Lumiprobe
Life science solutions

Annexin V-AF Apoptosis Detection Kits with Propidium Iodide manual

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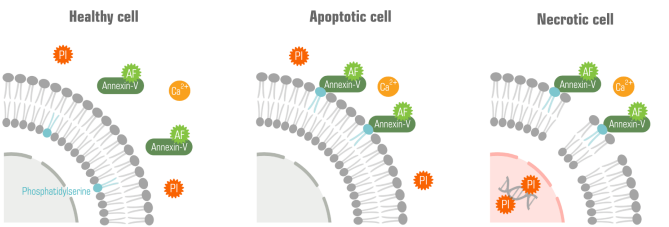
Annexin V (or Annexin A5) is a member of the phospholipid-binding annexin family of intracellular proteins. In flow cytometry and fluorescent microscopy, Annexin V is commonly used to detect apoptotic cells by its ability to specifically bind to phosphatidylserine (PS) in a calcium-dependent manner. The method was first reported by Koopman et al. (1994) [1].

In healthy cells, PS is normally retained in the inner leaflet of the plasma membrane. The early stage of apoptosis is accompanied by the loss of membrane phospholipid asymmetry, resulting in the exposure of PS at the cell surface. This translocation is mediated by the activation of Xkr8 scramblase after its cleavage by effector caspase-3. In conjunction with vital dyes such as Propidium Iodide (PI), fluorochrome-labeled Annexin V can distinguish between healthy (Annexin$^-$PI$^-$), apoptotic (Annexin$^+$PI$^-$), and necrotic (Annexin$^+$PI$^+$) cells [2], and conduct a quantitative analysis of cell death as a result of apoptosis and/or necrosis.



Propidium Iodide is a membrane-impermeable DNA stain allowing to differentiate necrotic, apoptotic, and healthy cells based on membrane integrity. After DNA binding, the dye emits in the orange-red channel. Its absorption max. is at 535 nm, the emission max. is at 617 nm.

Each **Apoptosis Detection Kit** contains all necessary reagents for labeling apoptotic and necrotic cells with AF 488- or AF 647-conjugated Annexin V and Propidium Iodide.

Kit components

Kit component	Count			
	11172 10 assays	21172 50 assays	14172 10 assays	24172 50 assays
11515, Annexin V-AF 488 conjugate, 1 ug	1	—	—	—
21515, Annexin V-AF 488 conjugate, 5 ug	—	1	—	—
12515, Annexin V-AF 647 conjugate, 1 ug	—	—	1	—
22515, Annexin V-AF 647 conjugate, 5 ug	—	—	—	1
19010, Propidium Iodide, 100 uL, 0.1 mg/mL in water	1	1	1	1
83215, Annexin V Binding Buffer, 5x, 15 mL	1	1	1	1

Transportation: at room temperature for 1 week. Store at -20°C.

Shelf life 9 months.

Recommended final concentrations of Annexin V-AF conjugate are 2 to 5 µg/mL, depending on the studied cell culture. Before the experiment, it is necessary to test different dilutions of Annexin V-AF to determine the optimal concentration.

Staining with Propidium Iodide is *optional*. This step can be skipped if there is no need to investigate necrosis.

Important! *Annexin V can only be used as a marker of apoptosis in cells with an intact plasma membrane. If the integrity of the plasma membrane is violated, Annexin V can bind to PS inside the cell and give a false positive result.*

Preparation of solutions

1. Dissolve the content of the **Annexin V-AF lyophilized conjugate (11515, 12515)** tube in 50 µL of deionized water. / Dissolve the content of the **Annexin V-AF lyophilized conjugate (21515, 22515)** tube in 250 µL of deionized water.

   Important! *The dissolved conjugate should be stored protected from light at 2-8°C. In solution, the conjugate is stable for a month. For longterm use, it is recommended to prepare aliquots and store them at -20°C. Avoid re-freezing!*

2. Prepare the required volume of 1x Binding Buffer by mixing 1 part of **5x Binding Buffer** with 4 parts of deionized water.

Cell staining

1. Carefully remove adherent cells from the growth surface in a suitable manner. With suspension cells, start work from the next step.
2. Wash cells once with cold PBS (pH7.4) and once with 1x Binding Buffer.
3. Resuspend cells in cold 1x Binding Buffer.
4. Collect 100 µL of cell suspension (1×10^5 to 1×10^6 cells/mL) in a 1.5-mL microcentrifuge tube. For flow cytometry, it is necessary to prepare additional tubes with appropriate controls (see description of controls below).
5. Add 2-5 µL of Annexin V-AF solution to each tube, and incubate for 10-15 min at room temperature, protected from light.
6. Without preliminary wash, add 400 µL of 1x Binding Buffer to each tube.
7. *(Optional)* Add 5 µL Propidium Iodide to each tube. Gently mix the contents of the tube and incubate for 5 min at room temperature, protected from light.

   Important! *Do not wash the cells to remove Propidium Iodide. Propidium iodide must remain in the buffer during data collection.*

8. Stained cells should be stored at 2-8°C in a dark place until analysis.

Important! *Quantitative analysis by flow cytometry or fluorescence microscopy must be conducted within 4 hours from the start of the staining due to the negative effect of Propidium Iodide on cell viability.*

Flow cytometry

1. For cytofluorimetric analysis of apoptosis and necrosis, it is necessary to prepare the following controls: unstained cells (negative control for instrument setup); cells stained with Annexin V-AF only, and cells stained with Propidium Iodide only (to adjust compensation).
2. Analyze binding of Annexin V-AF 488 using the FITC emission signal detector. / Analyze binding of Annexin V-AF 647 using the Cy5/Alexa Fluor 647 emission signal detector.
3. Analyze Propidium Iodide staining using the phycoerythrin emission signal detector.

Fluorescence microscopy

1. Transfer a drop of the stained cell suspension onto a glass slide. Cover the cells with a coverslip.
2. Alternatively, adherent cells can be stained directly on a coverslip. After staining, invert the coverslip onto the slide to place the cells between the slide and the coverslip.
3. *(Optional)* After staining with Annexin V, cells can be washed with 1x binding buffer and fixed in 2% paraformaldehyde before imaging. Do not fix cells prior to incubation with Annexin V-AF, as any disruption of the cell membrane may cause non-specific binding of Annexin V to PS on the inner surface of the cell membrane.
4. Imaging of the cells with a fluorescent microscope is carried out with appropriate filter sets.

Staining result

- Early apoptotic cells having bound Annexin V-AF 488/Annexin V-AF 647 feature a green/deep-red colored the plasma membrane.
- Necrotic cells are stained red as a result of the penetration of Propidium Iodide into the cell.
- Apoptotic cells with impaired membrane integrity due to secondary necrosis are stained red (Propidium Iodide) with a green/deep-red halo (Annexin V-AF 488/Annexin V-AF 647) on the cell surface (plasma membrane).
- Viable cells remain unstained.

Links

[1] Koopman G., Reutelingsperger C.P., Kuijten G.A., Keehnen R.M., Pals S.T., van Oers M.H. Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood. 1994. 84(5). P.1415-20.

[2] Martin S.J., Reutelingsperger C.P., McGahon A.J., Rader J.A., van Schie R.C., LaFace D.M., Green D.R. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. J Exp Med. 1995. 182(5). P.1545-56.

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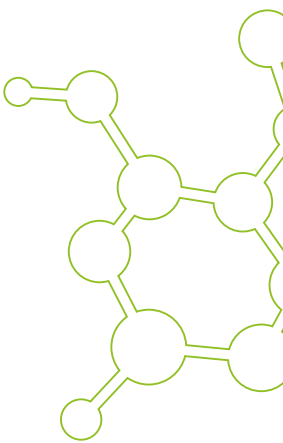

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