Caspase 3/7 and DAPI Double Staining Kit

Cat. No: E-CK-A833 **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-A163	DAPI Reagent (25µg/mL)	250 µL	500 µL	2~8℃/-20℃, shading light
	Manual		One Copy	

Storage

Caspase 3/7 Substrates (Green) and DAPI Reagent (25 µg/mL) can be stored for 1 year in the dark at 2~8℃.

Introduction

Elabscience® Caspase 3/7 and DAPI Double Staining Kit can be used for apoptosis detection 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.

Due to the loss of integrity of membrane, DAPI can enter late apoptotic or necrotic cells to stain DNA. DAPI co-staining of cells with Caspase 3/7 Substrates allows simultaneous differentiation of late-apoptotic cells, necrotic cells, and caspase 3/7 enzyme activity during different apoptotic periods.

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, sterile 1/2/15/50mL centrifuge tubes, pipette.

Reagent preparation

- Bring Caspase 3/7 Substrates (Green) and DAPI Reagent (25µg/mL) to room temperature in advance, and mix fully and centrifuge before use.

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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 PBS to resuspend the cells, add 1 μL of Caspase 3/7 Substrates (Green) and immediately mix fully.

（4） Incubate cells at 37℃ for 20~30 min with shading light. Then add 2 μL DAPI Reagent (25 μg/mL) to 200 μL cells and immediately mix fully, then incubate for 3~5 min at room temperature.

（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 Pacific Blue channel (DAPI, excitation/emission: 360/460 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 Caspase 3/7 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	Cell medium (without FBS)	Caspase 3/7 substrates (green) (1 mM)
Caspase 3/7 Working Solution (200 μL)	200 μL	1 μL
Caspase 3/7 Working Solution (1 mL)	1000 μL	5 μL
Caspase 3/7 Working Solution (2 mL)	2000 μL	10 μL

（3） Slowly add the Caspase 3/7 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） Then add 2 μL DAPI Reagent (25 μg/mL) directly, and mix fully, then incubate for 3~5 min at room temperature with shading light.

（5） After incubation, cells can be observed directly by fluorescence microscopy using FITC filter set (Caspase 3/7, excitation/emission: 490/535 nm) and DAPI filter set (DAPI, excitation/emission: 360/460 nm).

（6） For resuspend cells, add 1 μL of Caspase 3/7 Substrates (Green) to 200 μL cells (1~5×10^5) and immediately mix fully, incubate at 37℃ for 20~30 min, then add 2 μL DAPI Reagent (25 μg/mL) and mix fully, then incubate for 3~5 min at room temperature with shading light. 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

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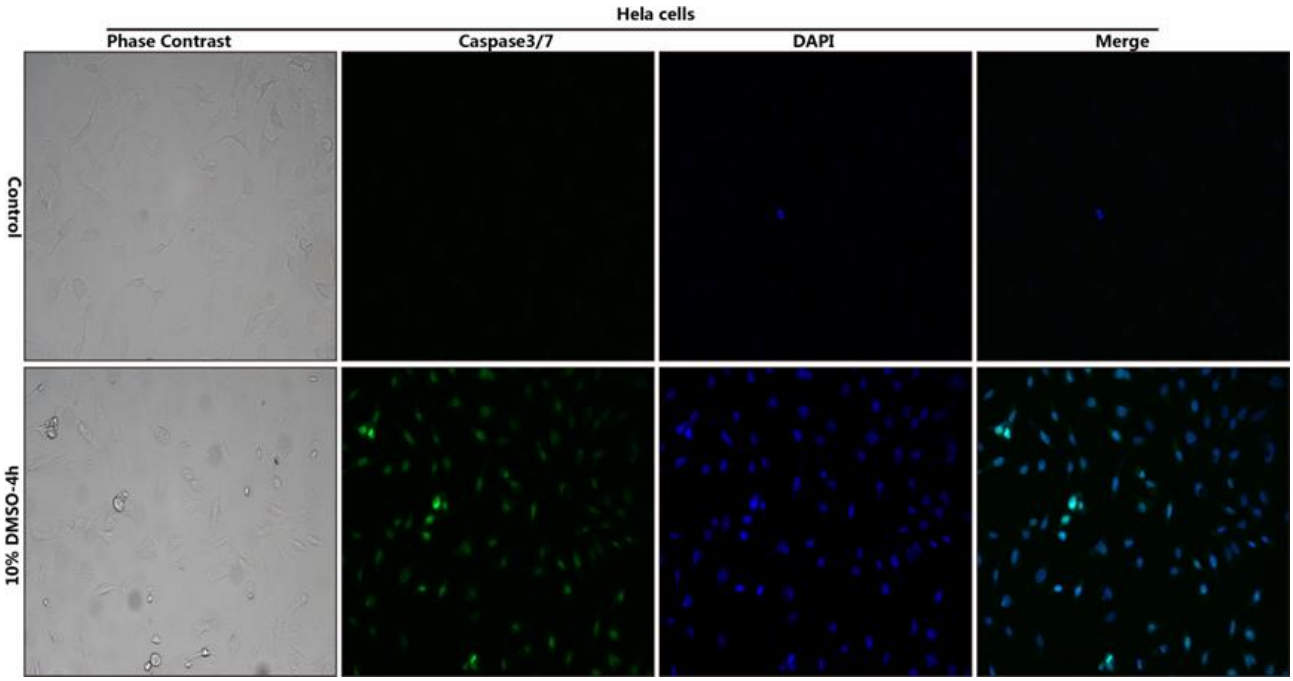
coverglass and observe the cells by fluorescence microscopy using FITC filter set and DAPI filter set.

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 DMSO.

10% DMSO-4h: Hela cells were treated with 10% DMSO for 4h.

HeLa cells stained with Caspase 3/7 Substrates (green) and DAPI Reagent (25 μg/mL) (blue).

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 DAPI Reagent (25 μg/mL) co-staining to determine the apoptotic process are suitable for living cells, not applicable to fixed cells.
4. This product has not been validated for live tissue staining.
5. 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.
6. The staining time of DAPI should be less than 15 min, otherwise it will cause an increase in fluorescence background value. If the subsequent microscope photography time is longer (more than 1 hour), DAPI can be diluted 200-500 times before staining.If the diluted DAPI solution was used, and the staining time can be appropriately extended to 1-2 h. In addition, to avoid high fluorescence background, after DAPI staining, wash with PBS and then observe or take photos under a microscope.

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