

Pacific Blue™ Annexin V

Catalog# / Size 640917 / 25 tests
640918 / 100 tests

Regulatory Status RUO

Other Names Annexin A5

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. Therefore, we recommend using our Helix NP™ Blue (Cat. No. 425305), Helix NP™ Green (Cat. No. 425303) or Helix NP™ NIR (Cat. No. 425301). 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.

Product Details

Reactivity All mammalian species

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

Preparation The purified protein was conjugated with Pacific Blue™ under optimal conditions.

Concentration Lot-specific (to obtain lot-specific concentration, please enter the lot number in our [Concentration and Expiration Lookup](#) or [Certificate of Analysis](#) online tools.)

Storage & Handling The Annexin V solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. Do not freeze.

Application [FC - Quality tested](#)

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 µl per 100,000 - million 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.

* Pacific Blue™ has a maximum emission of 455 nm when it is excited at 405 nm. Prior to using Pacific Blue™ conjugate for flow cytometric analysis, please verify your flow cytometer's capability of exciting and detecting the fluorochrome.

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Excitation Laser Violet Laser (405 nm)

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 1×10^6 cells/mL.
2. Transfer 100 µL of cell suspension in 5 mL test tube.
3. Add 5 µL of Pacific Blue™ Annexin V.
4. Add 10 µL of PI solution (Cat. No. 421301) or 7-AAD (Cat. No. 420403/420404).
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. No. 422201) to each tube. Analyze by flow cytometry.

For a better experience detecting apoptosis, we now recommend [Apotracker™](#). Cell staining with Apotracker™ is Calcium independent. Thus, no special buffers are required, and the protocol can be shortened for single-step co-staining with other reagents.

Application References

(PubMed link indicates
BioLegend citation)

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Product Citations

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RRID

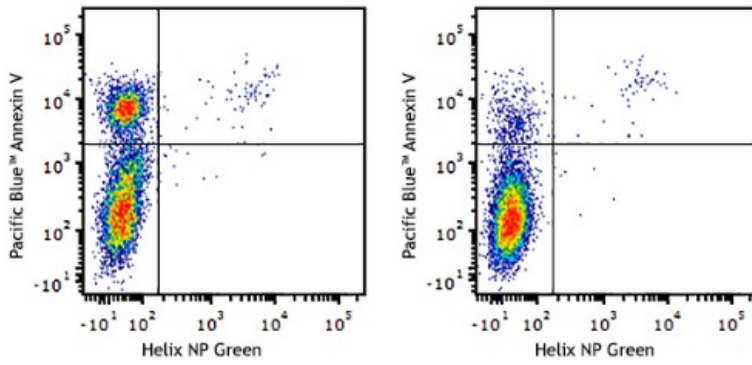
AB_1279046 (BioLegend Cat. No. 640917)
AB_1279044 (BioLegend Cat. No. 640918)

Antigen Details

Biology Area Cell Biology, Neuroscience

Gene ID [308](#)

Product Data



Human T leukemia cell line Jurkat, treated (left) or non-treated (right) with BioLegend's anti-human CD95 (EOS9.1) mAb (Cat. No. 305704) for 4 hours at 37°C, then stained with Annexin V-Pacific Blue™ for 15 minutes at 37°C in Annexin V Binding buffer. Helix NP Green (Cat. No. 425303 at 1.25 nM) was added 5 minutes at room temperature prior to running tubes.

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BioLegend Inc., 8999 BioLegend Way, San Diego, CA 92121 www.biolegend.com
 Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587