

**Annexin V, XFD488 conjugate \*XFD488 Same Structure to Alexa Fluor™ 488\***

Catalog number: 20092

Unit size: 100 tests

| Component                   | Storage                                    | Amount (Cat No. 20092) |
|-----------------------------|--|------------------------|
| Annexin V, XFD488 conjugate | Freeze (< -15 °C), Minimize light exposure | 100 tests              |

**OVERVIEW**

XFD488 is manufactured by AAT Bioquest, and it has the same chemical structure of Alexa Fluor® 488 (Alexa Fluor® is the trademark of ThermoFisher). 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. 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. 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). XFD488-Annexin V conjugate has the same molecule as the Alexa Fluor® 488-Annexin V. This probe is one of the most popular fluorescent dye-Annexin V conjugates used for monitoring cell apoptosis.

**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 CaCl<sub>2</sub>, 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<sup>5</sup> 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 membrane damage during cell detachment or harvesting. However, previous studies by Casiola-Rosen *et al.* and van Engeland *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.

## 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).

## DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email [info@aatbio.com](mailto:info@aatbio.com) if you have any questions.