

APOPTEST™-FITC (cat. no.: A700)

A700 contains enough product to perform 200 tests/assays at a testconcentration of 125 ng/test to detect the early apoptotic cell.

For routine apoptosis detection, 1000 tests/assays can be performed at a testconcentration of 25 ng/test.

Annexin V-FITC Kit: *100 µl Annexin V-FITC solution*
 10 ml 10 x concentrated buffer
 250 µg solid Propidium Iodide

Contents

- 1 vial containing 100 µl Annexin V-FITC solution.
- 6 vials containing 1.7 ml 10x concentrated binding buffer.
- 1 vial containing 250 µg solid propidium iodide

Features of 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.
- Quantity : 100 µl Annexin V-FITC solution.
- Concentration : 250 µg/ml annexin V-FITC
- Additives : None.

Biological properties

Annexin V-FITC binds to phosphatidylserine

Storage of Annexin V-FITC solution and the 10 fold concentrated buffer.

Store in the dark and refrigerated at 2-8°C.

Storage of the propidium iodide.

Store in the dark and refrigerated at 2-8°C.

Application

The APOPTEST™-FITC is designed to measure swiftly Apoptosis in a variety of suspended cell types by flow cytometry. Because there are no additives, that might interfere with cellular processes, the APOPTEST™-FITC can be applied for cell sorting of Apoptotic cells, which then can be further investigated without any interference by side-effects of additives.

**The APOPTEST™-FITC is to be used *in vitro* for research purposes only,
not for diagnostic or therapeutic procedures!**

The APOPTEST™-FITC Protocol (A700)

The APOPTEST™-FITC employs the property of Annexin V-FITC to bind to PS in the presence of Ca²⁺. Our conjugation protocol to form Annexin V with FITC of 1:1 stoichiometric complex has not changed the native phospholipid binding properties of Annexin V. Binding kinetics show a fast association of Annexin V-FITC with the phospholipid membrane if PS and Ca²⁺ are available. The APOPTEST™-FITC protocol is designed to measure easily and swiftly Apoptosis in a sample of suspended cells.

Materials

APOPTEST™-FITC (Annexin V-FITC solution and 10x concentrated binding buffer).

Propidium Iodide (250 µg solid propidium iodide).

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-ionised 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 10⁵ -10⁶ cells/ml.
4. Dilute the Annexin V-FITC solution 10 fold with cold diluted binding buffer.
5. Add 5 µl diluted Annexin V-FITC and 5 µl propidium iodide to 490 µl of the cell suspension prepared as given by step 3.
6. Keep the tube on ice and incubate for 10 minutes in the dark.
7. Then measure the cell sample by flow cytometry.

The flow cytometer is preferably set such that the Mean Fluorescence Intensity of the Annexin V 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 - 10^6 cells/ml. Proceed with step 4 as described above.

The incubation with Annexin V and Propidium Iodide is formulated to be carried out on ice to arrest further progress of the cells through the stages of life \Rightarrow apoptosis \Rightarrow secondary necrosis.

For rat thymocytes, we have shown that when kept on ice the population distribution (viable, apoptotic, secondary necrotic) remains stable for at least 6 hours.

<i>Testconcentration</i>	25 ng/test	125 ng/test
<i>Number of tests</i>	1000	200
<i>Application</i>	Routine apoptosis measurement	Early apoptosis measurement