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A Geno Technology, Inc. (USA) brand name

# Annexin V-Dye Apoptosis Assay

(Cat. # 786-1548, 786-1543, 786-1544)



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## INTRODUCTION

Apoptosis is a form of programmed cell death which is essential as a normal part of development in multicellular organisms. Apoptosis go awry in some disease states, such as Alzheimer's and cancer.

Necrosis on the other hand is a traumatic cell death resulting from injury. Compared to necrosis, apoptosis is regulated and shows characteristic morphological and biochemical changes, such as compaction and fragmentation of nuclear chromatin, shrinkage of cytoplasm and loss of membrane asymmetry. In normal healthy cells, phosphatidyl serine (PS) is located on the cytoplasmic surface of cell membrane, where, as in apoptotic cell, it's translocated to the outer leaflet of the plasma membrane.

Annexin V-Dye Apoptosis Assay is designed to detect apoptosis. It is based on a property of apoptotic cell, where during onset of apoptosis, cells translocate the membrane phosphatidyl serine from inner face of plasma membrane to cell surface. Annexin V protein has high binding affinity for phosphatidyl serine in the presence of calcium ions. Thus, Annexin V-FITC or other Annexin V-Dye bound cell is apoptotic and can be quantified by FACS. Furthermore, propidium iodide is supplied in kit to differentiate apoptotic cells from necrotic cells (Fig.1).

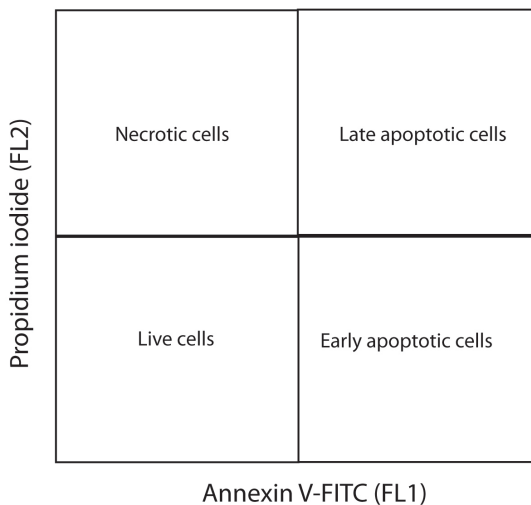


Fig.1: Sketch diagram of expected flow cytometry results using G-Biosciences Annexin V-FITC Apoptosis Assay

## ITEM(S) SUPPLIED

Description	Cat. # 786-1548 20 assays	Cat. # 786-1543 100 assays	Cat. # 786-1544 500 assays
Annexin V-FITC [100X] *	25 µl	105 µl	510 µl
Propidium Iodide Solution [1mg/ml]	100 µl	100 µl	100 µl
Annexin V-PS Binding Buffer [10X]	2 ml	10 ml	50 ml

\*Check table 1 for other Annexin V-Dye conjugates

## STORAGE CONDITIONS

The kit is shipped with blue ice. Store the kit at 4°C. The product is stable for at least 6 months from the date of receipt when stored properly.

## WARNING

Propidium Iodide Solution is considered a possible mutagen and should be handled carefully and disposed off as per local regulations. Wear protective clothing and gloves when handling this reagent.

## IMPORTANT INFORMATION

- Annexin V-FITC Apoptosis Assay is available in 20 assays (Cat. #786-1548), 100 assays (Cat. #786-1543) or 500 assays (Cat. # 786-1322) format with 100 µl assay volume. Other Annexin V-Dye conjugates also available are listed on table 1 with Catalog numbers.
- Annexin V-Dye Apoptosis Assay works on live cells only. It does not work on fixed cells.
- Annexin V-Dye and Propidium Iodide Solution is light sensitive. Do not expose to direct light when handling and staining cells. Incubations should be done in dark.
- Do not store Annexin V-Dye conjugates at -20°C. Store kit components at 4°C only.
- Suggested dilution for Annexin-Dye conjugate [100 X] is 1:100 dilution. Optimize the concentration as per requirement.

786-1548-350	Annexin V-Alexa Fluor®350 [100X]
786-1543-350	
786-1544-350	
786-1548-405	Annexin V-Alexa Fluor®405 [100X]
786-1543-405	
786-1544-405	
786-1548-488	Annexin V-Alexa Fluor®488 [100X]
786-1543-488	
786-1544-488	

786-1548-555	Annexin V-Alexa Fluor®555 [100X]
786-1543-555	
786-1544-555	
786-1548-594	Annexin V-Alexa Fluor®594 [100X]
786-1543-594	
786-1544-594	
786-1548-647	Annexin V-Alexa Fluor®647 [100X]
786-1543-647	
786-1544-647	

**Table 1:** List of Annexin V-Dye conjugates available with the kit

## WARNING

- Propidium Iodide is a potential mutagen. Handle the dye with care and dispose off the waste safely as per applicable local regulations.

## ADDITIONAL ITEMS REQUIRED

- Flow cytometer or fluorescence microscope.
- Cell Cultures.
- Apoptosis inducing agent for positive control (*optional*) such as Staurosporine (Cat. #786-1545)
- Drugs or compounds to be tested on cells.

## PREPARATION BEFORE USE

- Bring the kit components to room temperature.
- Prepare 1X Annexin V-PS Binding Buffer by adding 1 ml Annexin V-PS Binding Buffer [10X] to 9 ml of deionized water (1:9). Mix well.
- Prepare 100 µg/ml working solution of Propidium iodide by adding 10 µl of 1 mg/ml Propidium Iodide Solution to 90 µl of 1 X Annexin V-PS Binding Buffer. Remaining solution can be stored for later use at 4°C.
- Preparation of Annexin V -Dye /Propidium iodide staining solution immediately before assaying the cells for apoptosis as in Table 2. Table 2 is suitable for 48 assays. Keep the Annexin V- Dye /Propidium iodide staining solution protected from light.

**NOTE:** Suggested dilution for Annexin-Dye conjugate [100 X] is 1:100 dilution. Optimize the concentration as per requirement.

Reagents	Volume
1X Annexin V-PS Binding Buffer	5 ml
Annexin V-Dye Conjugate [100X]	50 µl
Propidium Iodide Working stock (100 µg/ml)	50 µl

**Table 2:** Annexin V -Dye conjugate/Propidium iodide staining solution

## PROTOCOL

### **Flow cytometry assay**

1. Culture cells and induce apoptosis or treat the cells with drug to be tested for apoptosis using the desired method.
2. Harvest the cells after incubation and determine the cell density.
3. Wash the cells once with 1X Annexin V-PS Binding Buffer.
4. Re-centrifuge the cells at 500 g for 5 minutes at room temperature.
5. Add 100 µl of Annexin V -Dye conjugate/Propidium iodide staining solution to each cell sample of around  $1 \times 10^5$  cells and resuspend the cells in it.
6. Incubate the cells for 15 minutes at room temperature in dark.
7. Add 400 µl of 1X Annexin V-PS Binding Buffer to cells and mix cells in buffer.
8. Keep the samples on ice and analyze immediately on flowcytometer using 488 nm excitation and 525 nm (FL1 channel) emission for Annexin-FITC and 730nm (FL2 channel) for Propidium iodide (Fig.1). For other Annexin V-Dye conjugates use the appropriate excitation and emission channel.

### **Fluorescence Microscopy assay**

1. Culture cells and induce apoptosis or treat the cells with drug to be tested for apoptosis using the desired method.
2. Harvest the cells after incubation and determine the cell density.
3. Wash the cells once with 1X Annexin V-PS Binding Buffer.
4. Re-centrifuge the cells at 500 g for 5 minutes at room temperature.
5. Add 100 µl of Annexin V-Dye conjugate/Propidium iodide staining solution to each cell sample of around  $1 \times 10^5$  cells and resuspend the cells in it.
6. Incubate the cells for 15 minutes at room temperature in dark.
7. Centrifuge cell samples at 500 g for 5 minutes.
8. Aspirate supernatant and resuspend cells in 1 X Annexin V-PS Binding Buffer.
9. Place cell suspension on glass slide and cover with coverslip or transfer to dish or chamber slide for imaging.

**NOTE:** *Cells should be analyzed under fluorescent microscope immediately.*

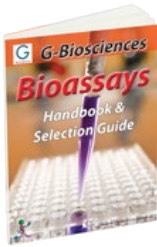
**NOTE:** *Early-stage apoptotic cells are stained by Annexin V -Dye conjugate*

## TROUBLESHOOTING

Issue	Suggested reason	Possible solution
No signal for Annexin V-Dye conjugate	Annexin V -Dye conjugate/Propidium iodide staining solution was not prepared properly	Prepare Annexin V-dye conjugate/Propidium iodide staining solution as given in protocol, protected from light.
	Cells lost during sample processing	Lower drug dose or treatment time
	Low fluorescence intensity of the dye	Increase the concentration of Annexin V-Dye Conjugate [100X] used
Strong staining observed for both Annexin V and propidium iodide including the negative controls	Cells are not healthy	Use healthy cells or decrease the staining time

## RELATED PRODUCTS

Download our Bioassays Handbook.



<http://info2.gbiosciences.com/complete-bioassay-handbook>

For other related products, visit our website at [www.GBiosciences.com](http://www.GBiosciences.com) or contact us.



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