

Annexin-V-FLUOS

Cat. No. 11 828 681 001 250 tests

Annexin-V-FLUOS Staining Kit

Cat. No. 11 858 777 001 50 tests
11 988 549 001 250 tests

Annexin-V-Alexa 568

Cat. No. 03 703 126 001 250 tests

Type	Direct fluorescence staining for flow cytometric or microscopic analysis
Useful for	Detection of apoptotic cells with membrane alterations (phosphatidylserine translocation); differentiation of apoptotic from necrotic cells
Samples	Cell lines (adherent or suspensions), freshly isolated cells
Method	Simultaneous staining of cell surface phosphatidylserine [with Annexin-V-FLUOS (green dye) or Annexin-V-Alexa 568 (red dye)] and necrotic cells (with propidium iodide)
Time	Approx. 15 min (after induction of apoptosis)

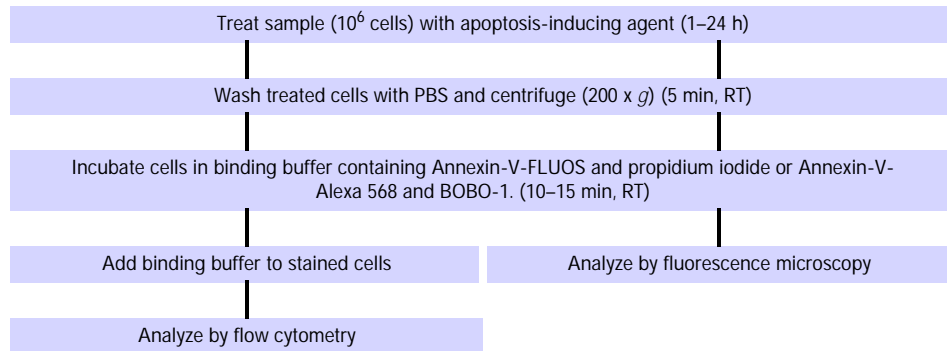
Significance of reagent and kit: Annexin-V is a phospholipid-binding protein with a high affinity for phosphatidylserine (PS). Detection of cell-surface PS with annexin-V thus serves as a marker for apoptotic cells. Analysis may be by flow cytometry or by fluorescence microscopy.

Test principle: Annexin-V-FLUOS (green dye) and Annexin-V-Alexa 568 (red dye) serves as a fluorescent probe for apoptotic cells. They will not bind normal, intact cells. However, since necrotic cells are leaky enough to give Annexin-V-FLUOS and Annexin-V-Alexa 568 access to inner membrane PS, apoptotic cells have to be differentiated from necrotic cells. Thus, the assay involves simultaneous staining with both Annexin-V-FLUOS (green) and the DNA stain propidium iodide (red) or Annexin-V-Alexa 568 (red) and BOBO-1 (green). Exclusion of propidium iodide or BOBO-1, coupled with binding of Annexin-V-FLUOS or Annexin-V-Alexa 568, indicates an apoptotic cell (Table 7). The procedure (Flow Chart 9) involves:

- 1 Washing suspended cells, then pelleting the cells.
- 2 Resuspending cells in a staining solution containing Annexin-V-FLUOS and propidium iodide or Annexin-V-Alexa 568 and BOBO-1.
Note: Cells may also be labeled with other membrane stains, such as a fluorescein-, phycoerythrin- or TRITC-labeled monoclonal antibody simultaneously.
- 3 Analyzing samples in a flow cytometer or under a fluorescence microscope.

	Normal cells	Apoptotic cells	Necrotic cells
Annexin-V staining	–	+	+
Propidium iodide staining	–	–	+
BOBO-1	–	–	+

▲ Table 7: Distinguishing apoptosis from necrosis using Annexin-V, propidium iodide, or BOBO-1.



▲ Flow Chart 9: Assay procedure, Annexin-V-FLUOS Staining Kit and Annexin-V-Alexa 568.

Specificity: Annexin-V-FLUOS and Annexin-V-Alexa 568 bind apoptotic cells and leaky necrotic cells. Propidium iodide and BOBO-1 are excluded from apoptotic and normal cells, but is taken up by necrotic cells.

Can be used to assay:

- Cell lines (adherent or suspensions)
- Freshly isolated cells

Reagent contents:

- Annexin-V-FLUOS solution, 50 x concentrated
- Annexin-V-Alexa 568, 50 x concentrated

Kit contents

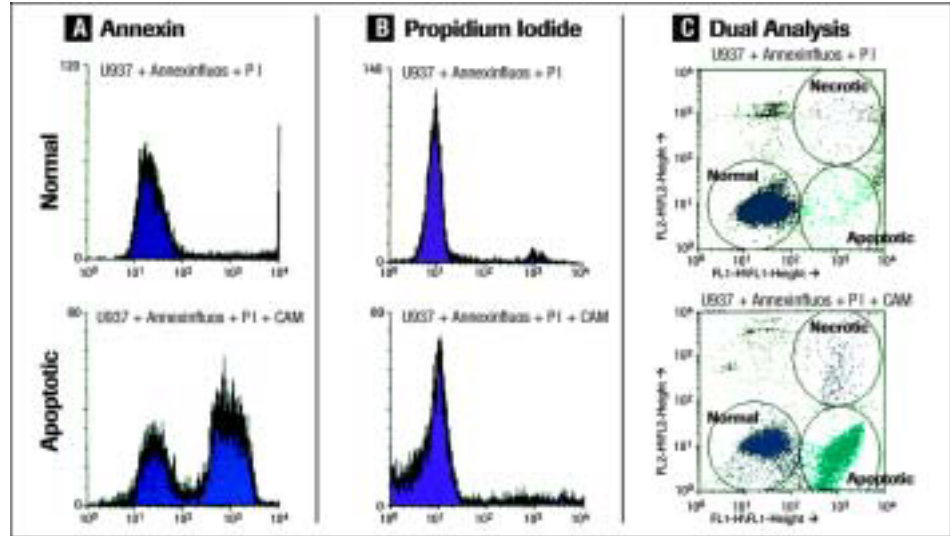
Annexin-V-FLUOS Staining Kit

1. Annexin-V-FLUOS, 50 x concentrated
2. Propidium iodide solution, 50 x concentrated
3. Binding buffer for flow cytometry, ready to use

Typical results: see Figures 33 and 34.

Other applications: For more examples of how the Annexin-V-FLUOS, Annexin-V-FLUOS Staining Kit and Annexin-V-Alexa 568 can be used in the lab, see Appendix, pages 138–141.

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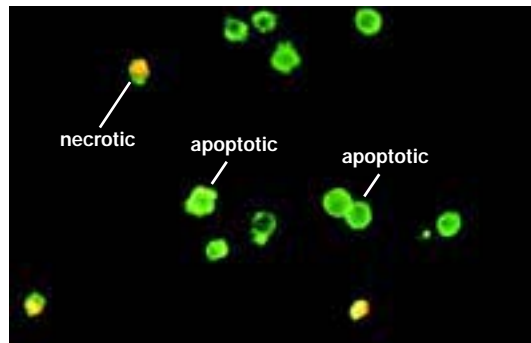
▲ Figure 33: Apoptotic and necrotic U937 cells identified in FACS analysis after staining with Annexin-V-FLUOS and propidium iodide (PI). Cells were then stained with the components of the Annexin-V-FLUOS Staining Kit and analyzed.

Panels A (upper and lower), single parameter analysis, Annexin-V-FLUOS only;

Panels B, single parameter analysis, propidium iodide (PI) only;

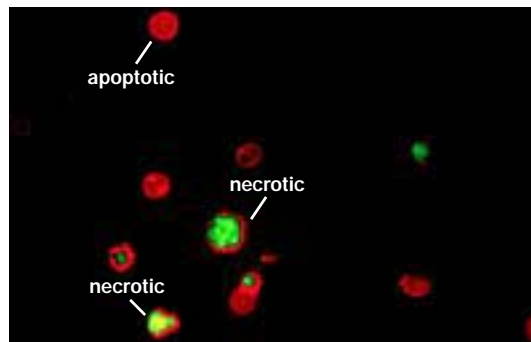
Panels C, dual parameter analysis, Annexin-V-FLUOS and propidium iodide. FL1, Annexin-V-FLUOS; FL2, propidium iodide.

Result: Flow cytometric analysis clearly differentiates normal (living) cells with low Annexin and low PI staining, apoptotic cells with high Annexin and low PI staining, and necrotic cells with high Annexin and high PI staining.



▲ Figure 34: Discrimination between apoptotic and necrotic U937 cells treated with camptothecin. Early-stage apoptosis detected with Annexin-V-FLUOS (green), and counterstained with propidium iodide (red cells).

Results: The apoptotic cells are visible in green and can be differentiated from necrotic cells by the propidium staining in figure 34. Necrotic cells take up propidium iodide and stain orange/green, while apoptotic cells stain green only.



▲ Figure 35: Discrimination between apoptotic and necrotic U937 cells treated with camptothecin (CAM) and stained with Annexin-V-Alexa 568 (red) and BOBO-1 (green).

Results: The apoptotic cells are visible in red and can be differentiated from necrotic cells by the BOBO-1 staining in figure 35. Necrotic cells take up BOBO-1 and stain green/red, while apoptotic cells stain red only.