

## Biotin Annexin V

**Catalog # / Size:** 3804520 / 100 tests  
3804695 / 300 tests

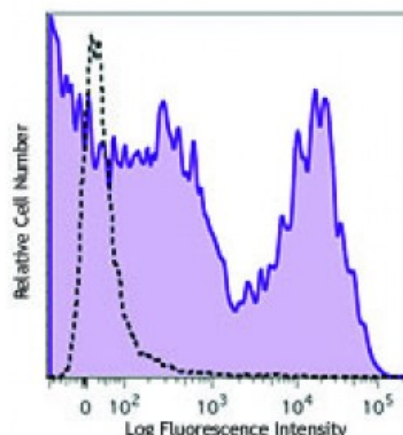
**Isotype:**

**Reactivity:** Human, Mouse, Non-human primate, Other, Rat

**Preparation:** The purified protein was conjugated with biotin under optimal conditions. The solution is free of unconjugated biotin and unconjugated antibody.

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

**Concentration:** Lot-specific



Human T-cell leukemia cell line, Jurkat, was treated with (filled histogram) or without (open histogram) LEAF™ purified anti-CD95 (clone EOS9.1) for 4 hours, then stained with biotinylated Annexin V, followed by Sav-PE.

## Applications:

**Applications:** Flow Cytometry

**Recommended Usage:** Each lot of this product is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 microL per 100,000 - million cells in a 100 microL volume of Annexin V Binding Buffer (Cat No. 422201). It is recommended that the reagent be titrated for optimal performance for each application.

### Application Notes: Annexin V Staining

1. Wash cells twice with cold BioLegend Cell Staining Buffer (Cat. No. 420201) and then resuspend cells in Annexin V Binding Buffer (Cat. No. 422201) at a concentration of  $1 \times 10^6$  cells/ml.
2. Transfer 100 microL of cell suspension in 5 ml test tube.
3. Add 5 microL of Biotin Annexin V.
4. Add 10 microL of PI solution (Cat. No. 421301) or 7-AAD (Cat. No. 420403/420404).
5. Gently vortex the cells, and incubate for 15 min at room temperature (25°C), in the dark.
6. Add 400 microL of Annexin V Binding Buffer (Cat. No. 422201) to each tube. Analyze by flow cytometry.

- Application References:**
1. Koopman G, et al. 1994. *Blood* 84:1415.
  2. Vermes I, et al. 1995. *J. Immunol. Methods* 184:39.
  3. Dachary-Prigent J, et al. 1993. *Blood* 81:2554.
  4. Sekine C, et al. 2009. *Int. Immunol.* 21:295. [PubMed](#)
  5. Grujic M, et al. 2010. *J. Immunol.* 185:1730. [PubMed](#)
  6. Hussain MS, et al. 2013. *Hum. Mol. Genet.* 22:5199. [PubMed](#)
  7. Feng Q, et al. 2014. *PLoS One.* 9:95927. [PubMed](#)

**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. 420403/420404) 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.