Annexin V Apoptosis detection Kits

	REF			
FITC/ PI	ANXVKF-100T	100 test	5 µl	CE IVD RUO
FITC/ 7-AAD	ANXVKF7-100T	100 test	5 µl	
PE/ 7-AAD	ANXVKPE-100T	100 test	5 µl	CE IVD
Dy634/ PI	ANXVKDY-100T	100 test	5 µl	RUO
CF-Blue/ PI	ANXVKCFB-100T	100 test	5 µl	RUO
CF-Blue/ 7-AAD	ANXVKCFB7-100T	100 test	5 µl	RUO

1. INTRODUCTION

Apoptosis, or programmed cell death, is characterized by a variety of morphological features. One of the earliest indicators of apoptosis is the translocation of the membrane phospholipid phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane. Once exposed to the extracellular environment, PS becomes available for binding to Annexin V, a Ca2+-dependent phospholipid-binding protein with high affinity for PS. The translocation of PS typically precedes other apoptotic processes, including loss of plasma membrane integrity, DNA fragmentation, and chromatin condensation. This early occurrence of PS exposure makes Annexin V an excellent tool for detecting apoptosis in its initial stages. Annexin V can be conjugated to various molecules for different detection methods, such as biotin for biochemical assays or fluorochromes for flow cytometry. These conjugations allow for easy and sensitive identification of cells undergoing early-stage apoptosis, particularly through flow cytometric analysis. (1-4)

2. PRODUCT DESCRIPTION

Staining with Annexin V is typically used to identify the loss of membrane integrity which accompanies the latest stages of cell death resulting from either apoptotic or necrotic processes. In conjunction with a viability dye allows to recognize early apoptotic cells from dead and damaged cells are permeable to viability dyes.

- **Tested application:** Flow Cytometry
- **Storage buffer**: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN3).
- **Recommended usage**: the intended of use is for the Identification of apoptotic cells by flow cytometry.
- **Presentation**: Liquid

- **Other Names:** Annexin A5
- **Gene ID:** 308
- **Molecular weight:** 35,9 kDa

3. REAGENTS PROVIDED

Each kit, according to the table below (Table 1), contains an annexin reagent conjugated to different fluorochromes, along with a cell viability reagent (PI or 7-AAD) compatible with the fluorochrome to which the annexin is conjugated. Additionally, it includes a buffer for sample resuspension, which provides the appropriate Ca^{2+} concentration to allow annexin V to specifically bind to PSThe kits are designed for flow cytometer compatibility between annexin-conjugated fluorochromes and viability dyes (Table 2).

Reference	Kit Components		
ANXVKF-100T	Annexin V FITC	Propidium Iodide (PI)	Binding Buffer 10X (BB10X)
ANXVKF7-100T	Annexin V FITC	7 aminoactinomycin (7-AAD)	Binding Buffer 10X (BB10X)
ANXVKPE-100T	Annexin V PE	7 aminoactinomycin (7-AAD)	Binding Buffer 10X (BB10X)
ANXVKDY-100T	Annexin V Dy634	Propidium Iodide (PI)	Binding Buffer 10X (BB10X)
ANXVKCFB-100T	Annexin V CF-Blue	Propidium Iodide (PI)	Binding Buffer 10X (BB10X)
ANXVKCFB7-100T	Annexin V CF-Blue	7 aminoactinomycin (7-AAD)	Binding Buffer 10X (BB10X)

Table 1: Description of the kit components by reference

Dye	Excitation laser Line (nm)	Max. Excitation peak (nm)	Max. Emission peak (nm)	Recommended Band Pass Filter (nm)
FITC	488 Blue Laser	495	519	530/30
R-PE	488,532,561 Blue Laser	496/564	578	585/42
Dy634	595,633,635,640,647 Red Laser	635	658	660/20
CF-Blue (Alternative to pacific Blue)	405 Violet Laser	405	450	450/50
Propidium Iodide (PI)	488,532,561 Blue Laser	351	617	585/42
7- aminoactinomycin (7-AAD)	488,532,561 Blue Laser	546	647	660/20

Table 2: Correlation between dye-conjugated Annexin V kit reagents, their excitation and emission characteristics, and the recommended laser in the flow cytometer.

4. APPROPIATE STORAGE AND HANDLING CONDITIONS

Store in the dark, refrigerated between 2 ºC and 8 ºC. DO NOT FREEZE. This reagent is stable until the expiry date stated on the vial label if kept at 2-8ºC. Do not use after the date indicated.

5. REAGENTS NO PROVIDED

- Wash solution: 20 Mm NaH2PO4, 150 NaCl, pH 7.2 + 0,09% Sodium azide (NaN3) + 0,5 % bovine serum albumin.
- 12x75mm Polystyrene Round Bottom Tubes (cytometer tubes).

6. EVIDENCE OF DETERIORATION

Reagents should not be used if any evidence of deterioration is observed. For more information, please contact our technical service: tech@immunostep.com

7. 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 on our website.
b. Protect from light. Use dim light during handling, incubation with cells and prior to analysis.
c. In the case of contact with skin, wash in plenty of water.
d. Do not use after the expiry date indicated on the vial. Reagents must not be used if the packaging shows clear evidence of deterioration.
e. Before starting the analysis, read the instructions carefully.Deviations from the recommended procedure could invalidate the analysis results.
f. When using a viability dye, do not leave the sample for more than a few minutes prior to flow cytometry acquisition. If you expect a longer delay before acquisition, it is better to leave the sample without the viability dye and add it just before acquisition.
g. Before acquiring the samples, it is necessary to make sure that the flow cytometer is calibrated and compensated.

8. PREPARATION OF REAGENTS

Annexin V Binding Buffer (Ref# BB10X-50ML) is a 10X concentrate. Prepare 1X Annexin V Binding Buffer by mixing 1 part of 10X binding buffer with 9 parts of distilled water. Wash solution (Ref# IMS0515) is a 10X concentrate. If crystallisation is observed in the concentrated buffer during storage,warm to 37°C and shake well before dilution. Prepare 1X Wash solution by mixing 1 part of 10X Wash solution with 9 parts of PBS, pH 7.4.

9. PROTOCOL

Annexin V dead cells Recommended Assay Procedure

1. A cell line that can be easily induced to undergo apoptosis, e.g. Jurkat cell line, should be used to obtain a positive control Annexin V staining. Induce apoptosis in cells using the desired method. Even in the absence of induced apoptosis, most cell populations will contain a minor percentage of cells that are positive for apoptosis. A negative control should be prepared by untreated cells that is used to define the basal level of apoptotic, necrotic and dead cells.
2. After apoptosis induction, obtain the cells by removing the culture medium. Centrifuge at 300xg for 5 minutes. Aspirate the supernatant without disturbing the cell pellet.
3. Harvest the cells after the apoptosis induction and wash cells twice by adding 2 ml of wash solution. Centrifuge at 300xg for 5 minutes and carefully aspirate the supernatants as not to touch the cell pellet.
4. Resuspend cells in 1 X Annexin V-binding buffer (Ref# BB10X) at a concentration 1 x 10 6 cells/ml.
5. Transfer 100 µL of cell suspension in 5 mL commonly used 12 x 75-mm flow cytometry assay tubes.
6. Add the suggested volume of dye conjugated-Annexin V (5 µL). Mix well and incubate cells for 15 minutes in the dark at room temperature (20-25ºC) or for 30 minutes at 4ºC.
7. Add a viability dye, such as PI (Ref# PI) or 7-AAD (Ref# 7AAD). This step is not mandatory, but is recommended.
8. Mix well and incubate cells for 5 minutes at room temperature (20-25ºC) in the dark.
9. After incubation period, add 400 µl of 1X Annexin-binding buffer (Ref# BB10X). Analyze by flow cytometry within one hour or store at 4ºC.

Annexin V expression in apoptotic peripheral blood lymphocytes

1. Separate mononuclear PMBC using a density gradient centrifugation protocol.
2. Induce apoptosis in leukocytes incubating 6 hours with an apoptosis inducting agent, e.g H 2 O 2 200µM. A negative control should be prepared by untreated cells that is used to define the basal level of apoptosis, necrosis and cell death.
3. Harvest the cells after the apoptosis induction and wash cells twice by adding 2 ml of wash solution. Centrifuge at 300 xg 5 minutes and carefully aspirate the supernatant so as not to touch the cell pellet.
4. Resuspend cells in 100 µl of Wash solution (Ref# IMS0515) at a concentration 1 x 10 6 cells/ml.
5. Add lymphocytes specific conjugated monoclonal antibody, e.g. CD19, and incubate for 15 minutes in the dark at room temperature (20-25ºC) or for 30 minutes at 4ºC.
6. Wash lymphocytes once with temperate wash solution and resuspend cells in 500 µl of 1 X Annexin-binding buffer (Ref# BB10X).
7. Add the suggested volume of dye conjugated-Annexin V (5 µL). Mix well and incubate cells for 15 minutes in the dark at room temperature (20-25ºC) or for 30 minutes at 4ºC.
8. Add a viability dye, such as PI (Ref# PI) or 7-AAD (Ref# 7AAD). This step is not mandatory, but is recommended.
9. Mix well and incubate cells for 5 minutes at room temperature (20-25ºC) in the dark.
10. After incubation period, add 400 µl of 1X Annexin-binding buffer (Ref# BB10X).
11. Analyze by flow cytometry within one hour or store at 4ºC.

10. FLOW CYTOMETRY ANALYSIS

These populations can be easily distinguished using a flow cytometer equipped with a 405 nm diode laser, a 488 nm argon ion laser, and a 633 nm HeNe laser for excitation. The choice of excitation wavelength—405 nm (diode laser), 488 nm (argon ion laser), or 633 nm (HeNe laser)—depends on the dye-conjugated Annexin V reagent used (Table 1).

A)

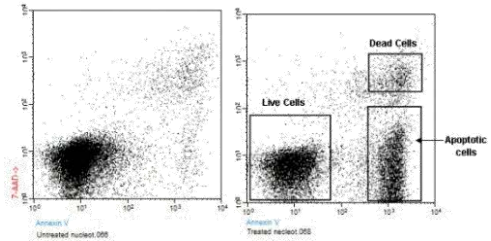

Figure 1. Jurkat cells (human T-cell leukaemia) treated with 6 µM camptothecin for four hours. (A) Analysis using the viability dye and comparing treated (right panel) and untreated (left panel).

11. TROUBLESHOOTING

a. Absence of Annexin V fluorence: apoptosis was not induced in the cells.
b. Elevated Annexin V stainability: apoptosis is an ongoing process so that cells stained with Annexin V shoukd 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. On adherent cells a good ieda is to remove supernatant with floating cells and replace media before adding drugs, or remove culture medium from cells, and immerse slide into cold (2-8C) 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 diution of the Binding BUffer, the fixation methodcan be optimized by using the CaCl2 in the 10X Binding Buffer to 25 MM (2,5 mM final concentration).

12. 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.

13. ADDITIONAL INFORMATION

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14. EXPLANATION OF SYMBOLS

	Fluorochrome
	Product reference
	Content for <n> analysis
	Regulatory Status
	Description
	Research Use Only
	Manufacturer

15. REFERENCES

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