Caspase 3/7 and Annexin V Double Staining Apoptosis Kit

Cat. No: E-CK-A831 **Size: 20 Assays/100 Assays**

Cat.	Products	20 Assays	100 Assays	Storage
E-CK-A483	Caspase 3/7 Substrates (Green) (1mM)	20 μL	100 μL	2~8℃, shading light
E-CK-A117	Annexin V-APC Reagent	100 μL	500 μL	2~8℃, shading light
E-CK-A151	Annexin V Binding Buffer (10×)	1.4 mL×2	11 mL	2~8℃
	Manual		One Copy	

Storage

The Caspase 3/7 Substrates (Green) and Annexin V-APC Reagent can be stored for 1 year in the dark at 2~8℃, The Annexin V Binding Buffer (10×) can be stored for 1 year at 2~8℃. The Annexin V-APC Reagent should avoid repeated freeze / thaw cycles.

Introduction

Elabscience® Caspase 3/7 and Annexin V Double Staining Apoptosis Kit is used to detect caspase 3/7 enzyme activity and apoptosis in suspension and adherent cells.

The Caspase 3/7 Substrates (Green) are based on novel fluorogenic DNA dyes that have been coupled to the caspase 3/7 recognition sequence (DEVD), which is both non-fluorescent and nonfunctional as a DNA dye. When it rapidly crosses cell membranes to enter the cytoplasm and cleaved by caspase 3/7 to form a high-affinity DNA dye that stains the nucleus bright green. Thus, the Caspase 3/7 substrates allow detection of caspase 3/7 activity and visualization of apoptotic nuclear morphology simultaneously.

Annexin V is a member of the annexin family, which binds to phosphatidylserine (PS) in a calcium-dependent manner. Annexin V-APC, the APC-conjugated format, binds specifically to the PS on the outer leaflet apoptotic cell membrane and can be detected with flow cytometry or fluorescence microscopy.

Elabscience® Caspase 3/7 and Annexin V Double Staining Apoptosis Kit includes Caspase 3/7 Substrates (Green) and Annexin V-APC Reagent, enables the user to detect two important apoptosis events, caspase 3/7 activity and phosphatidylserine (PS) translocation.

Detection Sample Types

☑ **Suspension Cells** ☑ **Adherent Cells**

Materials Not Supplied

1) **Reagents**

PBS, cell culture medium, fetal bovine serum.

2) **Instruments**

Flow cytometer, fluorescence microscopy, centrifuge.

3) **Materials**

Petri dish, centrifuge tubes, pipette.

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Reagent preparation

1) Bring caspase 3/7 Substrates (Green) to room temperature in advance and mix fully and centrifuge before use.
2) 1×Annexin V Binding Buffer: Dilute Annexin V Binding Buffer (10×) with deionized water to 1× Annexin V Binding Buffer before use.

Experimental Protocol

➢ **For flow cytometry**

（1） Collect the adherent or suspension cells and count the cells, take 1~5×10^5 cells, centrifuge at 250×g for 5 min, discard the supernatant.

（2） Add 1 mL PBS to resuspend the cells, centrifuge at 250×g for 5 min, discard the supernatant.

（3） Add 200 μL of 1×Annexin V Binding Buffer to resuspend the cells, add 1μL of Caspase 3/7 Substrates (Green) and 2μL Annexin V-APC Reagent and immediately mix fully.

（4） Incubate cells at 37℃ for 20~30 min with shading light.

（5） After incubation, cells can be analyzed directly by flow cytometry. Measure fluorescence in the FITC channel (Caspase 3/7,excitation/emission:490/535 nm) and APC channel (Annexin V, excitation/emission: 650/660 nm) .

Note: The stained cells should be carefully protected from light, placed at 4°C or ice bath, and conduct flow cytometry detection within 1 hour. Otherwise, it may lead to a decrease in cell viability, resulting in false positive results.

➢ **For fluorescence microscopy**

（1） Carefully aspirate the medium from adherent cells. Wash the cells with PBS and aspirate the PBS.

（2） Prepare the Working Solution according to the number of samples. Please refer to the table below (100 μL Working Solution per well for 96-well plates or 200 μL per well for 24-well plates)

Component	1×Annexin V Binding Buffer	Caspase 3/7 Substrate (Green) (1 mM)	Annexin V-APC Reagent
Working Solution (200 μL)	200 μL	1 μL	2 μL
Working Solution (1 mL)	1000 μL	5 μL	10 μL
Working Solution (2 mL)	2000 μL	10 μL	20 μL

（3） Slowly add the Working Solution to the wall, gently shake the plate to fully infiltrate the cells with the working solution and incubate for 20~30 min at 37℃ with shading light.

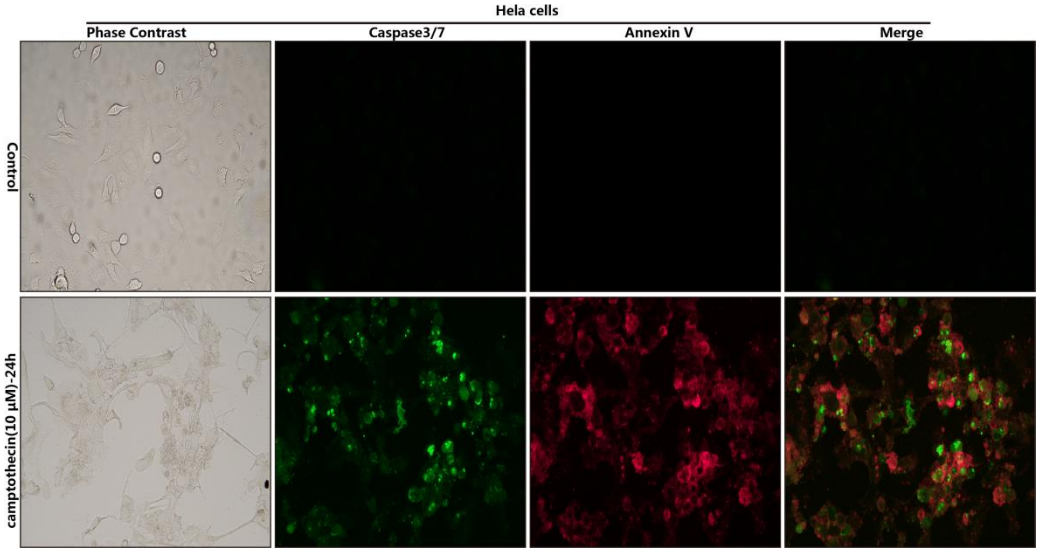
（4） After incubation, cells can be observed directly by fluorescence microscopy using FITC filter set (Caspase 3/7, excitation/emission: 490/535 nm) and Cy5 filter set (Annexin V, excitation/emission: 650/660 nm).

（5） For resuspend cells, add 1 μL of Caspase 3/7 Substrates (Green) and 2 μL Annexin V-APC to 200 μL cells (1~5×105) and immediately mix fully, incubate at 37℃ for 20~30 min, then centrifuge at 250×g for 5 min, aspirate part of the supernatant, and leave about 10~20 μL of final volume, gently mix the cells, then add the cell suspensions on the slides, cover with a coverglass and observe the cells by fluorescence microscopy using FITC filter set and Cy5

filter set.

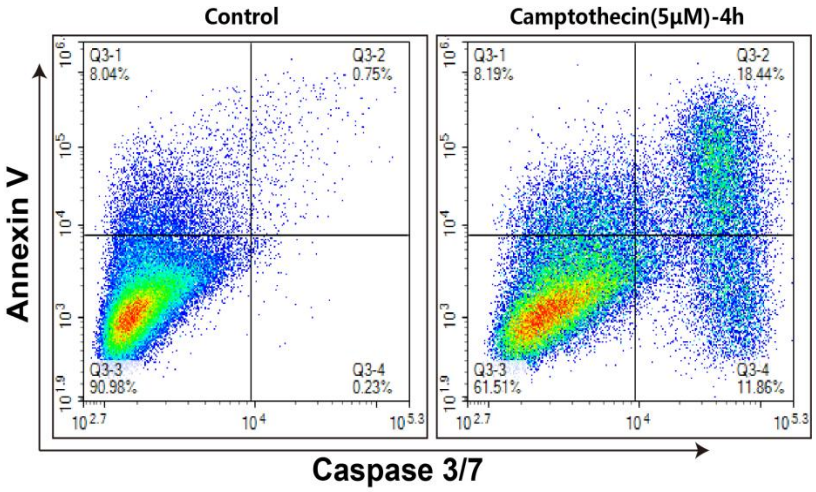
Note: The stained cells should be carefully protected from light, placed at 4°C or ice bath, and conduct flow cytometry detection within 1 hour. Otherwise, it may lead to a decrease in cell viability, resulting in false positive results.

Typical Results



Control: Normal Hela cells were not treated with camptothecin.

Camptothecin (10 μM)-24h: Hela cells were treated with 10 μM camptothecin for 24h.



MOLT-4 cells were cultured without (Left) or with (Right) 5 μM Camptothecin for 4 h. Annexin V-APC single-positive cells (Q3-1) were early apoptotic cells with phosphatidylserine translocation. Annexin V and Caspase 3/7 double-positive cells (Q3-2) were apoptotic cells with higher Caspase 3/7 activity in early to mid-late Stage. Caspase 3/7 single-positive cells (Q3-4) were early apoptotic cells with caspase 3/7 activation.

Cautions

1. This product is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. The Caspase 3/7 Substrates (Green) and Annexin V-APC Reagent co-staining to determine the apoptotic process are suitable for living cells, not applicable to fixed cells.
4. Mechanical damage caused by digestion of adherent cells should be avoided as much as possible. At the same time, trypsin solution should not contain EDTA as much as possible, because EDTA will affect the binding of Annexin V to phosphatidylserine.
5. If trypsin containing EDTA is used, cells should be washed thoroughly after harvesting to ensure that EDTA is removed.
6. Excessive acceleration and deceleration of centrifuge may cause cell loss. It is suggested to adjust the acceleration no more than 3 and deceleration no more than 2, that is, Acc≦3, Dec≦2.

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