Caspase 8 Activity Detection Substrate for Flow Cytometry

Cat. No: E-CK-A488 **Size: 20 Tests/100 Tests**

Cat.	Products	20 Tests	100 Tests	Storage
E-CK-A488	Caspase 8 Substrates(Green) (1mM)	20 μL	100 μL	-20°C, shading light
	Manual		One Copy	

Storage

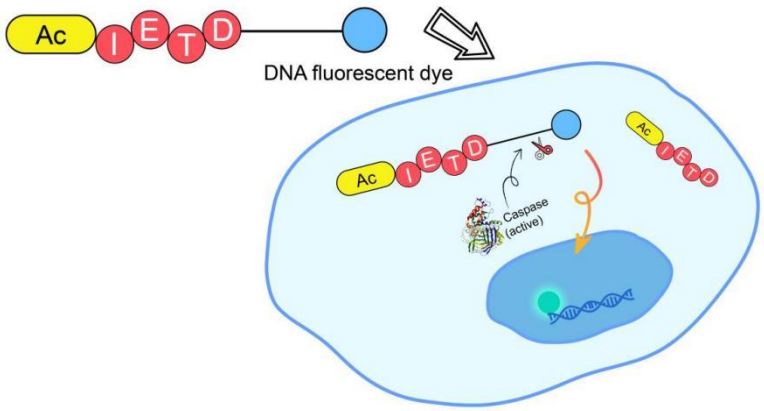
The Caspase 8 Substrates(Green)can be stored for 1 year in the dark at -20℃.

Introduction

Elabscience® Caspase 8 Substrates(Green) are used to monitor apoptosis in multiple cell models using a flow cytometry and fluorescence microscopy, it is a novel fluorogenic substrate with cell membrane permeability designed for the detection of activated caspases 8. The substrates can be used to detect caspase 8 activity in live cells without inhibiting or interfering apoptosis progression. It stains apoptotic cell nuclei with green fluorescence, for detection in the FITC channel in fluorescence microscopy or flow cytometry. Staining is also compatible with subsequent fixation and permeabilization for downstream immunostaining.

Detection Principle

The Caspase 8 Substrates (Green) are a novel fluorogenic dye that couple the caspase 8 recognition sequence (IETD) to a high affinity DNA dye, which have cell membrane permeability and can penetrate the plasma membrane into the cytoplasm. The substrate itself has no fluorescence and has a charge exclusion effect with DNA. During cell apoptosis, caspase-8 cleaves the substrate and releases high affinity DNA dyes, which bind to DNA to produce strong fluorescence, thereby detecting caspase-8 activity and visualizing the morphological changes of the cell nucleus during apoptosis.



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Detection Sample Types

☑ Adherent Cells

☑ Suspension Cells

Materials Not Supplied

1) Reagents

75% ethanol, cell culture medium and sterile PBS buffer

2) Instruments

Centrifuge, CO_2 incubator, inverted fluorescence microscope, flow cytometer, biosafety cabinet

3) Consumable materials

Petri dishes, pipette, 24-well plates, cell crawlers, microscope slides

Experimental Protocol

➢ For flow cytometry

(1) Collect the adherent or suspension cells and count the cells, take 2-5×10^5 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 400 μL of PBS to resuspend the cells, add 0.4 μL Caspase 8 Substrates(Green) and immediately mix fully.

(4) Incubate cells at 37°C for 30 min with shading light.

(5) After incubation, add 1 mL of PBS to resuspend the cells, centrifuge at 250×g for 5 min, discard the supernatant.

(6) Add 200 μL of PBS to resuspend the cells, and analyze by flow cytometry. Measure fluorescence in FITC channel (excitation/emission: 490/535 nm).

➢ For fluorescence microscopy

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

(2) Prepare the Caspase 8 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) / PBS	Caspase 8 Substrates(Green) (1 mM)
Caspase 8 Working Solution (200 μL)	200 μL	1 μL
Caspase 8 Working Solution (1 mL)	1000 μL	5 μL
Caspase 8 Working Solution (2 mL)	2000 μL	10 μL

(3) Slowly add the Caspase 8 Working Solution to the wall, gently shake the plate to fully infiltrate the cells with the working solution and incubate for 30-60min at 37°C with shading light.

(4) After incubation, cells can be observed directly by fluorescence microscopyin using FITC filter sets (excitation/emission: 490/535 nm).

(5) For resuspend cells, add 1 μL of Caspase 8 Substrates(Green) to 200 μL cells(2-5×10^5) and immediately

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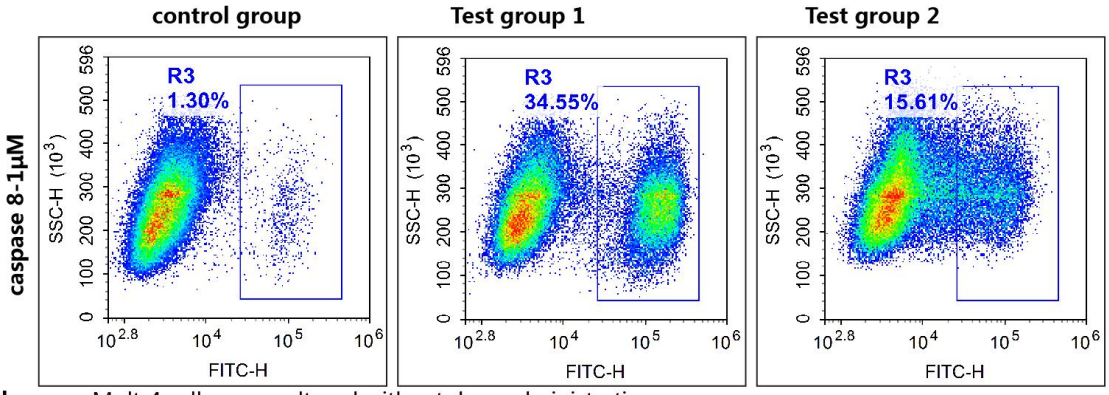
mix fully, incubate at 37°C for 30-60min, then add 1 mL PBS to resuspend the cells and centrifuge at 250×g for 5 min, aspirate part of the supernatant, and leave about 10-20 μL of final volume, gently mix the cells, then add the cell suspensions on the slides, cover with a coverglass and observe the cells by fluorescence microscopy using FITC filter set.

Note: The Caspase 8 Substrates (Green) is compatible with most of the PH-neutral cell medium without aldehyde groups. If the result after staining has a high fluorescence background value, then the working solution can be prepared with PBS and the cells should be washed for 1-3 times after incubation.

The Caspase 8 Substrates (Green) don't inhibit the apoptosis progression in live cells, it can be added at the same time within 1-3h after the addition of apoptosis-inducing reagents, and video-recorded or timed photographs can be taken to observe the real-time dynamic changes of caspase 8 enzyme activity.

Typical Results



control group: Molt-4 cells were cultured without drug administration;
Test group 1: Molt-4 cells were cultured with 5μM camptothecin for 3h;
Test group 2: Molt-4 cells were pretreated with 100 μM Z-VAD-FMK for 1h , and then cultured with 5μM camptothecin for 3h;

Figure 1: Molt-4 cells were stained with Caspase 6 Substrates (Green) and analyzed by flow cytometry.

Cautions

1. This product is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. Caspase 8 Substrates(Green)(1mM) is recommended to aliquot to smaller quantities and use out within 6 months, and avoid repeated freezing and thawing.
4. The staining can be fixed with 2-4% paraformaldehyde for 15-30 minutes at room temperature. Long term fixation may cause the signal to increase.
5. This product detects caspase8 enzyme activity in living cells, but not applicable to fixed cells.
6. This product was not been verified by staining of living tissue, and it can be used for the detection of caspase 8 activity in living cells, but cannot be used for the detection of fixed cells.
7. 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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