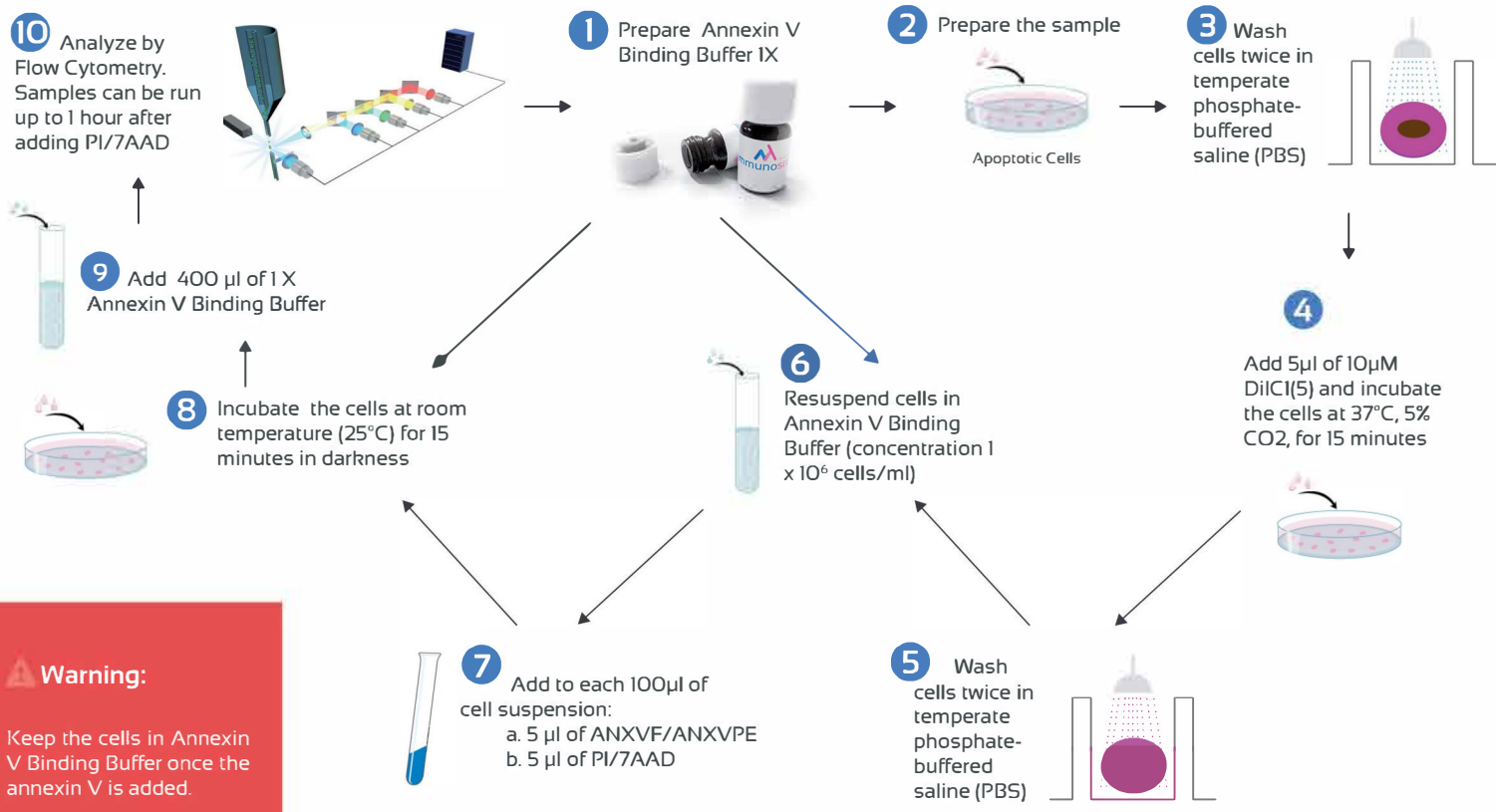


MITOCHONDRIAL ASSAYS PROTOCOL



Warning:

Keep the cells in Annexin V Binding Buffer once the annexin V is added.



More information

Please contact with our technical department, for request bulk and custom package size for Apoptosis Assays.

tech@immunostep.com

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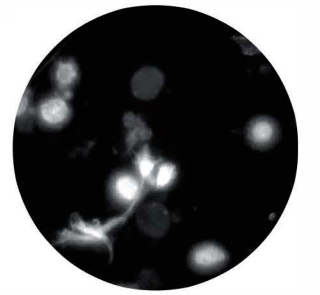
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Apoptosis Assays Detection of programmed cell death or apoptosis by flow cytometry



Apoptosis is a regulated process of cell death that occurs during embryonic development as well as maintenance of tissue homeostasis. The appearance of non-regulated apoptosis involves the possibility of different diseases, such as neurodegenerative disease and cancer.

ADVANTAGES

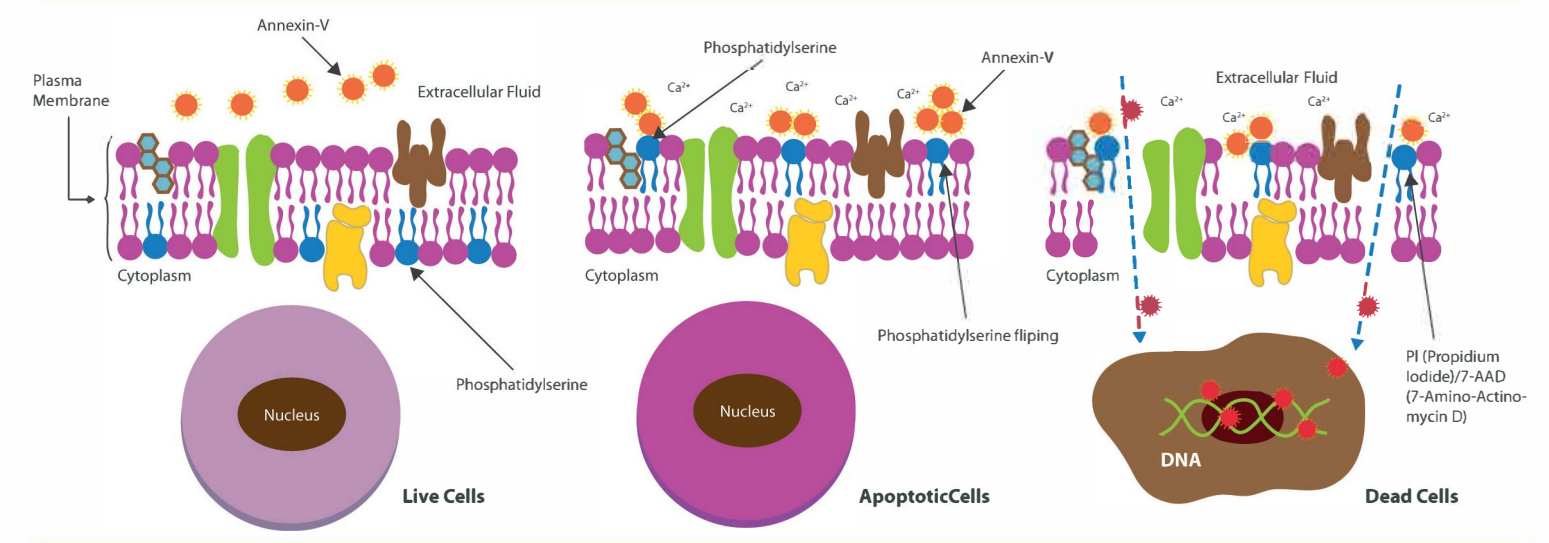
- Highly sensitive assays to detect early to late events.
- Large number of assays to detect different parameters.
- Very well validated in flow cytometry with many different type of cells (Jurkat, HL60, U266, whole blood, astrocytes, among many others) and many different type of drugs (Cytarabine, Camptothecin, Etoposide, Methotrexate, among many others).
- Different fluorescent options for multiple laser excitation sources.

APOPTOTIC MEMBRANE ASSAYS

PRINCIPLE OF MEMBRANE ASSAYS

During the earliest apoptosis, phosphatidylserine(PS), normally located on the cytoplasmic surface of the cell membrane, becomes exposed to the extracellular environment, causing changes in membrane asymmetry and permeability.

The human vascular anticoagulant, annexin V, is a 35-36KDa Ca²⁺ dependent phospholipids binding protein that has a high affinity for PS, and shows minimal binding to phosphatidylcholine and sphingomyelin.



Immunostep Apoptosis Detection kit offered by Immunostep provide the perfect way to identify and quantitate apoptotic cells on a single cell basis by flow cytometry.

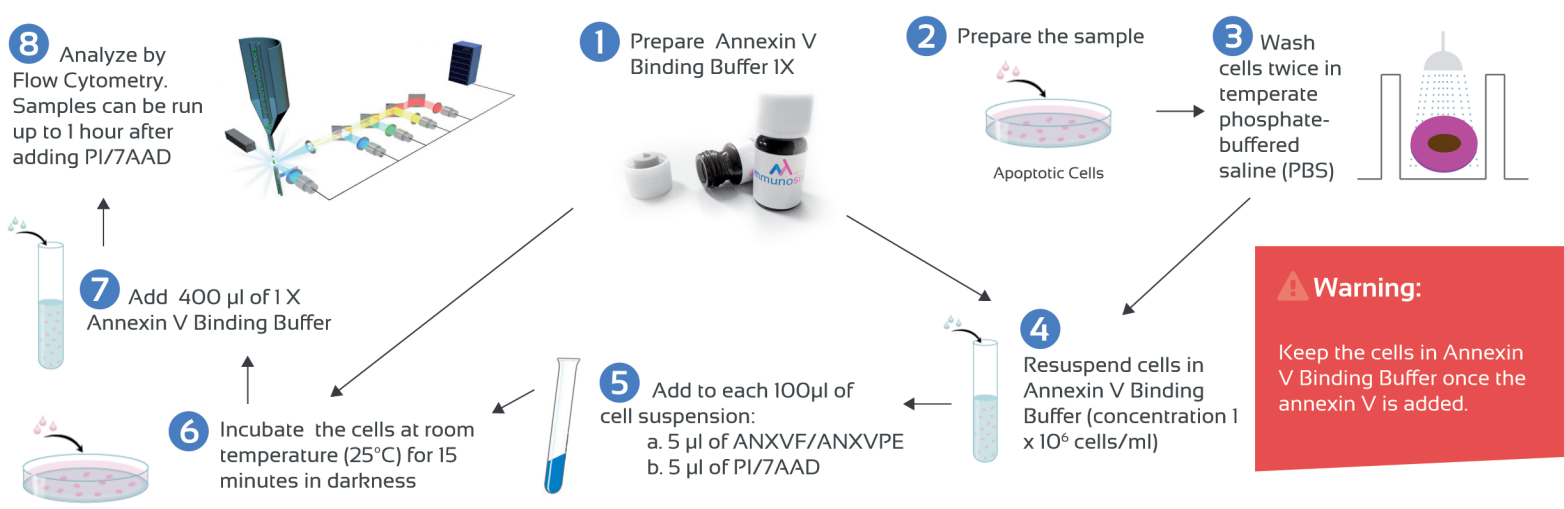
REAGENTS SUPPLIED WITH PLASMA MEMBRANA ASSAYS

Reference	Content of the kit	Images after analyzing
ANXVKF-100T FITC - Fluorescein Isothiocyanate (Blue Laser)	FITC Annexin V (ANXVF), 100 test, provided in Liquid form in buffer containing Antibody Stabilizer, PBS (PH 7,4)	
	Propidium Iodide Staining Solution (PI), 100 test in PBS (PH 7,4) or 7-Amino-Actinomycin D (7AAD), Staining Solution 100 test in PBS (PH 7,4)	
	Annexin V Binding Buffer 10X (BBIOX), 50 ml. 0,1M Hepes/NaOH (PH 7,4) 1,4 M NaCl, 25 mM CaCl2	
ANXVKPE-100T PE - R-Phycoerythrin (Blue Laser)	PE Annexin V (ANXVPE), 100 test, provided in Liquid form buffer containing Antibody Stabilizer, PBS (PH 7,2)	
	7-Amino-Actinomycin D. (7AAD) 500 µl in PBS and 0,09% NaN3 (sodium azide), pH 7,2	
	Annexin V Binding Buffer 10X (BBIOX), 50 ml. 0,1M Hepes/NaOH (PH 7,4) 1,4 M NaCl, 25 mM CaCl2	
ANXVKDY-100T Dy634 - Dyomics 634 (Red Laser)	Dy634 Annexin V (ANXVDY), 100 test, provided in liquid form in buffer containing Antibody Stabilizer, PBS, PH 7,4	
	Propidium Iodide Staining Solution (PI), 100 test in PBS (PH 7,4)	
	Annexin V Binding Buffer 10X (BBIOX), 50 ml. 0,1M Hepes/NaOH (PH 7,4) 1,4 M NaCl, 25 mM CaCl2	
ANXVKCFB-100T CF-Blue - CF405M (Violet Laser)	CF-Blue™ Annexin V (ANXVCFB), 100 test, provided in Liquid form in buffer containing Antibody Stabilizer, PBS (PH 7,2)	
	Propidium Iodide Staining Solution (PI) or 7-Amino-Actinomycin D (7AAD), Staining Solution 100 test in PBS (PH 7,4)	
	Annexin V Binding Buffer 10X (BBIOX), 50 ml. 0,1M Hepes/NaOH (PH 7,4) 1,4 M NaCl, 25 mM CaCl2	

Flow cytometry analysis of Jurkat cells (T-cell leukemia, human) treated with 6µM camptothecin for 4h at 37°C, 5% CO2.

*Browse our catalog for information on the individual components

PROTOCOL



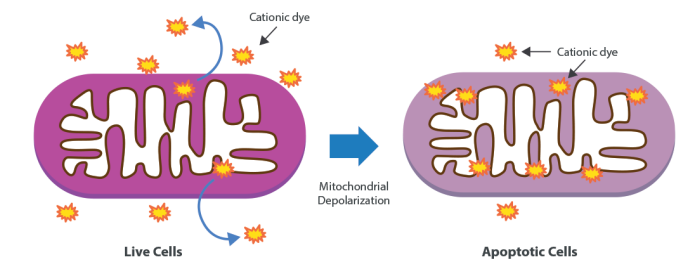
FLUOROCHROMES FOR APOPTOSIS ASSAYS

Abbreviation	Name	Excitation Laser Line (nm)	Maximum Excitation Peak (nm)	Maximum Emission Peak (nm)	Recommend Band Pass Filter (nm)
* CF-Blue™	CF405M	405 - Violet laser	405	450	450/50
FITC	Fluorescein Isothiocyanate	488 - Blue Laser	495	519	530/30
PE	R-Phycoerythrin	488, 532, 561 - Blue Laser	496, 564	578	585/42
* Dy634	Dyomics 634	595, 633, 635, 640, 647 Red Laser	635	658	660/20
PI	Propidium Iodide	488, 532, 561 - Blue Laser	351	617	585/42
7-AAD	7-Aminoactinomycin D	488, 532, 561 - Blue Laser	546	647	660/20
DiIC1(5)	1,1',3,3',3'-hexamethylindodicarbocyanine iodide (MitoStep)	595, 633, 635, 640, 647 Red Laser	638	658	660/20
*CF-Blue™	conjugated antibodies are provided under an agreement between Biotium Inc. and Immunostep				
Dy634	conjugated antibodies are provided under an agreement between Dyomics GmbH and Immunostep				

APOPTOTIC MITOCHONDRIAL ASSAYS

PRINCIPLE OF MITOCHONDRIAL ASSAYS

Depolarization of the sub mitochondrial and therefore changes in the mitochondrial membrane potential $\Delta\Psi$ have been described during early stages of apoptosis. Mitochondrial uptake of cationic dyes is a possible source of fluorescence variance.



MITOCHONDRIAL ASSAYS: Reagent supplied

Reference	Content of the kit	Excitation max (nm)	Emission max (nm)	Images after analyzing
MITO-100T MitoStep	DiIC1(5), 500 µl/10µM in DMSO	633	658	
KMAF-100T MitoStep + FITC Apoptosis Detection Kit	DiIC1(5), 500 µl/10µM in DMSO	633	658	
	ANXVKF - FITC Apoptosis Detection kit			
KMAPE-100T MitoStep + PE Apoptosis Detection Kit	DiIC1(5), 500 µl/10µM in DMSO	633	658	
	ANXVKPE - PE Apoptosis Detection kit			

*Jurkat cells *T/cell leukemia, human(treated with 6 µM Camptothecin for four hours /both panel.