SONY

Product Data Sheet

PE/Dazzle™ 594 Annexin V

Catalog # / Size: 3804780 / 100 tests
3804775 / 25 tests

Immunogen: Yeast-expressed, recombinant mouse GM-CSF

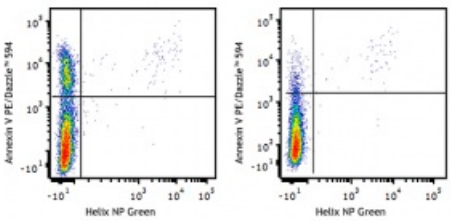
Reactivity: Human, Mouse, Non-human primate, Other, Rat

Preparation: The antibody was purified by affinity chromatography and conjugated with PE/Dazzle™ 594 under optimal conditions. The solution is free of unconjugated PE/Dazzle™ 594 and unconjugated antibody.

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

Workshop Number: V CD01.01

Concentration: Lot-specific



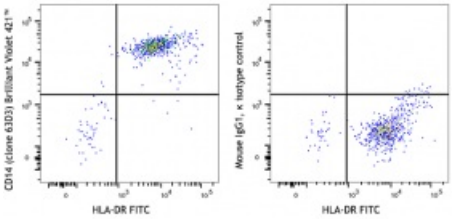
Human T leukemia cell line Jurkat, treated (left) or non-treated (right) with anti-human CD95 (EOS9.1) mAb for 4 hours at 37°C, then stained with Annexin V- PE/Dazzle™ 594 for 15 minutes at 37°C. Helix NP Green at 1.25µM final concentration was added before running sample.)

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 µl per 100,000 - million cells in a 100 µl volume of Annexin V Binding Buffer (Cat No. 2711005). It is recommended that the reagent be titrated for optimal performance for each application.

* PE/Dazzle™ 594 has a maximum excitation of 566 nm and a maximum emission of 610 nm.



Human peripheral blood monocytes were stained with HLA-DR FITC and Brilliant Violet 421™ anti-human CD14 (clone 63D3) (left) or Brilliant Violet 421™ mouse IgG1, κ isotype control (right).

Application Notes:

Annexin V Staining

1. Wash cells twice with cold Cell Staining Buffer (Cat. No. 2701005) and then resuspend cells in Annexin V Binding Buffer (Cat. No.2711005) at a concentration of $1x10^6$ cells/ml.
2. Transfer 100 µl of cell suspension in 5 ml test tube.
3. Add 5 µl of PE/Dazzle Annexin V.
4. Add 10 µl of PI solution (Cat. No. 2706505) or 7-AAD (Cat. No. 2702015/2702020).
5. Gently vortex the cells, and incubate for 15 min at room temperature (25°C), in the dark.
6. Add 400 µl of Annexin V Binding Buffer (Cat. No. 2711005) to each tube. Analyze by flow cytometry.

Application References:

1. Koopman G, *et al.* 1994. *Blood* 84:1415.
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
6. Hussain MS, *et al.* 2013. *Hum Mol Genet.* 22:5199. PubMed
7. Feng Q, *et al.* 2014. *PLoS One.* 9:95927. PubMed
8. Isobe T, *et al.* 2014. *eLife.* 3:1977. 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. 2711005) is recommended for use with Annexin V staining. Annexin V binding alone cannot differentiate between apoptotic cells and necrotic. 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.

Antigen References:

1. Blumberg RS, *et al.* 1995. *Immunol. Rev.* 147:5.
2. Calabi F, *et al.* 1991. *Tissue Antigens* 37:1.
3. Melian A, *et al.* 1996. *Curr. Opin. Immunol.* 8:82.