

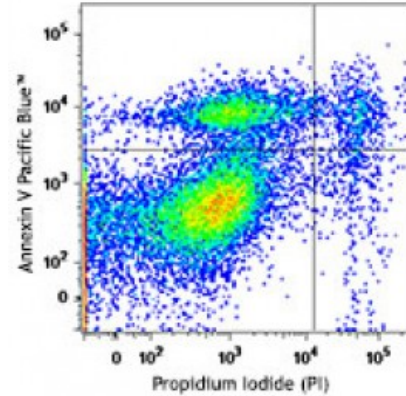
**Pacific Blue™ Annexin V Apoptosis Detection Kit with PI**

**Catalog # / Size:** 3804640 / 100 tests

**Isotype:**

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

**Concentration:** 0.2



Human T-cell leukemia cell line, Jurkat, treated (top) or non-treated (bottom) with LEAF™ purified anti-human CD95 (clone EOS9.1) for 4 hours, then stained with Pacific Blue™ Annexin V Apoptosis Detection Kit with PI.

**Applications:**

**Applications:** Flow Cytometry

**Recommended Usage:**

**Staining Procedure:**

1. Wash cells twice with cold Cell Staining Buffer, and then resuspend cells in Annexin V Binding Buffer at a concentration of 0.25-1.0 x 10<sup>7</sup> cells/ml.
2. Transfer 100 microL of cell suspension in a 5 ml test tube.
3. Add 5 microL of Pacific Blue™ Annexin V.
4. Add 10 microL of Propidium Iodide Solution.
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 to each tube. Analyze by flow cytometry with proper machine settings.

**Application Notes:**

**Materials Provided:**

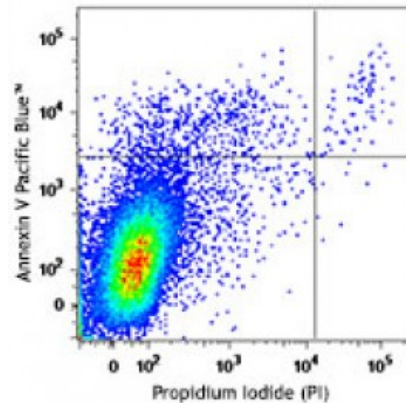
- 0.5 ml of Pacific Blue™ Annexin V
- 1 ml of Propidium Iodide Solution
- 50 ml of Annexin V Binding Buffer

**Materials Not Included:**

Cell Staining Buffer (Cat. No. 420201)

**Application References:**

1. Ranganathan P, *et al.* 2014. *J AM Soc Nephrol.* 25:239. [PubMed](#)



**Description:** Pacific Blue™ Annexin V Apoptosis Detection Kit with PI has been specifically designed for the identification of apoptotic and necrotic cells.

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 is recommended for use with Annexin V staining. Annexin V binding alone cannot differentiate between apoptotic and necrotic cells. To help distinguish between the necrotic and apoptotic cells we recommend use of our Propidium Iodide Solution (PI). Early apoptotic cells will exclude 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.

Propidium iodide is a fluorescent dye that binds to DNA. When excited by 488 nm laser light, it can be detected with in the PE/Texas Red® channel with a bandpass filter 610/10. It is commonly used in evaluation of cell viability or DNA content in cell cycle analysis by flow cytometry.