## **Product Data Sheet**

#### **Pacific Blue™ Annexin V**

**Catalog # / Size:** 3804585 / 25 tests

3804590 / 100 tests

Isotype:

Reactivity: Human, Mouse, Non-human

primate,Other,Rat

**Preparation:** The purified protein was conjugated

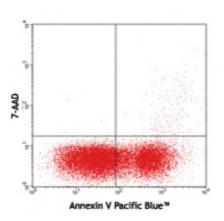
with Pacific Blue™ under optimal conditions. The solution is free of unconjugated Pacific Blue™.

Formulation: Phosphate-buffered solution, pH 7.2,

containing 0.09% sodium azide and

0.2% (w/v) BSA (origin USA).

Concentration: Lot-specific



### **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.

\* 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.

Application Notes:

**Annexin V Staining** 

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 1x10e6 cells/ml.
 Transfer 100 microL of cell suspension in 5 ml test tube.
 Add 5 microL of Pacific Blue ™

Annexin V.

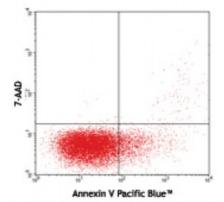
4. Add 10 microL of PI solution (cat #

421301) or 7-AAD (cat #

420403/420404).

5. Gently vortex the cells and incubate for 15 min at RT (25°C) in the dark.
6. Add 400 microL of Annexin V Binding Buffer (cat # 422201) to each tube.

Analyze by flow cytometry.



Human T-cell leukemia cell line, Jurkat, stimulated (4 hours) with (bottom) or without (top) LEAF™ purified anti-CD95, clone EOS9.1, then stained with Annexin V Pacific Blue™ and viability dye 7-AAD

# Application References:

- 1. Gordy C, et al. 2011 Blood 117:618. PubMed
- 2. Jia W, et al. 2011. J. Immunol. 186:5313. PubMed
- 3. Naegele M, et al. 2012. J. Neuroimmunol. 242:60. PubMed
- 4. Jenke AC, et al. 2013. PLoS One. 8:e55636. PubMed
- 5. Oh J, et al. 2013. J Exp Med. 210:1069. PubMed
- 6. Hasan S, et al. 2013. Blood. 122:1464. PubMed
- 7. Zhao BB, et al. 2013. PLoS One. 8:77008. PubMed
- 8. Mihaly SR, et al. 2014. PLoS One. 9:94982. PubMed
- 9. Miyazawa M, et al. 2014. Mol Biol Cell. 25:2116. PubMed
- 10. Yabas M, et al. 2014. J Biol Chem. 289:19531. PubMed
- 11. Burbulla LF, et al. 2014. Cell Death Dis. 5:1180. 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.