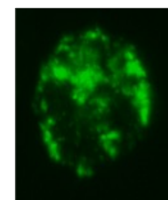
Annexin V - FluoProbes® 488

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

Product Information



Name :	**Annexin V - FluoProbes 488**
Catalog Number :	**FP- BH4140**, 500 µl (25µg/ml)
Contents	1 vial containing 500 µl Annexin-V-FluoProbes® 488 solution 3 vials containing 1.7 ml 10x concentrated binding buffer
Purity :	No unconjugated Annexin-V present as shown by Western blotting
Absorption / Emission :	$\lambda_{exc} \backslash \lambda_{em}$ = 493 / 518 nm

See other FluoProbes-AnnexinV products

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

The Annexin-V-FluoProbes® 488 Kit is to be used *in vitro* for research purposes only, not for diagnostic or therapeutic procedures

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 phoshatidylserine (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 fluorescence microscopy or 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 destilled water to obtain 1x binding buffer.
2. For **microscopy**, prepare freshly 1% and 4% paraformaldehyde in 1x binding buffer.
3. 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.

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4. Add 5 µl Annexin-V-FluoProbes® 488 to 100 µl of the cell suspension prepared as given by step 3.
5. Incubate for 15 minutes in the dark.
6. For **flow cytometry**, add 400 µl 1x binding buffer.
7. Wash the cells with 1x binding buffer (# FP-BU2080) or use a washing buffer (25 mM Hepes/NaOH pH 7.4, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$ and 2.5 mM $CaCl_2$; if necessary add 1 mg/ml BSA).
8. For **microscopy**, fix the cells in 4% paraformaldehyde during 30-60 minutes on ice in the dark. Transfer the cells to 1% paraformaldehyde. Keep the cells refrigerated in the dark until analysis
9. Analyse by fluorescence microscopy or flow cytometry.

AnnexinV can also be used for simultaneous apoptosis and immunophenotypic labeling of mammalian cells combined with caspase substrate (PhiPhiLux G2D2), 7-AAD (apply the protocol of FT-M19651 technical sheet)

Background: Apoptosis.
Apoptosis is a well-organized process of cell death by suicide. It is physiological and it forms a fundamental process to the healthy organisation of multicellular organisms (Wyllie *et al.* 1980). 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 (Kerr *et al.* 1972). 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 (Wyllie *et al.* 1984). 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 (Oberhammer *et al.* 1993) 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 (Savill 1996). The appearance of PS at the cell surface is one of the structural changes which occurs and which is recognized by phagocytes (Fadok *et al.* 1992a,b).

Background: 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 sofar, are thought to be responsible for this asymmetric distribution of PS. The molecular identitie(s) of the translocases have not been resolved sofar (Diaz and Schroit 1996).
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 (Verhoven et al. 1996, Zwaal and Schroit 1997). 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.

Background: Annexin-V.
Annexin-V is a phospholipid binding protein that belongs to the Annexin family (Van Heerde et al. 1995). 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 (Tait et al. 1989; Andree et al. 1990) and later for blood platelets, which expose PS at their cell surface under certain activating conditions (Thiagarajan and Tait 1992; Dachary-Prigent *et al.* 1993).
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 succesfully to measure apoptosis of various cell types by flow cytometry (Koopman et al. 1994, Homburg et al. 1995, Vermes et al. 1995, Martin et al. 1995, Van Engeland et al. 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 uncompromised. 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 et al. 1996).

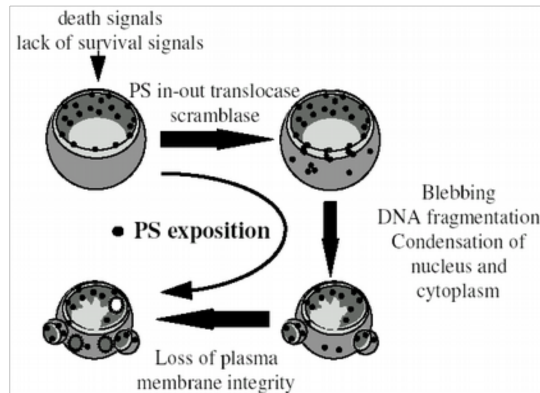

Figure 1: Schematic presentation of PS exposure during apoptosis

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 following example is an analysis of apoptotic cells by Confocal Scanning Laser Microscopy (CSLM. Cells are mixed with an amount of Annexin-V-FluoProbes® 488 in binding buffer. After an incubation period of 5-15 minutes the cells are washed with binding buffer and subsequently fixed with 4% paraformaldehyde prepared in binding buffer. The cells are then imaged with CSLM (see figure 2 below).
The figure below presents a comparison between Annexin-V-FITC and Annexin-V FluoProbes® 488
FluoPobes® 488 has a higher quantum yield and is more resistent to photobleaching as compared to FITC. These properties make FluoProbes® 488 superior as a probe for fluorecent analyses.

Bleaching time	Annexin V - FITC	Annexin V - FluoProbes® 488
0 minutes		

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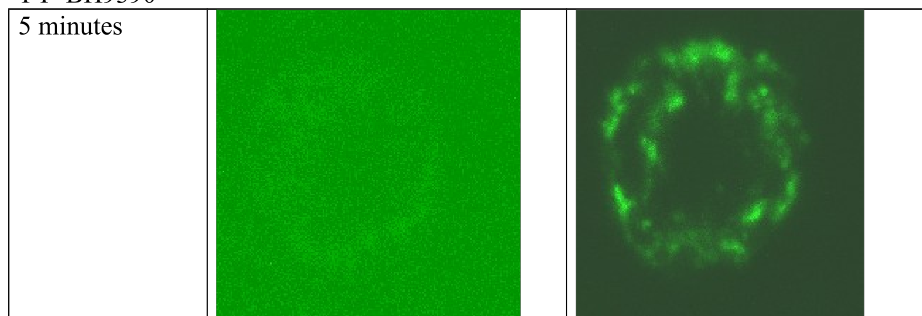

Figure 2: CSLM analysis of apoptotic cells using Annexin-V-FluoProbes® 488 and Annexin-V-FITC. The probes were challenged by a 5 minutes exposure time to the exciting laser beam. Only FluoProbes®488 remained fluorescent after this exposure time.

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

Table 1: Ubiquity of PS exposure during apoptosis as measured by APOPTEST™-FITC

Cell types	Initiating stimulus
Leukocytes	*Plasma membrane receptor/ligand*
neutrophils	lack of growth factors
T-lymphocytes	Fas/Fas ligand interaction
B-lymphocytes	*Intracellular receptor/ligand*
monocytes	glucocorticoids
Tissue cells	*Intracellular signalling*
endothelial cells	C2-ceramide
smooth muscle cells	staurosporine
fibroblasts	*Macromolecular synthesis*
neurons	actinomycin D
Tumors	cycloheximide
leukemic cells	*DNA*
carcinoma cells	inhibition topoisomerase II
	damage by UV irradiation

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Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com
Please inquire for higher quantities (availability, shipment conditions).
For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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

Related products:

- Annexin V binding buffer (5x), FP-EQI570
- Annexin V, recombinant #FP-JQ7610
- 7-Aminoactinomycin (7-AAD), FP-132303
- Propidium iodide, 1 mg/ml, FP-36774A
- Caspase Activity Assay Kit, Red fluorescence for flow cytometry, CJG060
- Annexin V- FluoProbes® 782, FP-DV7150

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