



## APOPTEST™-FITC kit for 100 tests

**CATALOG NUMBER:** MUB-8100F  
**SPECIES:** recombinant human Annexin-A5

### QUALITY CONTROL:

Annexin A5-conjugated to FITC (Annexin V-FITC)  
Annexin V-FITC : 1:1 stoichiometric complex.  
Purity: > 99% pure according to Fast Protein Liquid Chromatography.  
Quality: > 99% of the protein has full phospholipid binding properties according to ellipsometry

### BACKGROUND

APOPTEST™-FITC protocol is designed to measure easily and accurately programmed cell death (PCD), including apoptosis, in a sample of cells in suspension. During the process of PCD, the plasma membrane of cells start to expose phosphatidylserine at their outer surface before the membrane loses its integrity and allows propidium iodide to enter the cell (Ref 1-3). The APOPTEST™-FITC employs the property of Annexin V-FITC to bind to phosphatidylserine in the presence of  $Ca^{2+}$ . In addition, the use of propidium iodide in the assay allows to discriminate between viable cells (Annexin V-FITC negative and propidium iodide negative), apoptotic cells (Annexin V-FITC positive and propidium iodide negative) and (secondary) necrotic cells (Annexin V-FITC positive and propidium iodide positive) (Fig. 1).

### SPECIFICITY

The conjugation protocol to form Annexin A5 with FITC to a 1:1 stoichiometric complex has not changed the native phospholipid binding properties of Annexin A5. Binding kinetics show a fast association of Annexin V-FITC with the phospholipid membrane if phosphatidylserine and  $Ca^{2+}$  are available. In the assay, the binding buffer contains  $Ca^{2+}$  to allow the interaction between Annexin V-FITC and phosphatidylserine.

### PRODUCT

Each APOPTEST™-FITC kit for 100 tests contains:  
1 vial containing 100µl Recombinant Annexin V-FITC solution.  
3 vials containing 1.7 ml 10x concentrated binding buffer.  
1 vial containing 250µg red solid propidium iodide

### APPLICATION

The APOPTEST™-FITC kit is designed to measure PCD in cells in suspension. The procedures described in this datasheet allow to determine and quantify apoptosis with the flow cytometer. For other applications, please contact MUBio Products BV. For measuring apoptosis in adherent cells and

*in vivo*, we kindly advise you to use our APOPTEST™-Biotin (Ref. 4-7).

### PROCEDURE FOR FLOW CYTOMETRY

#### Materials

APOPTEST™-FITC kit

Cells in suspension

Ice

#### Apparatus

Flow cytometer

#### Method

1. Dilute the 10x concentrated binding buffer 10 fold with distilled water and place the diluted buffer on ice.
2. Dissolve propidium iodide in 1 ml de-ionized water (dH2O) at a final concentration of 250 µg/ml.
3. Wash the cells of interest with ice-cold culture medium or PBS and finally suspend them in ice-cold diluted binding buffer at  $10^5$  to  $10^6$  cells/ml.
4. Add 1 µl Annexin-V FITC and 2,5 µl propidium iodide to 96 µl cell suspension prepared as given by step 3.
5. Keep the tube on ice and incubate for 10 minutes in the dark.
6. Dilute the cell sample to 250 µl with 1x binding buffer.
7. Measure immediately (within 5 minutes).

The flow cytometer is preferably set such that the Mean Fluorescence Intensity of the Annexin V-FITC negative population is between 1 and 10. Optimal parameter settings can be found using a positive control. For a positive control, incubate the cells with 3% formaldehyde in buffer during 30 minutes on ice. Wash away the formaldehyde and suspend the cells in cold diluted binding buffer at  $10^5$  to  $10^6$  cells/ml. Proceed with step 4 as described above. We advise to incubate the samples with Annexin V-FITC and Propidium Iodide on ice to arrest further progress of the cells through the stages from viable to PCD and or (secondary) necrosis. For rat thymocytes, we have shown that when kept on ice the population distribution (viable, PCD, secondary necrotic) remains stable for at least 6 hours.

### SPECIES REACTIVITY

Annexin V-FITC binds to phosphatidylserine of all cell types (Ref. 6) and species tested (Ref. 7).

### STORAGE CONDITIONS

**Annexin V-FITC solution and the 10x concentrated binding buffer**

Store in the dark at 2-8°C.

#### Propidium iodide

Lyophilized propidium iodide should be stored in the

### WARNING and CAUTION

This product is intended FOR RESEARCH USE ONLY, and FOR TESTS IN VITRO, not for use in diagnostic or therapeutic procedures involving humans or animals. This datasheet is as accurate as reasonably achievable, but MUBio Products BV accepts no liability for any inaccuracies or omissions in this information.



dark at a temperature below 25°C. After dissolving in demineralized water, store the propidium iodide solution in the dark at 2-8°C.

#### Stability

When at 2-8°C, the product is stable at least until the expiry date printed on the vials.

## REFERENCES

1. G Koopman, CPM Reutelingsperger, GAM Kuijten, RMJ Keehnen, ST Pals, MHJ van Oers (1994) Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. *Blood* 84:1415–1420.
2. M van Engeland, LJ Nieland, FCS Ramaekers, B Schutte, CPM Reutelingsperger (1998) Annexin V-affinity assay: a review on an apoptosis detection system based on phosphatidylserine Cytometry 31: 1-9.
3. P Williamson, SM van den Eijnde, R Schlegel. Phosphatidylserine exposure and phagocytosis of apoptotic cells. In: *Methods in Cell Biol, Cell Death* (ed. L Schwartz). 2001, Vol 66, Chapter 15, pp. 339-365.
4. M van Engeland, FCS Ramaekers, B Schutte, CPM Reutelingsperger (1996) A novel assay to measure loss of plasma membrane asymmetry during apoptosis of adherent cells. *Cytometry* 24 : 131-9.
5. SM van den Eijnde, AJM Luijsterburg, L Boshart, CI De Zeeuw, JH van Dierendonck, CPM Reutelingsperger, C Vermeij-Keers (1997) In Situ Detection of Apoptosis During Embryogenesis With Annexin V: From Whole Mount to Ultrastructure. *Cytometry* 29:313–320.
6. SM van den Eijnde, L Boshart L, CPM Reutelingsperger, CI De Zeeuw, C Vermeij-Keers (1997) Phosphatidylserine plasma membrane asymmetry in vivo: A pancellular phenomenon which alters during apoptosis. *Cell Death and Differentiation* 4:311–317.
7. SM van den Eijnde, L Boshart, EH Baehrecke, CI De Zeeuw, CPM Reutelingsperger, C Vermeij-Keers (1998) Phosphatidylserine exposure by apoptotic cells is phylogenetically conserved. *Apoptosis* 3: 9-16.

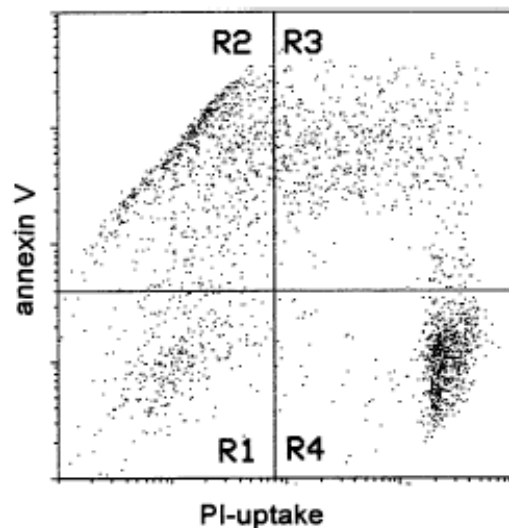


Fig. 1, adapted from reference number 2: Different labeling patterns in this assay identify the different cell populations, i.e., region R1: vital cells (PI-negative/annexin V-negative), region R2: apoptotic cells (PI-negative/annexin V-positive), region R3: dead cells (PI-positive/annexin V-positive) and region R4: damaged cells (PI-positive/annexin V-negative).

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