EXB0024
ANNEXIN V – FITC
100 tests

Intended Use

Annexin V -FITC is intended for detection of early apoptotic cells by flow cytometry.

Background

Apoptosis is a regulated cell death process characterized by morphological and biochemical features occurring at different stages. The translocation of phosphatidylserine (PS) from the inner side of the plasma membrane to the outer layer is one of the plasma membrane alterations during apoptosis. By this process PS becomes exposed on the external surface of the cell. Detection of such membrane changes by Annexin V binding to PS has been suggested as a suitable assay of early apoptotic cells.

Annexin V is a 35 kDa intracellular protein. It belongs to the family of proteins that bind to phosphatidylserine (PS) under a Ca^{2+}-dependent conditions. Fluorescently labeled Annexin V can be applied, with appropriate buffer (Annexin V Binding Buffer), for direct quantification of apoptotic cells using suitable protocols.

Since PS membrane translocation also occurs during necrosis, Annexin V is not an absolute marker of apoptosis. It is often used simultaneously with appropriate viability evaluation dyes such as propidium iodide (PI) or 7-aminoactinomycin D (7-AAD) to counterstain dead cells. PI and 7-AAD belong among the intercalating agents and fluorescent molecules. They pass through the plasmatic membrane of late apoptotic cells and necrotic cells and bind to chromosomal DNA. Plasmatic membrane of viable and early apoptotic cells is impermeable for vital dyes.

Reagents Provided

Annexin V - FITC

Quantity: 0.5 ml. Sufficient for 100 tests.

Preparation: Bacterially expressed Annexin V is purified to homogeneity (>99% by SDS-PAGE) and conjugated with fluorescein isothiocyanate (FITC) under optimum conditions. The reagent is free of unconjugated FITC.

Form: Ready-to-Use solution. No reconstitution is necessary. Reagent is provided in phosphate buffered saline (PBS) containing 15 mM sodium azide and 0.2% (w/v) high-grade protease free Bovine Serum Albumin (BSA) as a stabilizing agent.

Recommended Procedure

- Harvest cells intended for analysis by centrifugation (different cells may need different centrifugation conditions), discard supernatant. Resuspend cell pellet in cold PBS and wash cells by gentle shaking or by up-and-down mixing in a pipette tip. Re-centrifuge washed cells again and discard supernatant.
- Resuspend cell pellet in **1 x Annexin V Binding Buffer** and adjust cell density to 2 – 5 x 10^5 cells/ ml, preparing a sufficient volume of cell suspension (100 µl per test).
- Add **5µl** of **Annexin V – FITC** and **5µl** of **Propidium Iodide** to each 100µl of cell suspension and mix gently.
- Incubate stained cells for 15 minutes in dark at room temperature.
- After the incubation period, centrifuge cells and resuspend pellet in 100 µl of **1 x Annexin V Binding Buffer** (or in an appropriate volume according to a method of sample acquisition)
- Analyze the stained cells by flow cytometry as soon as possible.

Storage

Store in dark at 2-8°C. Do not freeze. Avoid prolonged exposure to light. Do not use after expiration date stamped on vial label. Short-term exposure to room temperature should not affect the quality of the reagent. However, if the reagent should be stored under any conditions other than those specified, the conditions must be verified by the user. Expiration date is stated on a vial label.

Other Apoptosis detection reagents:

	Description:	Quantity:
ED7044	ApoFlowEx® Kit	100 tests
EXB0023	Annexin V - Dyomics 647	100 tests
EXB0027	Annexin V – PE	100 tests
EXB0028	Annexin V – APC	100 tests
EXB0019	Annexin V Binding Buffer (10x)	50 ml
EXB0018	Propidium Iodide	100 tests
EXB0026	7-AAD Viability Staining Solution	400 tests

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