



## Annexin V - FluoProbes® 488

*To measure swiftly apoptosis in a variety of suspended cell types.*

### Product Information

<b>Name :</b>	<b>Annexin V - FluoProbes 488, Flow Cytometry Grade</b>
<b>Catalog Number :</b>	<a href="#">FP- BH9390</a> , 100 tests
<b>Contents</b>	1 vial containing Annexin-V-FluoProbes® 488 solution 4 vials containing 1.7 ml 10X concentrated binding buffer
<b>Purity :</b>	No unconjugated Annexin-V present as shown by Western blotting
<b>Absorption / Emission :</b>	$\lambda_{exc}/\lambda_{em} = 493 / 518 \text{ nm}$

See other [FluoProbes-AnnexinV products](#)

**Storage:** +4°C (stock solution and 10 fold diluted solution)  
**DO NOT FREEZE.** Protect from light and moisture.

### Introduction

The Annexin-V-FluoProbes® 488 Kit contains Annexin V, which detects apoptotic cells by the fact that early after the cell has decided to execute apoptosis it exposes phosphatidylserine (PS) at its plasma membrane surface. Annexin V recognizes the cell surface-exposed PS and binds with high affinity to it. Apoptotic cells are stained with a fluorescent conjugate of Annexin-V by a simple and quick one-step staining procedure. No fixation of the cells and no washing procedures are necessary. The stained cells can be measured by flow cytometry. Apoptotic cells are stained by Annexin-V-FluoProbes® 488 Kit before the dying cell changes morphology and cleaves its DNA (Vermes *et al.* 1995; Martin *et al.* 1995). The early detection and the ubiquity of apoptosis associated PS exposure makes Annexin-V- FluoProbes® 488 Kit in combination with its simple and rapid protocol a powerful tool to study apoptosis. The Annexin-V-FluoProbes® 488 Kit contains Annexin-V-FluoProbes® 488 and binding buffer, enough to perform at least 100 assays.

### Directions for use

- 1- Dilute the 10x concentrated binding buffer 10 fold with distilled water to obtain 1x binding buffer.
- 2- Wash the cells of interest with culture medium or PBS and finally suspend them in 1x binding buffer at  $10^6 - 10^7$  cells/ml.
- 3- Add 5 µl Annexin-V-FluoProbes® 488 to 100 µl of the cell suspension prepared as given by step 2.
- 4- Incubate for 15 minutes in the dark.
- 5- Add 400 µl 1x binding buffer.

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6- Wash cells with 1x binding buffer #FP-BU2080

Note: alternatively, use as washing buffer (25 mM Hepes/NaOH pH 7.4, 140 mM NaCl, 5 mM KCl, 1 mM MgCl<sub>2</sub> and 2.5 mM CaCl<sub>2</sub>; if necessary add 1 mg/ml BSA).

7- Measure the cell sample by flow cytometry.

AnnexinV can also be used for simultaneous apoptosis and immunophenotypic labeling of mammalian cells combined with PhiPhiLux G1D2, 7-AAD (apply the protocol of [FT-M19651](#) technical sheet)

## Technical information

### Apoptosis

Apoptosis is a well-organized process of cell death by suicide. It is physiological and it forms a fundamental process to the healthy organization of multicellular organisms <sup>(Wyllie *et al.* 1980)</sup>. Recently it has been recognized that apoptosis or aberrant forms of apoptosis are involved in the pathophysiology of diseases such as congenital malformations, infections, malignancies, lymphoproliferative disorders, neurodegenerative disorders, alopecia, psoriasis and atherosclerosis. Apoptosis was first discovered in tissues on basis of its morphological appearance <sup>(Kerr *et al.* 1972)</sup>. Gradually the morphological criteria for apoptosis, like cell shrinkage, nuclear condensation and pyknosis, were extended with biochemical criteria like the cleavage of DNA between the nucleosomes resulting in the ladder appearance of DNA on agarose gels <sup>(Wyllie *et al.* 1984)</sup>. Until recently this typical feature was considered as the Hallmark for apoptosis. However, not all cells in apoptosis appeared to cleave their DNA strands between the nucleosomes <sup>(Oberhammer *et al.* 1993)</sup> and those which did, cleaved not until late in the apoptotic pathway. New insights in the apoptotic process have come up with new parameters, which can be used to detect and measure apoptosis. One of these parameters is the phosphatidylserine (PS). During apoptosis the cell changes the structure of its plasma membrane (PM) to signal its suicide to the environment. Phagocytes pick up this signal and remove the dying cell by phagocytosis <sup>(Savill 1996)</sup>. The appearance of PS at the cell surface is one of the structural changes which occurs and which is recognized by phagocytes <sup>(Fadok *et al.* 1992a,b)</sup>.

### Phosphatidylserine (PS).

The viable cell treats PS in a specific manner by localizing PS predominantly in the membrane leaflet facing the cytosol. The outer leaflet of the PM facing the environment is almost devoid of PS. Aminophospholipid translocases, the activity of which has been measured in all viable cell types tested so far, are thought to be responsible for this asymmetric distribution of PS. The molecular identity(ies) of the translocases have not been resolved so far <sup>(Diaz and Schroit 1996)</sup>.

During apoptosis the equilibrium distribution of PS changes by an increased appearance of PS in the outer leaflet of the PM. It has been postulated that as yet unknown specific intrinsic membrane proteins facilitate this movement <sup>(Verhoven *et al.* 1996, Zwaal and Schroit 1997)</sup>. This exposed PS serves the function of removal of the dying cell from the tissues. This functionality and the fact that specific molecular machineries regulate the localisation of PS in the plasma membrane strongly suggest that cell surface exposed PS constitutes a functionally important part of the apoptotic pathway.

### Annexin-V properties

Annexin-V is a phospholipid binding protein that belongs to the Annexin family <sup>(Van Heerde *et al.* 1995)</sup>. In the presence of calcium ions it exhibits a high affinity for binding selectively to phosphatidylserine (PS). Annexin-V displays very low affinity for phospholipid species like phosphatidylethanolamine, sphingomyelin and phosphatidylcholine. This was firstly demonstrated for model membranes <sup>(Tait *et al.* 1989; Andree *et al.* 1990)</sup> and later for blood platelets, which expose PS at their cell surface under certain activating conditions <sup>(Thiagarajan and Tait 1992; Dachary-Prigent *et al.* 1993)</sup>. It is this phospholipid binding property which makes Annexin-V a powerful and selective tool to detect apoptotic cells.

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## Detection of apoptosis using Annexin-V.

Annexin-V was used successfully to measure apoptosis of various cell types by flow cytometry (Koopman 1994, Homburg 1995, Vermes 1995, Martin 1995, Van Engeland 1996). These studies showed that PS exposure starts soon after the cell has decided to execute apoptosis. The cell does so well before other features of apoptosis, like nuclear condensation and DNA cleavage, become visible. Cell surface exposure of PS also proceeds while the integrity of the plasma membrane remains un-compromised. These findings strongly suggest that specific machineries are being activated to translocate PS from the inner to the outer leaflet of the plasma membrane during the early phase of apoptosis. Once exposed at the surface PS remains there during the rest of the apoptotic pathway (see figure 1). Recently it was suggested that cell surface exposure of PS occurs downstream of the point of no return (Castedo 1996).

As depicted by the figure the different phenotypes of apoptosis are characterized by the cell surface exposure of PS. Execution of apoptosis *in vitro* is eventually accompanied by a loss of plasma membrane integrity during the late phase. This phenotype is designated as secondary necrotic.

When conjugated to a fluorochrome Annexin-V (Annexin-V-FluoProbes® 488) is a powerful tool to measure apoptosis by flow cytometry and fluorescence microscopy.

The phospholipid binding properties of Annexin-V allows a simple and quick protocol. Next is an example of a flow cytometric assay. Cells are mixed with an amount of Annexin-V-FluoProbes® 488 in binding buffer. After an incubation period of 5-15 minutes the cells can be analyzed by flow cytometry. In order to discriminate between the various phenotypes of apoptosis the vital dye propidium iodide is included to the assay mixture. By the combination of Annexin-V-FluoProbes® 488 and propidium iodide three distinct phenotypes become distinguishable. i) the viable population, the ii) the apoptotic population and iii) the secondary necrotic population (see figure 2).

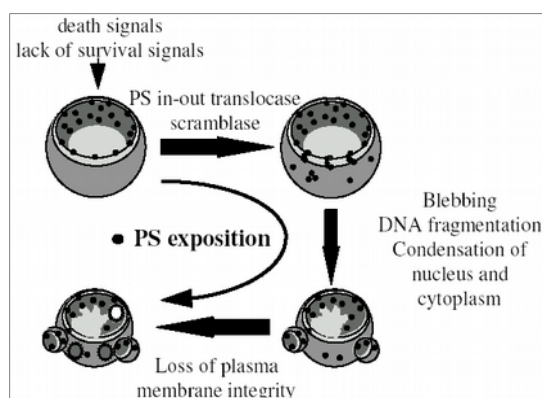


Figure 1: Schematic presentation of PS exposure during apoptosis

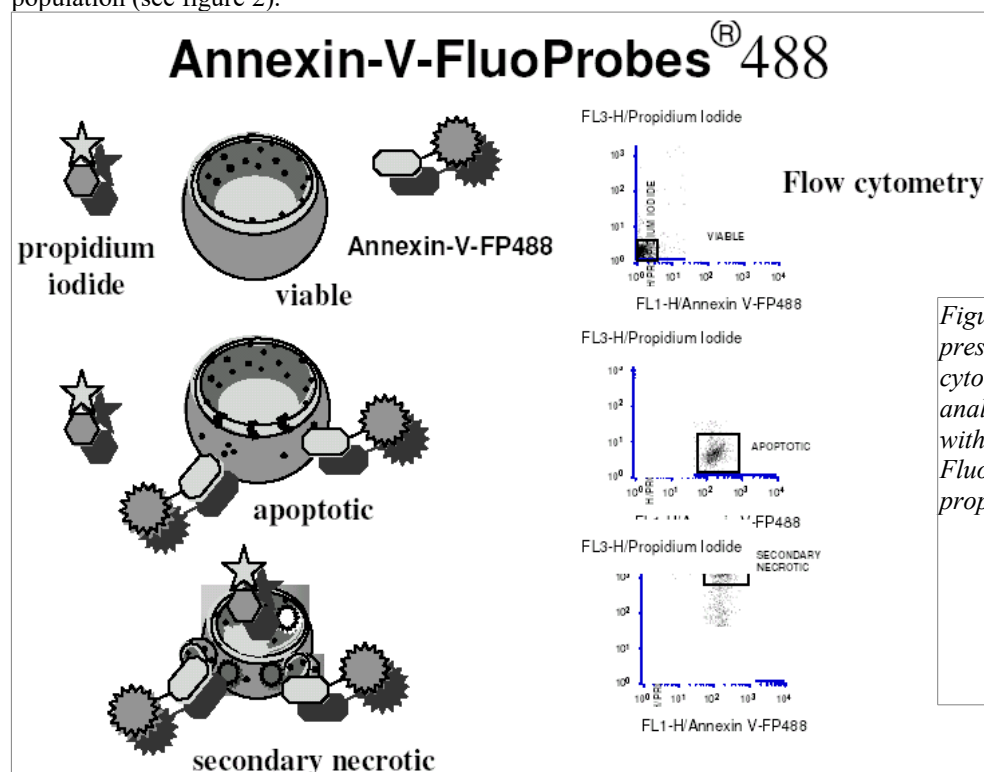


Figure 2: Schematic presentation of the flow cytometric result of analyzing rat thymocytes with Annexin-V-FluoProbes® 488 and propidium iodide).

The phenomenon of PS exposure appears to be ubiquitous in the sense that all cell types tested so far exhibit this phenomenon under the action of all apoptosis inducing stimuli used so far (see table 1) (Martin *et al.* 1995).

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**Table 1:** Ubiquity of PS exposure during apoptosis

<i>Cell Types</i>	<i>Initiating stimulus</i>
<u>Leukocytes</u>	<u>Plasma membrane receptor/ligand</u>
neutrophils	lack of growth factors
T-lymphocytes	Fas/Fas ligand interaction
B-lymphocytes	<u>Intracelulare receptor/ligand</u>
monocytes	glucocorticoids
<u>Tissue cells</u>	<u>Intracellular signalling</u>
endothelial cells	C2-ceramide
smooth muscle cells	<u>Macromolecular synthesis</u>
smooth muscle cells	actinomycin D
neurons	cycloheximide
<u>Tumors</u>	<u>DNA</u>
leukemia cells	inhibition topoisomerase II
carcinoma cells	damage by UV irradiation

## References

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## Ordering information

**To be used *in vitro* for research purposes only, not for diagnostic or therapeutic procedures!**

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## Related products

- AnnexinV binding buffer (10x), [FP-BU2080](#)
- AnnexinV, recombinant #[FP-JQ7610](#)
- 7-Aminoactinomycin (7-AAD), [FP-132303](#)
- Propidium iodide, 1 mg/ml, [FP-36774A](#)
- [Annexin V- FluoProbes® 488](#), FCM Grade, FP-BH4140, *Microscopy* Grade, FP-BH9390, or *in vivo* Grade, FP-BY6820
- [Annexin V- FluoProbes® 782](#), FP-DV7150

See [Products Highlights](#), [BioSciences Innovations catalogue](#) and [e-search tool](#).

Catalog size quantities and prices may be found at <http://www.interchim.com>

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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