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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
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ANNEXIN V FITC
Apoptosis detection kit

Fluorocrhome	Reference	Test
FITC	ANXVKF-100T	100 test

IVD

CE

immunostep we know how

INTRODUCTION.

Apoptosis is a regulated process of cell death that occurs during embryonic development as well as maintenance of tissue homeostasis. Inappropriately regulated apoptosis is implicated in different disease states, such as neurodegeneration disease and cancer. The apoptosis program is characterized by morphologic features, including loos of plasma membrane asymmetry and attachment, condensation off the cytoplasm and nucleus, and compaction and fragmentation of the nuclear chromatin. In normal viable cells, phospholipids are asymmetrically distributed between inner and outer leaflets of the plasma membrane whit phosphatidylcholine and sphingomyelin exposed on the external leaflet of the lipid bilayer, and phosphatidylserine predominantly observed on the inner surface facing the cytosol.

Exposure of PS on the external surface of the cell membrane has been reported in apoptotic cells, this occurs in the early phases of apoptotic cell dearth during which the cell membrane remains intact. In leukocyte apoptosis, PS on the outer surface of the cell marks the cell for recognition and phagocytosis by macrophages. The human vascular anticoagulant, annexin V, is a 35-36 kDa Ca^{2+} dependent phospholipids binding protein that has a high affinity for PS, and shows minimal binding to phosphatidylcholine and sphingomyeline. Changes in PS asymmetry, which is analyzed by measuring annexin V binding to the cell membrane, were detected before morphological changes associated with apoptosis have occurred and before membrane integrity has been lost. Annexin V labelled with FITC (green fluorescence) can identify and quantitate apoptotic cells on a single-cell basis by flow cytometry. Staining cells simultaneously with Annexin V –FITC and the non-vital dye propidium iodide (red fluorescence) allows (bivariate analysis) the discrimination of intact cells (Annexin V-FITC negative, PI negative), early apoptotic (Annexin V-FITC positive, PI negative) and late apoptotic or necrotic cells (Annexin V-FITC positive, PI positive).

MATERIALS

Annexin V-FITC, 100 tests, recommended for use in flow cytometry. The conjugate is provided in liquid form in buffer containing Antibody Stabilizer, PBS, PH 7,4.

Propidium Iodide Staining Solution. 100 test in PBS (pH 7,4)

Annexin V Binding Buffer, 10 X, 50 ml. 0,1M Hepes/NaOH (pH 7,4) 1,4 M NaCl, 25 mM $CaCl_2$.

Reference	Excitation laser Line (nm)	Max. Excitation peak (nm)	Max. Emission peak (nm)	Recommended Band Pass Filter (nm)
ANXVF-	488 Blue Laser	495	519	530/30
PI	488,532,561 Blue Laser	351	617	585/42

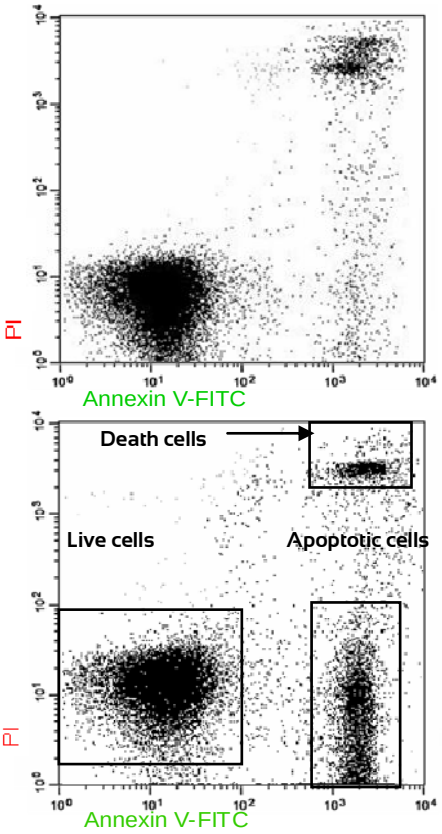

Figure 1. Jurkat cells (T-cell leukemia, human) treated with 6 µM camptothecin for four hours (bottom panel) or untreated (top panel).

1. RECOMMENDATIONS AND WARNINGS ⚠

a) The reagents contain sodium azide. In acid conditions, it is transformed into hydrazoic acid, a highly toxic compound. Azide compounds must be diluted in running water before being discarded. These conditions are recommended so as to avoid deposits in plumbing, where explosive conditions could develop. The safety data sheet (SDS) is available online at www.immunostep.com
b) Avoid microbial contamination of the reagent.
c) Protect from light. Use dim light during handling, incubation with cells and prior to analysis.
d) Never mouth pipette.
e) In the case of contact with skin, wash in plenty of water.
f) The samples should be handled in the same way as those capable of transmitting infection. Appropriate handling procedures should be guaranteed.
g) Do not use after the expiry date indicated on the vial.
h) Deviations from the recommended procedure could invalidate the analysis results.
i) FOR *IN VITRO* DIAGNOSTIC USE.
j) For professional use only.
k) Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.

STORAGE

Store in the dark at 2-8 °C. Do not use after expiration date stamped on vial. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the product is suspected, contact our Technical Services. tech@immunostep.com
DO NOT FREEZE. Protect the fluorescent conjugates from the light.

STAINING CELLS PROTOCOL WITH ANNEXINA-FITC FLOW CYTOMETRY

1. Prepare Annexin V Binding Buffer: 10 mM Hepes/NaOH (pH 7,4) 140 mM NaCl, 2,5 mM $CaCl_2$. .
2. Induce apoptosis in cells using the desired method. A negative control should be prepared by untreated cells, that is used to define the basal level of apoptotic and necrotic or dead cells.
3. Harvest the cells after the apoptosis induction and wash in temperate phosphate-buffered saline (PBS).
4. Wash cells twice with temperate PBS and resuspend cells in 1 X Annexin-binding buffer at a concentration 1 x 10^6 cells/ml.
5. Add 5 µl of the Annexin V-FITC and 5 µl of PI, to each 100 µl of cell suspension (up 1x10^5 cells).
6. Incubate the cells at room temperature for 15 minutes in the dark.
7. After incubation period, add 400 µl of 1X Annexin-binding buffer. Analyze by flow cytometry within one hour.

EXAMPLE PROTOCOL FOR ANNEXIN V EXPRESSION IN APOPTOTIC PERIPHERAL BLOOD LYMPHOCYTES

1. MN-Cells (Mononuclear cells) have been separated by Ficoll, from peripheral blood.
2. Apoptosis induction in leukocytes incubating 6 hours with H_2O_2 200µM.
3. 1 million cells have been harvested after the apoptosis induction. The supernatant was removed by centrifugation.
4. Added 100uL of PBS and 20uL of the CD19 APC(Ref. CD19APCA3-B1 - IMMUNOSTEP) and incubated 15 min.
5. The cells was washed once with temperate PBS and the cells was resuspend in 1 X Annexin-binding buffer 0,5 ml.
6. Added 5 µl of the Annexin V-FITC and 5 µl of PI, to each 100 µl of cell suspension (up 1x10^5 cells).
7. The cells was incubated for 15 minutes at room temperature, and have been analyzed by flow cytometry

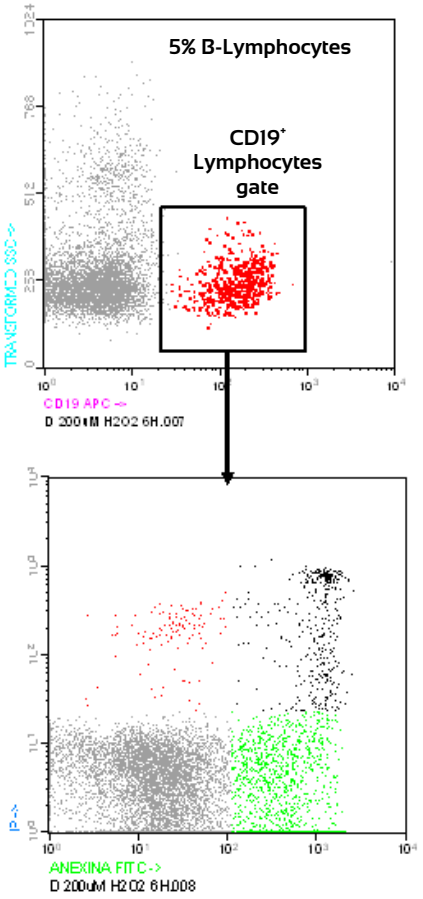

Figure 2. MN-Cells Treated with H2O2 200µM 6H
Live Cells: 76%
Apoptotic Cells: 16%
Dead and Late Apoptotic Cells: 6%
Necrótic Cells: 2%

CONTROLS TO SET UP FLOW CYTOMETER COMPENSATION AND QUADRANTS.

1. Unstained cells.
2. Cells stained with Annexin V- FITC alone (no PI).
3. Cells stained with PI alone (no Annexin V- FITC).

PERFORMANCE TEST

Evaluation of the functioning with different inducting drugs of the apoptosis and in different cellular types:

A. <u>Assay n°1</u>

<u>Cellular type</u>: HL-60 (Human promyelocytic leukemia cells).

<u>Drugs</u>:

- Citarabina (AraC)
- Camptotecina.
- Etoposido (VP-19)
- Metotrex

Drugs	Cel. Apopt Media (%)	Cel. Apopt Max (%)	Cel. Apopt Min (%)	Typical Des.	n
AraC	27,59	31,62	25,74	2,16	6
Campotecina	44,70	48,23	40,51	2,67	6
VP-19	38,99	44,47	34,51	3,25	6
Metotrexato	2,38	2,96	2,00	0,38	6
Not treated	1,36	1,62	1,16	0,23	2

B. Assay n°2

Cellular Type : Jurkat (Human T-cell lymphoblasts)

Drugs:

- Citarabina (AraC)
- Camptotecina.
- Etoposido (VP-19)
- Metotrexato

Drugs	Cel. Apopt Media (%)	Cel. Apopt Max (%)	Cel. Apopt Min (%)	Typical Des.	n
AraC	4,87	5,45	4,20	0,49	6
Campotecina	25,62	27,95	23,88	1,81	25
VP-19	20,11	21,43	18,84	0,93	6
Metotrexato	2,18	2,82	1,80	0,34	6
Not treated	1,63	1,78	1,45	0,16	2

C. Assay n°3

Cellular type: U266 (Human myeloma cell line)

Drugs: Zalypsis® at different concentrations (5,10 y 50 nM) and incubation times (24, 48 hours).

Incubation time: 24 h

Concentration Zalypsis	%viable	%apoptotic	%necrotic	n
Basal	68,84	24,19	5,82	1
5 nM	36,59	52,86	10,13	1
10 nM	12,82	76,53	10,62	1
50 nM	16,92	72,77	10,14	1

Incubation time: 48h

Concentration Zalypsis	%viable	%apoptotic	%necrotic	n
Basal	77,17	16,41	2,86	1
5 nM	14,73	57,4	27,22	1
10 nM	6,34	62,08	30,21	1
50 nM	6,82	52,5	34,32	1

TROUBLESHOOTING

a. Absence of Annexin V-FITC fluorescence: apoptosis was not induced in the cells.

b. Elevated Annexin V-FITC stainability: apoptosis is an ongoing process so that cells stained with Annexin V should not be kept for prolonged times before measurement.

c. Adherents cells may be released from their substrate by using trypsin. Trypsinized cells can be affected in the integrity of the plasma membrane [5]. On adherent cells a good idea is to remove supernatant with floating cells and replace media before adding drugs, or remove culture medium from cells, and immerse slide into cold (2-8 °C) 1X PBS.

d. Target cells that have been stained with Conventional annexin V-FITC/PI kit and then fixed with 1% PFA or methanol; they can give a signal quenching. In this case, probably, you have used an excessive dilution of the Binding Buffer, the fixation method can be optimized by using the $CaCl_2$ in the 10X Binding Buffer to 25 mM (2,5 mM final concentration).

WARRANTY

Warranted only to conform to the quantity and contents stated on the label or in the product labelling at the time of delivery to the customer. Immunostep disclaims hereby other warranties. Immunostep's sole liability is limited to either the replacement of the products or refund of the purchase price.

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