence microscope.

Storage and Handling Conditions

The fluorescent annexin V conjugates are stored in a PBS buffer solution containing 0.1% bovine serum albumin (BSA) with a pH of 7.4. To ensure their stability, it is recommended that the solutions be stored at a temperature of -20°C and protected from light. Avoid exposing the fluorescent conjugates to repeated freeze-thaw cycles as this can have a negative effect on their integrity. These solutions can be stored for at least 6 months under the recommended conditions.

KEY PARAMETERS

Flow cytometer

Emission	530/30 nm filter
Excitation	488 nm laser
Instrument specification(s)	FITC channel

Fluorescence microscope

Emission	FITC filter set
Excitation	FITC filter set
Recommended plate	Black wall/clear bottom

SAMPLE EXPERIMENTAL PROTOCOL

Prepare and Incubate Cells with Annexin V Conjugate

1. Prepare an Annexin V-binding assay buffer: 10 mM HEPES, 140 mM NaCl, and 2.5 mM CaCl2, pH 7.4.
2. Treat cells with test compounds for a desired period of time (e.g., 4-6 hours for Jurkat cells treated with staurosporine) to induce apoptosis.
3. Centrifuge the cells to get 1-5×10^5 cells/tube.
4. Resuspend cells in 200 µL of the Annexin V-binding assay buffer from Step 1.
5. Add 2 µL of the Annexin V conjugate to the cells.

   Optional: Add a dead cell stain such as Propidium Iodide (Cat No. 17585) into the cells for necrosis cells.
6. Incubate at room temperature for 30 to 60 minutes, protected from light.
7. Add 300 µL of the Annexin V-binding assay buffer (from Step 1) to increase volume before analyzing the cells with a flow cytometer or fluorescence microscope.
8. Monitor the fluorescence intensity by using a flow cytometer or a fluorescence microscope.

Flow Cytometer Protocol

1. Quantify Annexin V conjugates binding by using a flow cytometer with appropriate filters.

   Note: It is not common to perform Annexin V binding flow cytometric analysis on adherent cells due to the possibility of

However, previous studies by Casiola-Rosen *et al.* and van Engelend *et al.* (refer to Refs 1 and 2) have demonstrated methods for using Annexin V in flow cytometry on adherent cell types.

Fluorescence Microscope Protocol

1. Pipette the cell suspension from Step 6, rinse 1-2 times with Annexin V-binding assay buffer (Step 1), and then resuspend the cells with the Annexin V-binding assay buffer (Step 1).

2. Add the cells on a glass slide that is covered with a glass cover slip.

   Note: For adherent cells, it is recommended to grow the cells directly on a cover slip.

3. After incubation with Annexin V conjugate (Step 6), rinse 1-2 times with Annexin V-binding assay buffer (Step 1), and add Annexin V-binding assay buffer (Step 1) back to the cover slip.

4. Invert the cover slip on a glass slide and visualize the cells. The cells can also be fixed in 2% formaldehyde after incubation with Annexin V conjugate and visualized under a microscope with the appropriate filter set.

EXAMPLE DATA ANALYSIS AND FIGURES

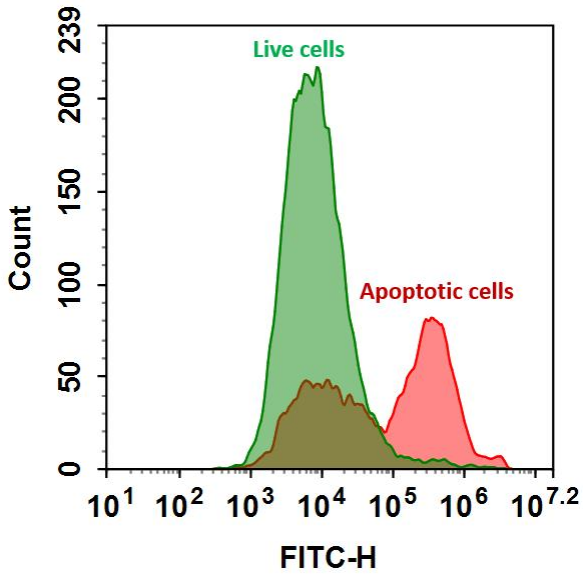

Figure 1. Binding activity of FITC-Annexin V conjugate to phosphatidylserine (PS) residues in Jurkat cells. Jurkat cells were treated without (Green) or with 1 µM staurosporine (Red) at 37°C for 4 hours and then labeled with FITC-Annexin V conjugate for 30 minutes. The fluorescence intensity was measured using an ACEA NovoCyte flow cytometer in the FITC channel.

APPENDIX

References

1. Pascal Clerc, Pauline Jeanjean, Nicolas Halalli, Michel Gougeon, Bernard Pipy, Julian Carrey, Daniel Fourmy, Véronique Gigoux. Journal of Controlled Release (2017).

2. Hanshaw RG, Lakshmi C, Lambert TN, Johnson JR, Smith BD. Chembiochem, 6, 2214. (2005).

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