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## **I. DESCRIPTION**

**The Annexin V-EGFP Apoptosis Detection Kit** (Cat. No. L00288) is a new product from GenScript for assaying apoptosis cells. Most of the phosphatidylserines (PS) in cell membrane phospholipids translocate from the inner surface to the outer surface during the early stages of apoptosis. Once the PS are on the outer surface, they can be detected easily by staining then with an enhanced green fluorescent protein (EGFP) fused with annexin V, a protein that has strong natural affinity with PS. In addition, propidium iodide (PI) is a nucleic dye. It can not be through cellular membrane directly, but it can pass through the membranes of cells in middle and late period of apoptosis and stain their nuclei red. Detection can be analyzed by flow cytometry and fluorescence microscopy with a FITC filter. EGFP is brighter and much more photo-stable than other fluorescent reagents, such as FITC.

## **II. KEY FEATURES**

- **Simplified Procedure:** This kit is precise and easy to use.
- **High Precision:** This kit can detect cells in the early period of apoptosis and accurately differentiate and compute the ratio of normal cells, apoptosis cells, and necrotic cells.
- **Enhanced Stability:** EGFP has strong signals that are hard to quench.

## **III. KIT CONTENTS**

**The Annexin V-EGFP Apoptosis Detection Kit** (L00288) is designed for use with enhanced green fluorescent protein (EGFP) fused with annexin V and propidium iodide. Each kit contains enough reagents for a hundred apoptosis assays.

Components	Cat. No. L00288 (100 Assays)
Annexin V-EGFP	500 µl
Binding Buffer	25 mlx2
Propidium Iodide (PI)	500 µl



#### IV. STORAGE

Store the kit at 4°C. Annexin V-EGFP and propidium iodide must be protected from light. The kit will remain stable for one year.

#### V. Annexin V-EGFP Apoptosis Detection Kit PROTOCOL

##### A. Incubation of suspension cells with Annexin V-EGFP

1. Induce apoptosis by any desired method.
2. Collect  $\approx 5 \times 10^5$  suspension cells by centrifugation at 2000 rpm for five minutes, or collect adherent cells by using trypsin without EDTA.
3. Wash cells twice with PBS (centrifugation at 2000 rpm for five minutes).
4. Resuspend cells in 500  $\mu$ l Binding Buffer.
5. Add 5  $\mu$ l of annexin V-EGFP and 5  $\mu$ l of propidium iodide (PI), and mix, respectively.
6. Incubate at room temperature for 5 to 15 minutes, away from light. Depending on your method of analysis, proceed to either section C or D.

##### B. Incubation of Adherent Cells with Annexin V-EGFP

For adherent cells, there are two methods.

###### a. Digestion

1. Collect  $\approx 5 \times 10^5$  adherent cells by using trypsin without EDTA. After the cells are digested, keep them in the culture medium with serum to prevent overdigestion.
2. Repeat steps A3-A5.

###### b. Direct Observation

1. Culture cells on a coverslip and induce apoptosis directly with the proper inducer. Use a negative control.
2. Rinse samples twice with PBS.
3. Add 5  $\mu$ l annexin V-EGFP and 5  $\mu$ l propidium iodide to 500  $\mu$ l Binding Buffer and mix.
4. Evenly apply the abovementioned reagent to the coverslip.
5. Incubate wet, protected from light, for five minutes.

##### C. Detection by Fluorescence Microscopy

1. Place the stained cells from step A.5 or step Bb5 on a glass slide. Cover the cells with a glass coverslip.
2. Observe the cells under a fluorescence microscope using a dual filter for FITC and rhodamine. Cells that have been bound by annexin V-EGFP will appear to have green plasma membranes. Cells that have lost membrane integrity will appear to have red (PI) throughout their nuclei and a halo of green (EGFP) on the cell surfaces (plasma membranes).

##### D. Quantification by Flow Cytometry

Analyze annexin V-EGFP binding by flow cytometry (Ex =488 nm; Em =530 nm) using an FITC signal detector (usually FL1) and PI staining by the phycoerythrin emission signal detector (usually FL2).

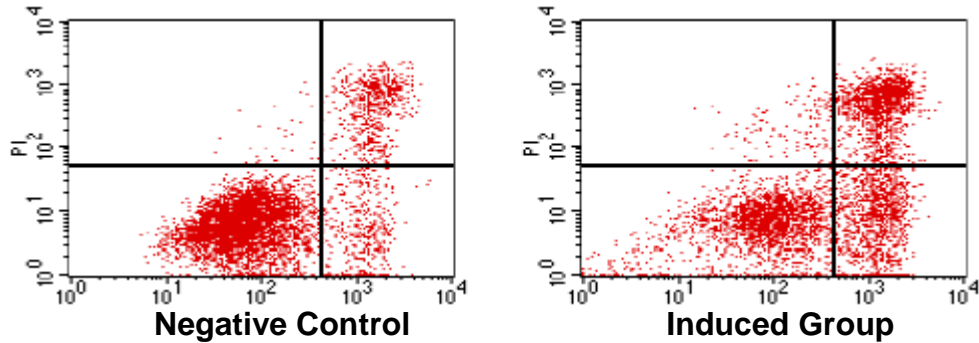
Fluorescence Balance: Use normal cells as the control. Balance fluorescence to reduce spectrum overlapping and set the orientation of the system.



## VI. Examples

P388 cells were induced apoptosis by H<sub>2</sub>O<sub>2</sub>, and the detections were performed in accordance with the procedures described above. The results are as follows:

### Observation by Flow Cytometry



## VII. Ordering Information

Annexin V-EGFP Apoptosis Detection Kit, Cat. No. L00288

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