

**Brilliant Violet 421™ Annexin V**

**Catalog # / Size:** 3804615 / 25 tests  
3804620 / 100 tests

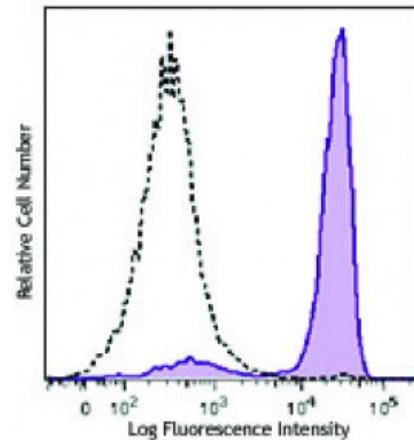
**Isotype:**

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

**Preparation:** The protein was purified by affinity chromatography and conjugated with Brilliant Violet 421™ under optimal conditions. The solution is free of unconjugated Brilliant Violet 421™ and unconjugated antibody.

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

**Concentration:** Lot-specific



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 Brilliant Violet 421™.

**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.

Brilliant Violet 421™ excites at 405 nm and emits at 421 nm. The standard bandpass filter 450/50 nm is recommended for detection. Brilliant Violet 421™ is a trademark of Sirigen Group Ltd.

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**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 Brilliant Violet™ 421 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** 1. Koopman G, et al. 1994. *Blood* 84:1415.
- References:** 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.* [PubMed](#)  
5. Grujic M, et al. 2010. *J. Immunol.* 185:1730. [PubMed](#)
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**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.