The Phosphatidyl Serine Detection™ kit to measure apoptosis

Apoptosis or programmed cell death (PCD) is a genetically encoded cell elimination program which ensures an equilibrium between cell proliferation and cell death and by which damaged or unwanted cells are eliminated. Therefore it is important to stress that apoptosis is a normal physiological process. Without continuous signaling by growth factors, hormones or cytokines, all cells undergo PCD. Aberrations in the mechanism of apoptosis occur in congenital defects, malignancies, auto-immune diseases, immune deficiency syndromes and in degenerative conditions (1,2).

The Phosphatidyl Serine Detection kit provides a rapid and reliable method for the detection of apoptosis by flow cytometry. The method enables detection at the single-cell level (3) and also allows the distinction between apoptosis and necrosis. During the early stages of apoptosis, Phosphatidyl Serine (PS) becomes exposed on the outside of the cell membrane. In vivo, this is a signal to phagocytes to engulf the dying cell before it loses its plasma membrane integrity and releases inflammatory mediators into the surroundings. This early stage of apoptosis can be specifically detected by PS binding proteins. During the early stage of apoptosis, the cell membrane is intact and the cells exclude propidium iodide (PI). Later, during the apoptotic process in vitro, the membrane becomes porous and PI becomes associated with DNA in the nucleus. The uptake of PI is an indication of necrosis. In a single cell suspension, cells can be analyzed using the Phosphatidyl Serine Detection™ kit, which contains the PS binding protein, Annexin V FITC or Annexin V PE, plus calcium buffer and PI. (See table 1 below.) The binding of Annexin V FITC to Phosphatidyl Serine is illustrated in Figure 1.

Figure 1
Flow cytometry analysis of peripheral blood lymphocytes labeled with Annexin V FITC and PI after overnight incubation with dexamethasone. Apoptotic cells exclude PI and express Phosphatidyl Serine. Necrotic or dead cells are permeable for PI which associates with nuclear DNA and is visible as red fluorescence.
The dot plot shows three populations: viable cells (lower left), apoptotic cells (lower right) and apoptotic cells which have lost plasma membrane integrity (upper right).

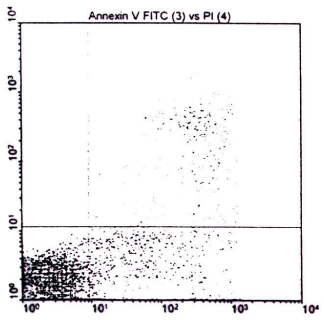

Figure 2
Dual staining with Annexin V and a monoclonal antibody to the membrane antigen CD3. Isolated lymphocytes were treated overnight with dexamethasone and subsequently labeled with Annexin V FITC and CD3 PE.

Upper left = viable CD3 positive lymphocytes.
Upper right = CD3 positive lymphocytes which have begun to undergo apoptosis.

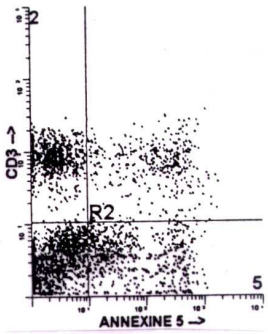

Table 1 - IQ Products Phosphatidyl Serine Detection Kit

• Phosphatidyl Serine Detection Kit (Annexin V FITC) for 100 tests	**Product code: IQP-116F**
• Phosphatidyl Serine Detection Kit (Annexin V PE) for 100 tests	**Product code: IQP-116R**
These kits contain the following:	
Annexin V (FITC or PE)	(1 ml / 100 tests)
Propidium iodide solution	(1 ml / 100 tests)
Calcium buffer	(5 x 5 ml)
Protocol for detection of Phosphatidyl Serine by flow cytometry	(100 tests)

Protocol I: Detection of Phosphatidyl Serine after culture with apoptosis inducing agents

1. Prepare a suspension of the cells to be studied. Adjust to 1.0 x 10^6 cells per ml.
2. Add 1 ml cell suspension to one well of a 24 well plate.
3. Prepare a 100 µm dexamethasone* solution to induce apoptosis. Weigh 3.4 mg of dexamethasone and dissolve in 1 ml methanol.
4. Add 10 µl dexamethasone solution to the cell suspension.
5. Incubate for 1 or 2 day(s) at 37 °C in a CO_2 incubator.
6. Dilute the calcium buffer 20 times in demi water and keep at 4 °C.
7. Remove the cells from the 24 well plate and wash with calcium buffer.
8. Re-adjust the cell concentration to 1.0 x 10^6 cells per ml.
9. Add 10 µl Annexin V FITC to 100 µl cell suspension and incubate for 20 minutes at 4 °C.
10. Wash the cells again with calcium buffer.
11. Add 10 µ propidium iodide and incubate for at least 10 minutes at 4 °C.
11. Keep the cells at 4 °C until ready to analyze by flow cytometer.

* = dexamethasone: Sigma D1756

Protocol II: Detection of Phosphatidyl Serine in unstimulated cells (or ex vivo)

1. Dilute the calcium buffer in demi water (20 x) and store at 4 °C.
2. Wash the cells to be measured in calcium buffer and re-adjust the cell concentration to 1.0 x 10^6 cells per ml in calcium buffer.
3. Add 10 µl Annexin V FITC or Annexin V PE to 100 µl cell suspension.
4. Incubate for 20 minutes on ice, in the dark.
5. Wash the cells with calcium buffer.
6. Add 10 µl Propidium iodide and incubate for at least 10 minutes on ice.
7. Keep the cells at 4 °C until ready to analyze by flow cytometer.

Protocol III: Dual staining: Phosphatidyl Serine plus membrane antigen

1. Follow the protocol as described above for stimulated or unstimulated cells.
2. Add the FITC or PE labeled monoclonal antibody to the membrane antigen at the same time as the Annexin V.

Notes:

- The Phosphatidyl Serine detection assay detects apoptotic cells well before DNA-based assays, since externalization of PS occurs prior to the nuclear changes associated with apoptosis.
- Normally, PI uptake is a sign of death by necrosis. In vivo, apoptotic cells are removed by phagocytic cells before losing membrane integrity and would normally not show PI uptake.
- Annexin V is a 35 kDa single chain protein which is dependent on Ca^{2+} for its binding to Phosphatidyl Serine.
- It is recommended that the Phosphatidyl Serine detection assay should be performed on ice in order to prevent further progression from viable cells to apoptotic cells or necrosis.
- The Phosphatidyl Serine detection assay has been found to detect apoptosis in a wide variety of cell suspensions, including peripheral blood lymphocytes (3), thymocytes (4) and germinal center B cells (5).

References:

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3. Vermes et al. 1995. A novel assay for apoptosis: Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labeled Annexin V. J. Immunol. Methods. 184. 39-51
4. Martin, S.J., et al., 1995. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus. Inhibition by over expression of Bcl-2 and Abl.. J.Exp. Med. 182, 1545 - 1557
5. Koopman et al. 1994. Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood. 84, 1415-1420

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