MitoStep™ + Apoptosis Detection Kit

	REF	Σ	
FITC	KMAF-100T	100 test	RUO
PE	KMAPE-100T	100 test	

1. PRODUCT DESCRIPTION

Tested application: flow cytometry

Species reactivity: All mammalian

Storage buffer: aqueous buffered solution containing protein stabilizer and 0.09% sodium azide (NaN3). Recommended usage: Immunostep's Annexin V, is intended for the identification and enumeration of apoptotic cells. This reagent is effective for direct immunofluorescence staining for flow cytometric analysis using ≤1 x 105 cells in 100 µl volume of Annexin V Binding Buffer.

Presentation: liquid

Reagent provided: 100 test (5µl/test)

Reference	Excitation laser Line (nm)	Max. Excitation peak (nm)	Max. Emission peak (nm)	Recommended Band Pass Filter (nm)
ANXVF-200T	488 Blue Laser	495	519	530/30
ANXVPE-200T	488,532,561 Blue Laser	496/564	578	585/42
7-AAD	488,532,561 Blue Laser	546	647	660/20
PI	488,532,561 Blue Laser	351	617	585/42
DiICI(5)	595,633,635, 640,647 Red Laser	638	658	660/20

Large description: Apoptosis is characterized by a variety of morphological features. One of the earliest indications 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, binding sites on PS become available for Annexin V, Ca2+ -dependent, phospholipid binding protein with a high affinity for PS. The translocation of PS precedes other apoptotic processes such as loss of plasma membrane integrity, DNA fragmentation, and chromatin condensation. As such, Annexin V can be conjugated to biotin or to a fluorochrome, and used for the easy, flow cytometric identification of cells in the early stages of apoptosis.

Membrane potential (ΔΨ) is generated and maintained by concentration gradients of ions such as sodium, potassium, chloride, and hydrogen. MitoStep uses a cationic dye DiICI(5) (1,1´,3,3,3´-hexamethylindodicarbo-cyanine idodide) for the study of mitochondrial ΔΨ.
During the apoptosis occurs depolarization of the membrane and as a result there is an increase in cells with less DiICI(5) fluorescence.(1-7)

2. PROTOCOL

Staining cells protocol with DiICI(5), Annexin V and Non-Viable cells solutions (PI and 7-AAD).

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.
4. Wash cells twice with temperate PBS and resuspend cells in temperate phosphate-buffered saline (PBS) at a concentration 1 x 106 cells/ml.
5. Add 5 µl of 10µM DiICI(5).
6. ncubate the cells at 37 °C, 5% CO2, for 15 minutes.
7. Wash cells twice with temperate PBS and resuspend cells in 1 X Annexin-binding buffer at a concentration 1 x 10 6 cells/ml.
8. Add 5 µl of the Annexin V-FITC and 5 µl of PI, to each 100µl of cell suspension.
9. Incubate the cells at room temperature for 15 minutes at room temperature (25°C) in the dark.
10. After incubation period, add 400 µl of 1X Annexin-binding buffer. Analyze by flow cytometry within one hour.

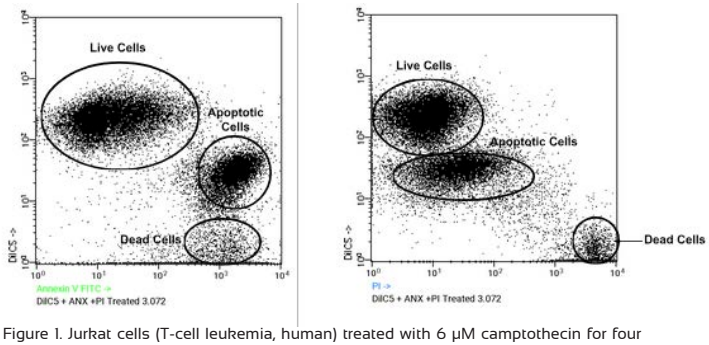

Figure 1. Jurkat cells (T-cell leukemia, human) treated with 6 µM camptothecin for four hours (both panel).

Possible combinations:

PRODUCT	LASER EXCITATION WAVELENGTH (NM)	EMIT (NM)
DiICI(5)	633	658
Annexin V-PE	488	575
7-AAD	488	645

PRODUCT	LASER EXCITATION WAVELENGTH (NM)	EMIT (NM)
DiICI(5)	633	658
Annexin V- FITC	488	520
PI	488 & 595	617

3. 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.

4. REFERENCES

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3. Homburg CH, de Haas M, von dem Borne AE, Verhoeven AJ, Reutelingsperger CP, Roos D. Human neutrophils lose their surface Fc gamma RIII and acquire Annexin V binding sites during apoptosis in vitro. Blood1995 Jan 15;85(2):532-40.
4. Vermes I, Haanen C, Steffens-Nakken H, Reutelingsperger C. A novel assay for apoptosis. Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. J Immunol Methods1995 Jul 17;184(1):39-51.
5. Darzynkiewicz Z, Bedner E, Traganos F. Difficulties and pitfalls in analysis of apoptosis. Methods Cell Biol2001;63:527-46.
6. Howard M. Shapiro. Membrane Potential Estimation by Flow Cytometry. Methods 21, 271-279 (2000).
7. Perez-Andres M, Benito JJ, Rodriguez-Fernandez E, Corradetti B, Primo D, Manzano JL, et al. Bisursodeoxycholate(ethylenediamine)platinum(II): a new autofluorescent compound. Cytotoxic activity and cell cycle analysis in ovarian and hematological cell lines. Dalton Trans2008 Nov 28(44):6159-64.
8. Herrero-Martin D, Osuna D, Ordonez JL, Sevillano V, Martins AS, Mackintosh C, et al. Stable interference of EWS-FLI1 in an Ewing sarcoma cell line impairs IGF-1/IGF-1R signalling and reveals TOPK as a new target. Br J Cancer2009 Jul 7;101(1):80-90.

5. EXPLANATION OF SYMBOLS

	Form
REF	Catalog reference
Σ	Contains sufficient for <n> test
	Quantity per test
	Regulatory Status
RUO	Research Use Only
	Manufacturer

6. MANUFACTURED BY:

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