

# Product Data Sheet

## Alexa Fluor® 647 Annexin V

**Catalog # / Size:** 640911 / 25 tests  
640912 / 100 tests

**Reactivity:** All mammalian species

**Preparation:** The purified protein was conjugated with Alexa Fluor® 647 under optimal conditions. The solution is free of unconjugated Alexa Fluor® 647.

**Formulation:** Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and 0.2% (w/v) BSA (origin USA).

**Storage:** The Annexin V solution should be stored undiluted at 4°C, and protected from prolonged exposure to light. Do not freeze.

## Applications:

**Applications:** FC - Quality tested

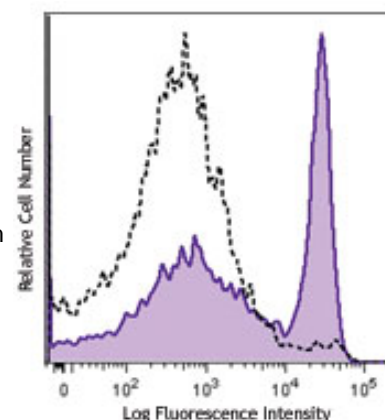
**Recommended Usage:** Each lot of this product is quality control tested by staining of apoptotic cells and analyzing by flow cytometry. The suggested use of this reagent is 5 µl per 1X10<sup>5</sup> cells in a 100 µl volume of Annexin V Binding Buffer (cat. no. 422201). It is recommended that the reagent be titrated for optimal performance for each application. \* Alexa Fluor® 647 has a maximum emission of 668 nm when it is excited at 633nm / 635nm.  
\*\* Alexa Fluor® is a registered trademark of Molecular Probes, Inc. Alexa Fluor® dye antibody conjugates are sold under license from Molecular Probes, Inc. for research use only, except for use in combination with microarrays and high content screening, and are covered by pending and issued patents.

### Application Notes: Annexin V Staining

1. Wash cells twice with cold BioLegend cell staining buffer (cat # 420201) and then resuspend cells in Annexin V Binding Buffer (cat # 422201) at a concentration of 1x10<sup>6</sup> cells/ml.
2. Transfer 100 µl of cell suspension in 5 ml test tube.
3. Add 5 µl of Alexa Fluor® 647 Annexin V.
4. Add 10 µl of PI solution (cat # 421301) or 7-AAD (cat # 420401).
5. Gently vortex the cells and incubate for 15 min at RT (25°C) in the dark.
6. Add 400 µl of Annexin V Binding Buffer (cat # 422201) to each tube. Analyze by flow cytometry.

### Application References:

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8. Liu F, *et al.* 2011. *Cancer Res.* 71:6807. PubMed.
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Human T-cell leukemia cell line, Jurkat, was stimulated (4 hours) with (filled histogram) or without (open histogram) LEAF™ purified anti-CD95 (clone EOS9.1), then stained with Annexin V Alexa Fluor® 647.

**Description:** Annexin V (or Annexin A5) is a member of the annexin family of intracellular proteins that binds to phosphatidylserine (PS) in a calcium-dependent manner. PS is normally only found on the intracellular leaflet of the plasma membrane in healthy cells, but during early apoptosis, membrane asymmetry is lost and PS translocates to the external leaflet. Fluorochrome-labeled Annexin V can then be used to specifically target and identify apoptotic cells. Annexin V Binding Buffer (cat. no. 422201) is recommended for use with Annexin V staining. Annexin V binding alone cannot differentiate between apoptotic cells and necrotic. So, we recommend using our 7-AAD Viability Staining Solution (cat. no. 420401/420402) or Propidium Iodide Solution (cat. no. 421301). Early apoptotic cells will exclude 7-AAD and PI, while late stage apoptotic cells and necrotic cells will stain positively, due to the passage of these dyes into the nucleus where they bind to DNA.

**Related Products:** Product  
Annexin V Binding Buffer

**Clone**

**Application**  
FC, IF



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Propidium Iodide Solution  
7-AAD Viability Staining Solution

FC  
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