Product Information

SuperView™ 488 Caspase-3 Assay Kit for Live Cells

Catalog Number: S6007S, S6007L

Product Size: 25T, 100T

Contents:

Component	S6007S (25T)	S6007L (100T)
A. SuperView™ 488 Caspase-3 Substrate, 0.2 mM in DMSO	125 µL	500 µL
B. Caspase-3 inhibitor Ac-DEVD-CHO, 2 mM in DMSO	20 µL	100 µL

Parameters

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

Storage

Store at 4°C. Component A should be protected from light. Expiration date marked on the outer packing.

Description

This kit contains caspase-3 substrate and a caspase-3 inhibitor Ac-DEVD-CHO. It 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 without inhibiting the whole cell apoptosis process.

Unlike conventional caspase assays, SuperView™ 488 Caspase-3 Substrate detects activity of caspase-3 within individual intact cells without inhibiting it. The substrate consists of a fluorogenic DNA dye and a DEVD substrate moiety specific for caspase-3. The substrate, which is both non-fluorescent and nonfunctional as a DNA dye, rapidly crosses cell membranes to enter the cytoplasm, where it is cleaved by caspase-3 to form a high-affinity DNA dye that stains the nucleus bright green. Thus, the SuperView™ 488 Caspase-3 Substrate is bi-functional, allowing detection of intracellular activity of caspase-3 and visualization of changes in nuclear morphology during apoptosis. The fluorescent staining produced in response to activity of caspase-3 is compatible with subsequent fixation and permeabilization for immunostaining.

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:

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

B. Positive control: cells induced to undergo apoptosis;

C. Inhibitor control: cells induced to undergo apoptosis and incubated with caspase-3 inhibitor prior to addition of SuperView™ 488 Caspase-3 Substrate.

3. Ac-DEVD-CHO caspase-3 inhibitor controls:

The caspase-3/7 inhibitor Ac-DEVD-CHO included in the kit can be used to confirm the caspase-3/7 dependence of SuperView™ 488 fluorescence signal. For inhibitor control samples, the final concentration of inhibitor should be at least 2-fold higher than the final substrate concentration (for example, use 10 µM Ac-DEVD-CHO when using 5 µM SuperView™ 488 substrate). Incubate samples with Ac-DEVD-CHO for 15-30 minutes at room temperature before adding substrate, and include inhibitor during incubation with the substrate. Ac-DEVD-CHO is a reversible competitive inhibitor. In some cell types, effective caspase-3/7 inhibition may require the use of an irreversible caspase-3/7 inhibitor such as Z-DEVD-FMK, or may require addition of inhibitor before or during apoptosis induction.

4. 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 buffer.

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

(5) For inhibitor control samples only, treat with Ac-DEVD-CHO (see above).

(6) Add 5 µL of 0.2 mM SuperView™ 488 substrate stock solution to tube and mix well to obtain a final SuperView® 488 substrate concentration of 5 µM (see Assay Optimization).

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

(8) 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).

5. For fluorescence microscopy:

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

(2) For inhibitor control samples only, treat with Ac-DEVD-CHO (see above).

(3) Replace medium with fresh medium or PBS containing 5 µM SuperView™ 488 substrate stock solution (see Assay Optimization). For inhibitor controls, inhibitor should be included during incubation with substrate.

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

(5) 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).

6. 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 desired 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 inhibitor control samples only, treat with Ac-DEVD-CHO(see above).

(4) 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.

(5) Incubate cells at room temperature for 15-30 minutes,

protected from light.

(6) 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.

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