

## ANNEXIN V-FITC APOPTOSIS ASSAY KIT



**ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.**

**FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

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### PRODUCT INFORMATION:

NAME	ANNEXIN V-FITC APOPTOSIS ASSAY
Catalog No.	SK00482-06
Formulation	100 Tests
Lot No.	
Sample Type	Apoptotic cells
Storage	2 – 8 °C
This kit contains sufficient materials to run 100 tests provided that assay is run according to protocol.	

### INTRODUCTION

This Annexin V-FITC Assay Kit contains the necessary components required for the detection of apoptotic cells by flow cytometry. Annexin V-FITC is a fluorescent probe which binds to phosphatidylserine in the presence of calcium at the onset of apoptosis.

The assay consists of binding annexin V-FITC to phosphatidylserine in the membrane of cells at the beginning of apoptosis, and the binding of propidium iodide to the cellular DNA in cells where the membrane has been totally compromised. After incubating the cells, Annexin V-FITC is detected as a green fluorescence and propidium iodide is detected as a red fluorescence with flow cytometry.

### REAGENTS PROVIDED:

DESCRIPTION	CODE	QUANTITY
Annexin V-FITC Conjugate – 400 µl	482-06-01	2 vials (100 tests)
Propidium Iodide Solution – 800 µl	482-06-02	2 vials (100 tests)
10x Binding Buffer – 1.7 ml	482-06-03	9 vials (100 tests)

### STORAGE

Store at 2 – 8 °C for up to 12 months

**ADDITIONAL MATERIALS REQUIRED**

- Dulbecco's phosphate buffered saline (DPBS), 0.2 µm filtered
- 12 x 75 mm test tubes
- Cells to undergo apoptosis
- Serological centrifuge
- Incubator at 37 °C, 5% CO<sub>2</sub>
- Flow cytometer

**PRECAUTION**

This kit is for research use only. Annexin V is a product of human origin; handle carefully. This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

**PREPARATION**

Allow kit components to reach room temperature before use.

**1x Binding Buffer:** Dilute 1 ml of 10x Binding Buffer with 9 ml of deionized water.

**ASSAY PROTOCOL**

**Bring all reagents to room temperature before the start of the assay. Perform the experiment using aseptic technique.**

1. Induce apoptosis of cells by desired method.
2. Collect cells by centrifugation.
3. Resuspend cells in 1x Binding Buffer at a concentration of  $\sim 1 \times 10^6$  cells/ml.
4. Add 500 µl of apoptotic cell suspension to a plastic 12 x 75 mm test tube.
5. Add 5 µl of Annexin V-FITC Conjugate and 10 µl of Propidium Iodide Solution to each cell suspension.
6. Incubate the tubes at room temperature for exactly 10 minutes and protect from light.
7. Determine the fluorescence of the cells immediately with a flow cytometer (Ex = 488 nm ; Em = 530 nm). Cells, which are early in the apoptotic process will stain with the Annexin V-FITC conjugate alone. Live cells will show no staining by either Propidium Iodide solution or Annexin V-FITC conjugate. Necrotic cells will be stained by both the Propidium Iodide solution and Annexin V-FITC conjugate.