Annexin V-iFluor® 680 conjugate

Catalog number: 20075
Unit size: 100 tests

Component	Storage	Amount (Cat No. 20075)
Annexin V-iFluor® 680 conjugate	Freeze (< -15 °C), Minimize light exposure	100 tests

OVERVIEW

Annexins are a family of proteins that bind to phospholipid membranes in the presence of calcium. Annexin V is a valuable tool for studying cell apoptosis. It is used as a probe to detect cells which have expressed phosphatidylserine on the cell surface, a feature found in apoptosis as well as other forms of cell death. There are a variety of parameters that can be used for monitoring cell viability. Annexin V-dye conjugates are widely used to monitor cell apoptosis through measuring the translocation of phosphatidylserine (PS). In apoptosis, PS is transferred to the outer leaflet of the plasma membrane. The appearance of phosphatidylserine on the cell surface is a universal indicator of the initial/intermediate stages of cell apoptosis and can be detected before morphological changes can be observed. This fluorescent Annexin V conjugate has spectral properties similar to Alexa Fluor® 680 (Alexa Fluor® 680 is the trademark of Invitrogen) and Cy5.5® (Cy5.5® is the trademark of GE Healthcare).

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	710/50 nm filter
Excitation	633 or 640 nm laser
Instrument specification(s)	Cy5.5 channel

Fluorescence microscope

Emission	Cy5 filter set
Excitation	Cy5 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 Nuclear Green™ DCS1 (Cat No. 17550) 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 membrane damage during cell detachment or harvesting. 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

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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

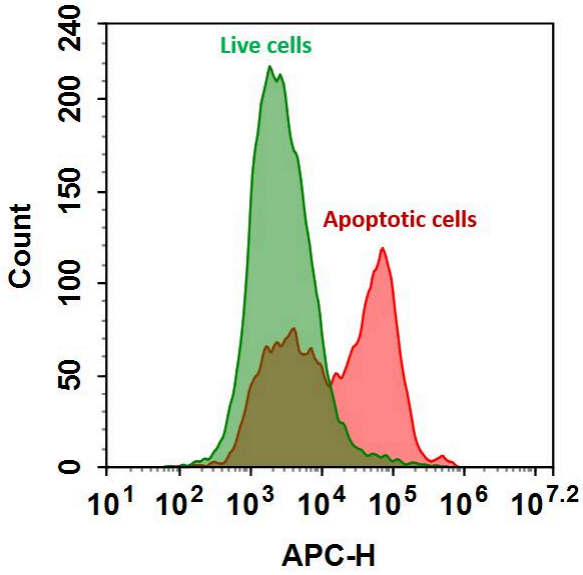

Figure 1. The detection of binding activity of Annexin V-iFluor® 680 conjugate to phosphatidylserine in Jurkat cells. Jurkat cells were treated without (Green) or with 1 μM staurosporine (Red) at 37 ºC for 4 hours and then labeled with Annexin V-iFluor® 680 conjugate for 30 minutes. Fluorescence intensity was measured using an ACEA NovoCyte flow cytometer in the APC 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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