

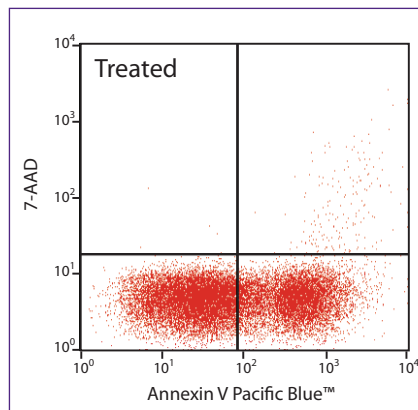
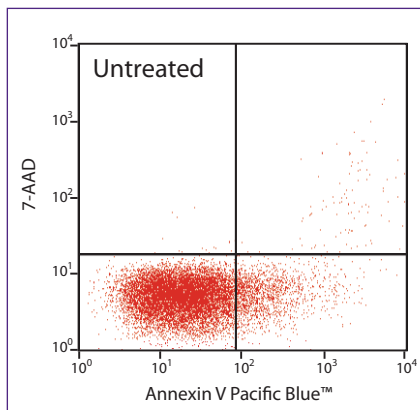
Reagents for Cell Death Detection

Annexin V

Annexin V

Annexin V belongs to the annexin family of intracellular proteins, that bind to phosphatidylserine (PS) in a calcium-dependent manner. In viable cells, PS is located on the inner leaflet of the plasma membrane. While maintaining an intact plasma membrane, cells undergoing early apoptosis will undergo a loss of membrane asymmetry involving the translocation of PS to the external leaflet. Fluorescently labelled Annexin V may be used to specifically target and identify cells engaged in the early stages of apoptosis.

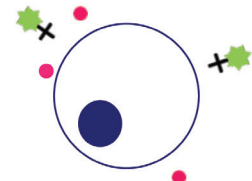
During late apoptosis or necrosis, plasma membrane integrity is lost, therefore facilitating the access of cell impermeant DNA dyes such as Propidium Iodide (PI) and 7-Aminoactinomycin D (7-AAD). The dual staining of cells with Annexin V and either PI or 7-AAD will effectively resolve viable, early apoptotic and late apoptotic/necrotic cell populations by flow cytometry. Early apoptotic cells will exclude 7-AAD and PI, staining positive for Annexin V only, while late-stage apoptotic and necrotic cells will exhibit positive staining, due to the passage of these dyes into the cell nucleus.



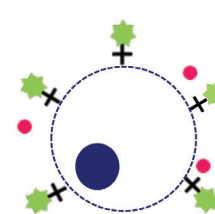
Human T-cell leukaemia cell line, Jurkat, un-stimulated (left) or stimulated for four hours (right) with LEAF™-purified anti-Fas (CD95) antibody, clone EOS9.1, then stained with Annexin V-Pacific Blue™ and the viability dye 7-AAD.

Characterisation of apoptosis using the Annexin V assay:

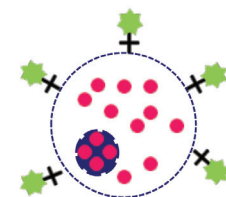
A. Viable cells



B. Apoptotic cells



C. Late apoptotic or necrotic cells



★ Annexin V
+ 7-AAD or PI

- A. Viable cells: Negatively stained for Annexin V and 7-AAD/PI
- B. Apoptotic cells: Positive staining for Annexin V. Negative for 7-AAD/PI.
- C. Late apoptotic or necrotic cells: Dual positive for both Annexin V and 7-AAD/PI.

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Suggested Protocol:

1. Harvest the cells . If using adherent cells, retain the media and combine with the detached cells prior to step 2.
2. Wash the cells twice using chilled Cell Staining Buffer. Re-suspend the pellet in Annexin V Binding Buffer at a concentration of 1×10^6 cells per ml.
3. Transfer 100 μ l cell suspension to a 5 ml test tube.
4. Add 5 μ l Annexin V conjugate.
5. Add 10 μ l of either 7-AAD or PI.
6. Gently mix and incubate for 15 minutes at room temperature, in the dark.
7. Add 400 μ l Annexin V Binding Buffer to each tube and analyse immediately by flow cytometry.

Tubes 3 and 4 are for setting compensation and ideally need to contain a proportion of cells that are apoptotic. Therefore, these tubes should be treated with an appropriate agent to induce apoptosis, e.g. camptothecin or staurosporine. Alternatively, to generate a positive population, the cells may be fixed for 30 minutes on ice, using 3% paraformaldehyde, prior to staining.

Controls:

1. Unstained cells
2. Viable cells stained with Annexin V conjugate and 7-AAD or PI
3. Annexin V-positive single colour sample
4. 7-AAD or PI-positive single colour sample

Product List (For latest product updates visit: biolegend.com)

Description	Size	Cat. No.
Annexin V FITC	25 tests 100 tests	640905 640906
Annexin V Alexa Fluor® 647	25 tests 100 tests	640911 640912
Annexin V Pacific Blue™	25 tests 100 tests	640917 640918
Cell Staining Buffer (FBS)	500 ml	420201
Annexin V Binding Buffer	100 ml	422201
Propidium Iodide Solution	2 ml	421301
7-AAD Viability Staining Solution	200 tests 500 tests	420403 420404

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