

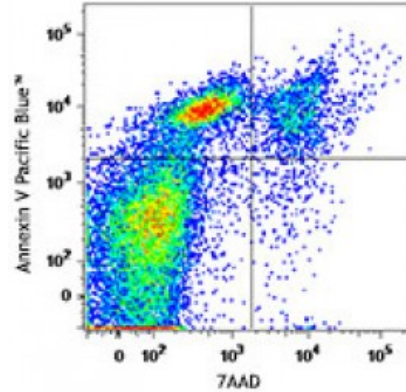
Pacific Blue™ Annexin V Apoptosis Detection Kit with 7-AAD

Catalog # / Size: 3804630 / 100 tests

Isotype:

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

Concentration: Lot-specific



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 7-AAD.

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 $0.25-1.0 \times 10^7$ 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 5 microL of 7-AAD Viability Staining 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:

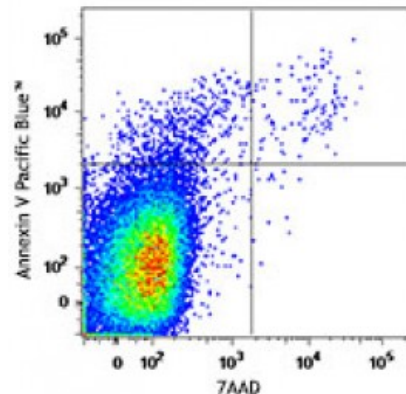
- 0.5 ml of Pacific Blue™ Annexin V
- 0.5 ml of 7-AAD Viability Staining Solution
- 50 ml of Annexin V Binding Buffer

Materials Not Included:

Cell Staining Buffer (Cat. No. 420201)

Application References:

1. Maciel E, *et al.* 2014. *Arch Biochem Biophys.* 548:38. [PubMed](#)



Description: Pacific Blue™ Annexin V Apoptosis Detection Kit with 7-AAD 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 7-amino-actinomycin D (7-AAD) solution. Early apoptotic cells will exclude 7-AAD, while late stage apoptotic cells will stain positively, due to the passage of these dyes into the nucleus where they bind to DNA.

7-AAD (7-amino-actinomycin D) has a high DNA binding constant and is efficiently excluded by intact cells. It is useful for DNA analysis and dead cell discrimination during flow cytometric analysis. When excited by 488 laser light, 7-AAD fluorescence is detected in the far red range of the spectrum (650 nm long-pass filter).