Product Information

SuperView™ 488 Caspase-3 substrate, 1 mM in DMSO

Catalog Number: S1005

Product Size: 100 μL

Parameters

SuperView™ 488 Ex/Em: 500/530 nm (with DNA)

Storage

Store at -20℃. Keep dry and away from light. Expiration date marked on the outer packing.

Description

SuperView™ 488 Caspase-3 Substrate based on caspase-3/7 provides a convenient tool for profiling apoptotic cell population based on caspase-3 activity using fluorescence microscopy or flow cytometry.

Compared with other fluorescence substrates or inhibitors of caspase based on FLICA analysis, SuperView™ 488 Caspase-3 Substrate can detect activity of caspase-3/7 without inhibiting the whole cell apoptosis process.

Substrate is made of DNA fluorescence dye coupling the caspase-3/7 DEVD recognition sequence. Substrate is initially non-fluorescent and penetrates the cell membrane into the cell Cytoplasm. In apoptotic cells, caspase-3/7 cleaves Substrate and releases high affinity Harmonic DNA staining, this dye migrates to the nucleus to mark the DNA and emit.

Bright green fluorescence. Therefore, SuperView™ 488 Caspase-3 Substrate is dual-functional, which can not only detect the activity of caspase-3/7, but also visualize the nucleus morphological changes in the progress of apoptosis. SuperView™ 488 staining can be fixed in formaldehyde and compatible with subsequent immunostaining experiments.

SuperView™ 488 Caspase-3 Substrate is dissolved in DMSO or PBS. PBS form can be used for Cells sensitive to DMSO toxicity.Cells are not sensitive to DMSO,Substrate is dissolved in DMSO form can enhance dyeing effect.

Protocol

1. Assay optimization:

SuperView™ 488 substrate can be incubated with cells for extended periods for time course studies. Optimization of cell density, substrate concentration, and inhibitor concentration may be required. Optimal substrate concentration may vary between 1-10 μM. Cell can be incubated with substrate in culture medium or PBS. For adherent cells, we recommend removing medium and replacing with fresh medium containing substrate because high background can result in the area where concentrated substrate is added to the well. Media change or washing after incubation with substrate is optional.

2. Controls:

We recommend that you perform the following controls:

A. Negative control: cells not induced to undergo apoptosis;

B. Positive control: cells induced to undergo apoptosis;

3. For flow cytometry:

(1) Induce apoptosis by desired methods. Untreated cell sample is needed as a control.

(2) For adherent cells, detach cells from culture substrate using trypsin or another cell dissociation method prior to performing the SuperView™ 488 Caspase-3 Assay.

(3) Resuspend cells at a density of 10^6 cells/mL in medium or

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Tel:0512-88965152
Web:www. uelandy.com

buffer.

(4) Pipette 0.2 mL cell suspension into a flow cytometry test tube.

(5) Add 1 μL of 1 mM SuperView™ 488 substrate stock solution to test tube and mix well to obtain a final SuperView® 488 substrate concentration of 5 μM (see Assay Optimization).

(6) Incubate cells at room temperature for 15-30 minutes, protected from light.

(7) Add 300 μL medium or PBS to each tube and analyze by flow cytometry. Measure fluorescence in the green detection channel (excitation/emission: 485/515 nm).

4. For fluorescence microscopy:

(1) Induce apoptosis by desired methods. Untreated cell sample is needed as a control.

(2) Replace medium with fresh medium or PBS containing 5 μM SuperView™ 488 substrate stock solution (see Assay Optimization).

(3) Incubate cells with substrate at room temperature for 30 minutes or longer.

(4) Cells can observed directly in medium containing substrate. For endpoint analysis, wash cells with PBS and observe cells by fluorescence microscopy in PBS using filter sets for green fluorescence (Ex/Em: 485/515 nm).

5. For fluorescence microplate reader:

(1) Grow adherent cells in a black 96-well plate; for suspension cells, adjust density to 10^6 cells/mL and pipette 0.2 mL cell suspension into each well.

(2) Induce apoptosis in cells by suitable methods. Untreated cell sample is needed as a control.

Note: cells may be treated in tubes or flasks and then aliquoted into plate wells for assay.

(3) For suspension cells, add substrate directly to wells and mix well. For adherent cells, replace medium with fresh medium or PBS containing 5 μM SuperView™ 488 substrate (see Assay Optimization). For Ac-DEVD-CHO inhibitor controls, inhibitor should be present during incubation with substrate.

(4) Incubate cells at room temperature for 15-30 minutes, protected from light.

(5) For suspension cells, gently shake plate to resuspend cells. Read fluorescence on a plate reader at settings close to 488 nm excitation and 520 nm emission cut-off. Bottom read is recommended for adherent cells. Inaccurate readings may result from variability in density of adherent cells.

Notes

1. Cells can be counterstained with Hoechst 33342 at a final concentration of 1 μM to stain nucleus with blue fluorescence (Ex/Em: 346/460 nm)

2. SuperView™ 488 staining is formaldehyde-fixable and not compatible with methanol fixation.

3. Formaldehyde-fixed SuperView™ 488-stained cells can be permeabilized with 0.1% Triton X-100 for subsequent immunostaining; however, staining brightness may be diminished after permeabilization and washing.

Q&A

Questions	Answers
How is the stability?	The stability of the substance is very good, and users report that the product is placed at 37°C for 4-5 days, and the effect is still very good.
When is it added to the cell?	The substance can be added to cells in the early and late stages of the experiment. It does not affect the process of apoptosis and can monitor the activity of caspase-3 in real time.

Can it be used for tissue staining?	The company has not carried out live tissue staining experiments, and there are reports in the literature that it can be used for embryonic tissue or three-dimensional cultured cells.
Can it be used for subsequent immunostaining?	Can. It is recommended to fix with 2-4% paraformaldehyde at room temperature for 10-15 min. If the fixation time is too long, the signal will decrease.
How is the specificity?	Similar to other caspase-3 substrates, SuperView™ Caspase-3 Substrate is based on the DEVD caspase-3 consensus sequence that can be cleaved by Caspase-7, other caspases may also cleave due to overlapping substrate-specific sequences DEVD substrate.
Which cells does it apply to?	SuperView™ Caspase-3 Substrate has been reported in a variety of primary cells and immortalized cells in the scientific literature.

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