

## Annexin V-EGFP/7-AAD Apoptosis Kit

**Catalog No.:** K070

**Size:** 20T/50T/100T

### Kit Summary:

Detection method- Flow cytometry (Ex = 488 nm; Em = 530 nm) and fluorescence microscopy

Sample type- Living cells (suspension and adherent)

Species reactivity- Mammalian

Applications- Detect early/middle stages of apoptosis; differentiate apoptosis from necrosis.

### Kit components:

Reagents	20T	50T	100T
Annexin V-EGFP	100µL	250µL	500µL
1X Binding Buffer	10mL	25mL	50mL
7-AAD	100µL	250µL	500µL

### Storage:

2-8°C for 12 months. Annexin V-EGFP and 7-AAD need to be stored away from light.

**Shipping Conditions:** ice pack

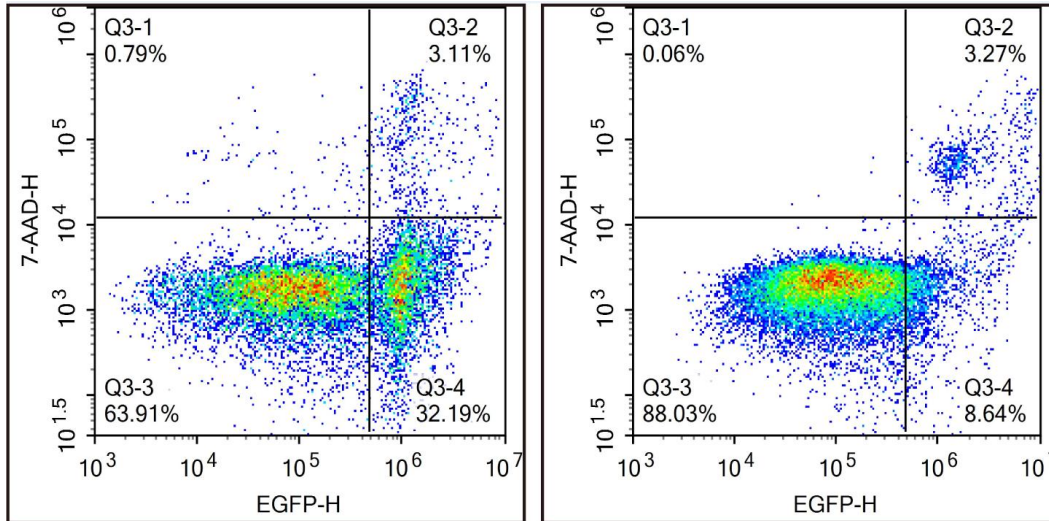
### Description:

Annexin V-EGFP/7-AAD apoptosis detection kit can be used to detect apoptosis in suspension cells and adherent cells.

Annexin V is a calcium-dependent phosphatidylserine binding protein with a high affinity for phosphatidylserine PS. Annexin V labelled EGFP can bind to the membrane of early apoptotic cells by means of the PS exposed outside the cells. Apoptosis can be detected by flow cytometry or fluorescence microscopy.

7-AAD binds specifically to double-stranded DNA and produces strong fluorescence, which is normally unable to penetrate cell membranes. Due to late apoptotic or necrotic cell membrane loss of integrity, 7-AAD can enter cells to stain DNA and, when used in combination with Annexin V, distinguish cells at different stages of apoptosis.

The following figure shows the apoptosis effect of Jurkat cells induced by camptothecin detected by this kit.



Jurkat cells were treated with 5 $\mu$ M Camptothecin (left) or untreated (right) for 4 h. After staining with this kit, fluorescence detection was performed by flow cytometry. Annexin V-EGFP monopositive cells are early apoptotic cells, Annexin V-EGFP and 7-AAD double positive cells are necrotic or late apoptotic cells, and 7-AAD monopositive cells are nude cells.

### Annexin V-EGFP/7-AAD Assay Protocol:

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

1. Induce apoptosis by desired method. Centrifuge at 300 g for 5 min, discard the supernatant, collect the cells, gently suspend the cells with PBS and count them.
2. Collect 1-5 x 10<sup>5</sup> cells by centrifugation, and the supernatant was discarded. The the cells were washed with PBS once, the supernatant was abandoned after centrifugation.
3. Resuspend cells in 500  $\mu$ l of 1X Binding Buffer.
4. Add 5  $\mu$ l of Annexin V-EGFP and 5  $\mu$ l of 7-ADD.
5. Incubate at room temperature for 15 min in the dark.

Proceed to B or C below depending on method of analysis.

#### B. Quantification by Flow Cytometry

Analyze Annexin V-EGFP binding by flow cytometry (Ex = 488 nm; Em = 530 nm) using EGFP signal detector (usually FL1) and 7-AAD staining by the PerCP detector (usually FL3). For adherent cells, gently trypsinize and wash cells once with serum-containing media before incubation with Annexin V-EGFP (A.3-5).

### C. Detection by Fluorescence Microscopy

Place the cell suspension from Step A.5 on a glass slide. Cover the cells with a glass coverslip.

For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (A.5), invert coverslip on glass slide and visualize cells.

#### Note:

1. It should be detected as soon as possible after staining. Too long a time may lead to an increase in the number of apoptotic or necrotic cells.
2. When detecting adherent cells, the suspension cells generated after inducing apoptosis should be collected and detected together with the adherent cells collected later.
3. Mechanical damage caused by digestive adherent cells should be avoided as much as possible. At the same time, the digestive juices of pancreatic enzymes should be as free of EDTA as possible, as EDTA affects Annexin V binding to phosphatidylserine.
4. If EDTA-containing pancreatic enzymes are used, the cells should be thoroughly washed after collection to ensure that EDTA is removed.
5. Fluorescent substances are prone to quenching, in the fluorescence observation, as far as possible to shorten the observation time, while in the operation and storage process also try to pay attention to the preservation of light.
6. For your safety and health, please wear a lab coat and disposable gloves.
7. This kit is for scientific research only.