Product Manual

Sulforhodamine multiCaspase activity kit

BML-AK115

100 Tests

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Product Manual

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Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

Enzo Life Sciences' Sulforhodamine multiCaspase activity kit enables researchers to quantitate apoptosis by measuring intracellular caspase activity in vitro. The SR-VAD-FMK reagent is a non-cytotoxic red fluorescent inhibitor that covalently binds with active caspase enzymes.

This kit is a powerful method to assess cell death by detecting apoptosis in vitro. Just add the SR-VAD-FMK reagent directly to the cell culture media, incubate, and wash. This inhibitor is cell-permeant and will efficiently diffuse in and out of all cells. If there is an active caspase enzyme inside the cell, it will covalently bind[2] with the SR-VAD-FMK and retain the red fluorescent signal within the cell. Since non-apoptotic cells lack active caspases, these cells quickly return to their non-fluorescent status. There is no interference from pro-caspases or inactive forms of the enzymes. The SR-VAD-FMK reagent measures the intracellular process of apoptosis instead of a side-effect, such as the turn-over of phosphatidyl serine, and eliminates the incidence of false positives that often plagues both Annexin V and TUNEL staining. If the treatment is causing cell death via apoptosis, apoptotic cells will have an elevated level of caspase activity relative to non-apoptotic cells or negative control cells. Therefore, apoptotic cells will retain a higher concentration of the SR-VAD-FMK reagent and fluoresce brighter than non-apoptotic cells.

Apoptosis, or programmed cell death, is an evolutionarily conserved biochemical mechanism that allows cells to die in a controlled and organized manner. This death process is essential for normal cellular differentiation and tissue homeostasis within multicellular organisms[1]. It is centered on a cascade of proteolytic enzymes called caspases (Cysteinyl-directed Aspartate-Specific ProteASE1) that are triggered in response to pro-apoptotic signals. Once activated, caspases cleave protein substrates leading to the eventual disassembly of the cell[7]. Caspase enzymes play a central role as executioners in the apoptotic cell death process[5] and have been identified in organisms ranging from C. elegans to humans. Mammalian caspases play distinct roles in both apoptosis and inflammation. In apoptosis, effector caspases (-3, -6, and -7) are responsible for proteolytic cleavages that lead to cell disassembly. Initiator caspases (-8, -9, and -10) regulate apoptosis upstream. Caspase-1 is associated with inflammasomes and takes on the role of a key housekeeping enzyme in its conversion of pro-IL-1ß protein into the active IL-1ß cytokine[9, 10, 11].

Like the majority of other proteases, caspases are synthesized as pro-form precursors that undergo proteolytic maturation, either autocatalytically or in a cascade by enzymes with similar

specificity[3]. Active caspase enzymes consist of two large (~20 kDa) and two small (~10 kDa) subunits that non-covalently associate to form a two heterodimer, tetrameric active caspase[4, 10, 11].

Caspase enzymes cleave proteins. They specifically recognize a 3 or 4 amino acid sequence that must include an aspartic acid (D) residue in the P1 position[6]. This C–terminal residue is the target for the cleavage reaction at the carbonyl end[8, 4]. The SR-VAD-FMK probe contains a 3 amino acid sequence that is targeted by different activated caspases. This sequence is sandwiched between a red fluorescent label, sulforhodamine (SR), and a fluoromethyl ketone (FMK) caspase active site reactive group. SR is ideal for intracellular detection of enzymatic activity due to its small size, allowing it to easily cross the cell membrane. Larger red dyes like phycoerythrin are not as cell membrane permeant. A caspase enzyme cannot cleave SR-VAD-FMK; instead, it forms an irreversible covalent bond with the target amino acid sequence and becomes inhibited from further enzymatic activity.

SR-VAD-FMK has been known to detect caspase activity in human, rabbit, rat, mice, drosophila, squid, paramecium, and yeast cell lines, among others. The SR-VAD-FMK reagent can be used to label suspension or adherent cells, and thin tissue sections. After labeling, cells can be fixed or frozen. This inhibitor works well to label tissues that will be paraffin-embedded.

Cells labeled with SR-VAD-FMK can be counter-stained with other reagents such as the vital stain 7-AAD to distinguish apoptosis from necrosis. Nuclear morphology may be concurrently observed using Hoechst, a blue DNA binding dye, which is included. SR-VAD-FMK maximally excites at 565 nm and emits at 590-600 nm. Cells can be viewed directly through a fluorescence microscope, or the fluorescence intensity can be quantified using a fluorescence plate reader or flow cytometer. The best results in flow cytometry are obtained using a laser that can efficiently excite the SR fluorophore such as a yellow 561 nm laser. It is possible to observe meaningful results using an argon laser at 488 nm, although it may only detect 10% of the signal in FL-2.

REAGENTS PROVIDED AND STORAGE

Protect from light

Avoid freeze / thaw cycles

Store the unopened kit and each unopened component at 2-8°C until the expiration date. Once reconstituted with DMSO, use SR-VAD-FMK immediately, or store at ≤-20°C for 6 months protected from light and thawed no more than twice during that time.

Reagent	Cat. No	Quantity
SR-VAD-FMK Reagent, lyophilized	BML-KI164	4 vials
10X Apoptosis Wash Buffer	BML-KI186	60 ml
Fixative	BML-KI187	5 ml
Hoechst Stain	BML-KI189	1 ml

OTHER MATERIALS NEEDED

1. DMSO, 50 µl per vial to reconstitute SR-VAD-FMK
2. Di$H_2$0, 135-540 ml to dilute the wash buffer
3. Phosphate buffered saline (PBS) pH 7.4, up to 100 ml, to dilute SR-VAD-FMK and handle cells
4. Cultured cells or tissues treated with the experimental conditions ready to be labeled
5. Reagents to induce apoptosis and create controls, such as staurosporine (Prod. No. ALX-380-014) or camptothecin (Prod. No. ALX-350-015)
6. Hemocytometer
7. Centrifuge at <400g
8. 15 ml polystyrene centrifuge tubes (1 per sample)
9. DAPI (Prod. No. BML-AP402)
10. Detection equipment: SR-VAD-FMK excites at 550 nm and emits at 590-600 nm. Hoechst stain can be seen using a UV filter with excitation at 365 nm and emission at 480 nm (Section 6). Use filter pairings that best approximate these settings.
    - Fluorescence microscope
    - Fluorescence plate reader
    - Flow cytometer

SAFETY WARNINGS AND PRECAUTIONS

- Use gloves while handling the SR-VAD-FMK reagent and the fixative.
- Dispose of all liquid components down the sink and flush with copious amounts of water. Solid components may be tossed in standard trash bins.

METHODS AND PROCEDURES

Experimental Preparation

Staining apoptotic cells with the SR-VAD-FMK kit can usually be completed within a few hours. However, the SR-VAD-FMK kit is used with living cells, which require periodic maintenance and cultivation several days in advance. In addition, once the proper number of cells has been cultivated, time must be allotted for the experimental treatment or apoptosis induction process which typically requires a 2-6 hour incubation at 37°C based on the cell line and concentration. Create cell populations, such as:

- Cells that were exposed to the experimental condition or treatment
- A placebo population of cells that received a blank treatment instead of the experimental treatment

As SR-VAD-FMK detects caspase-mediated apoptosis, plan the experiment so that SR-VAD-FMK will be diluted and administered at the time when caspases are expected to be activated in the cells. The recommended volume of 30X SR-VAD-FMK is 10 µl per 300 µl of cells at 3-5 x 10^5 cells/ml, but the amount may vary based on the experimental conditions and the instrument used for analysis. Each investigator should adjust the amount of SR-VAD-FMK to accommodate the particular cell line and research conditions.

Controls

At least two sets of controls should be run:

1. Positive control cells induced to undergo apoptosis
2. Negative control cells not induced to undergo apoptosis

The induced positive cell population and negative control cell population tubes should come from a common pool of cells and contain similar quantities of cells. Create negative controls by culturing an equal volume of non-induced cells for every labeling

condition. For example, if labeling with SR-VAD-FMK and Hoechst stain (which is optional), make eight populations:

1&2. Unlabeled: induced and non-induced

3&4. SR-VAD-FMK-labeled: induced and non-induced

5&6. SR-VAD-FMK-labeled and Hoechst-labeled: induced and non-induced

7&8. Hoechst-labeled: induced and non-induced

Apoptosis Induction

The induction of apoptosis varies significantly with each cell line and concentration. Prior to commencing the experiment, determine a reproducible method for obtaining a positive control by triggering caspase activity. For example, apoptosis may be induced with 2-4 µg/ml camptothecin (Prod. No. ALX-350-015) for >4 hours; or 1-2 µM staurosporine (Prod. No. ALX-380-014) for >4 hours.

Preparation of SR-VAD-FMK

SR-VAD-FMK is supplied as a lyophilized powder that may be slightly visible as an iridescent sheen inside the vial. Protect from light and use gloves when handling. Because the 30X SR-VAD-FMK solution must be used immediately, prepare it just before staining.

1. Reconstitute each vial of SR-VAD-FMK with 50 µl DMSO to form the 150X stock. The stock solution should be colorless or pink. Once reconstituted, it may be stored at ≤-20°C for 6 months protected from light and thawed no more than twice during that time.

2. Immediately prior to addition to the samples and controls, dilute SR-VAD-FMK 1:5 by adding 200 µl PBS to each vial to form the 30X SR-VAD-FMK solution. Use 30X SR-VAD-FMK within 30 minutes of dilution into aqueous buffers.

Preparation of 1X Apoptosis Wash Buffer

The Apoptosis Wash Buffer (Prod. No. BML-KI186) is used to dilute SR-VAD-FMK and wash cells. It contains mammalian proteins to stabilize cells stained with SR-VAD-FMK and sodium azide to retard contamination (1X Apoptosis Wash Buffer contains 0.01% w/v sodium azide). Cell media may be used instead of Apoptosis Wash Buffer to wash cells.

1. 10X Apoptosis Wash Buffer may form precipitates during cold storage. If this happens, gently warm it until all crystals have dissolved. Do not boil.

2. Dilute 10X Apoptosis Wash Buffer 1:10 in diH2O. For example, add 15 ml 10X Apoptosis Wash Buffer to 135 ml diH2O for a total of 150 ml. 1X Apoptosis Wash Buffer may be stored at 2-8°C and used within 1 week or frozen and used within 6 months.

Hoechst 33342 Stain

Warning: Hoechst 33342 is a mutagen. It may be irritating to respiratory system and skin. Gloves, protective clothing, and eyewear are strongly recommended. When disposing, flush sink with copious amounts of water; see MSDS for further information.

Hoechst 33342 (Prod. No. BML-KI189) is a cell-permeant nuclear stain that emits blue fluorescence when bound to double stranded DNA. It is used to stain the nuclei of living or fixed cells blue and is often used to distinguish condensed, pyknotic nuclei in apoptotic cells. When bound to nucleic acids, the maximum absorption is 350 nm, and its maximum emission is 480 nm. It is revealed under a microscope using a UV-filter with excitation at 365 nm and emission at 480 nm. Hoechst stain is provided ready-to-use at 200 µg/ml. Hoechst stain can be used with SR-VAD-FMK to label the nuclei of live, dying, and apoptotic cells.

Fixative

Warning: Fixative is toxic: danger exists of very serious irreversible effects through inhalation, contact with skin, or if swallowed. Gloves, protective clothing, and eyewear are strongly recommended. When disposing, flush sink with copious amounts of water; see MSDS for further information.

Fixative (Prod. No. BML-KI187) is a formaldehyde solution designed to cross-link intracellular components. It will not interfere with the sulforhodamine (SR) label, unlike the use of absolute ethanol- or methanol-based fixatives, which will inactivate the SR-VAD-FMK label.

If the stained cell populations cannot be evaluated immediately after labeling with SR-VAD-FMK add fixative at a 1:10 ratio. For example, add 100 µl fixative to 900 µl cells. Never add the fixative until the staining and final wash steps have been completed. Fixed cells may be stored on ice or at 4°C up to 24 hours.

Staining Protocol for Suspension Cells

Prepare experimental cell and control cell populations. Cell concentration should be 3-5 x 10^5 cells/ml and should not exceed 10^6 cells/ml, as cells cultivated in excess of this concentration may begin to naturally enter apoptosis. Just prior to staining with SR-VAD-FMK, cells may need to be concentrated to 2-5 x 10^6 cells/ml as both microscopy and plate reader analysis methods (Sections 9 and 10) require high cell concentrations. Start with a larger volume of cells at 3-5 x 10^5 cells/ml (which is a typical density for cell culture) and then concentrate cells and resuspend to 300 µl when ready for SR-VAD-FMK staining.

1. Expose cells to the experimental and control conditions. At least two sets of controls should be run (refer to Controls section):

    a. Positive control cells induced to undergo apoptosis.
    b. Negative control cells not induced to undergo apoptosis.

If analyzing with a flow cytometer, set aside two populations to create instrument controls:

    a. Induced cells stained with SR-VAD-FMK.
    b. Non-induced cells stained with SR-VAD-FMK.

2. If analyzing with a fluorescence microscope or plate reader, concentrate cells to 2-5 x 10^6 cells/ml just prior to SR-VAD-FMK staining. Fluorescence microscopy requires an excess of 2 x 10^6 cells/ml to obtain 5-20 cells per image field. Flow cytometry can efficiently analzye a 4-5 fold lower concentration of cells (3-5 x 10^5 cells/ml).

3. Transfer 290 µl cells into fresh tubes.

4. Add 10 µl 30X SR-VAD-FMK solution, forming a final volume of 300 µl. If different cell volumes were used, add 30X SR-VAD-FMK at a ratio of 1:30. Mix by slightly flicking the tubes. The amount of SR-VAD-FMK should be optimized for each cell line and experimental condition.

5. Incubate cells at 37°C protected from light. The incubation period may range from 30 minutes to several hours and should be optimized for each cell line and experimental condition. As cells may settle on the bottom of the tubes, gently resuspend them by swirling cells every 20 minutes to ensure an even distribution of SR-VAD-FMK.

6. If cells are to be analyzed with a microscope, cells may be dually stained with Hoechst. Add Hoechst at 0.5% v/v and incubate 5 minutes at 37°C. For example, if the cell suspension is at 300 µl, add 1.5 µl Hoechst.

7. Add 2 ml 1X Apoptosis Wash Buffer and gently mix.

8. Centrifuge at <400 X g for 5 minutes at RT.

9. Carefully remove and discard supernatants. Gently vortex the pellets to disrupt clumping. Resuspend in 1 ml 1X Apoptosis Wash Buffer and gently mix.

10. Centrifuge cells at <400 X g for 5 minutes at RT.

11. Carefully remove and discard supernatants. Gently vortex pellets to disrupt clumping.

Microscopy Analysis of Suspension Cells

Follow Staining Protocol for Suspension Cells, Steps 1-11.

1. Resuspend cells in 300-500 µl 1X Apoptosis Wash Buffer and place on ice. At this point, the cells may be stained with other dyes or fixed for future viewing (Step 2), or observed immediately (Step 3).

2. If not viewing immediately, cells may be fixed for viewing up to 24 hours later. Add fixative at a ratio of 1:10 (30 µl if resuspended in 300 µl).

    a. Incubate 15 minutes at RT in the dark.
    b. Place cells on a microscope slide and let dry.
    c. Briefly wash cells with PBS.
    d. Cover cells with mounting media and coverslip.
    e. Store slides at 2-8°C up to 24 hours.

3. To view cells, place 1 drop of cell suspension on a microscope slide and cover with a coverslip.

4. Observe cells with a fluorescence microscope using a bandpass filter (excitation 550 nm, emission >580 nm) to view red fluorescence. Cells bearing active caspase enzymes covalently coupled to SR-VAD-FMK appear red. Hoechst stain can be seen using a UV-filter with excitation at 365 nm and emission at 480 nm.

Fluorescence Plate Reader Analysis of Suspension Cells

Follow Staining Protocol for Suspension Cells, Steps 1-11, but omit Hoechst staining in Step 6.

1. Resuspend cells in 500 µl PBS.

2. Determine the concentration and compare the cell density of each sample. The non-induced population may have more cells than the induced population, as some apoptotic cells in the induced samples may be lost during the wash steps. Adjust the volume of the cell suspensions to equalize the cell density. When ready to read, cells should be >3 x 10^6 cells/ml.

3. Pipette 100 µl per well into a black microtiter plate. Do not use clear plates. Analyze at least 2 aliquots per sample. Avoid bubbles.

4. Perform an endpoint read. Set the excitation wavelength at 550 nm and the emission wavelength to 595 nm; use a cut-off filter of 570 nm. SR-VAD-FMK has an optimal excitation range of 550 nm and emission range of 590-600 nm

Flow Cytometry Analysis

Sulforhodamine B is ideal for intracellular detection of enzymatic activity due to its small size, allowing it to easily cross the cell membrane, unlike larger red dyes such as phycoerythrin, which are not as cell membrane permeant.

SR- works well on flow cytometry if excited optimally. Sulforhodamine B is maximally excited at 565 nm and emits between 590-600 nm. Flow cytometry is best performed using a laser that can efficiently excite the fluorophore (e.g. a yellow-green 561 nm laser). Use of a traditional argon 488 laser is not recommended. The blue light (488 nm) excitation wavelength falls well below the optimal sulforhodamine B excitation curve. It may still be possible to observe meaningful results if the sulforhodamine B-conjugated reagent is the only dye in the assay. Use the emission filter associated with the FL-2 channel usually used for phycoerythrin.

Greater issues arise when applying compensation for another fluorophore (for example, green fluorescein). Because the sulforhodamine B signal is not optimized with a 488 nm excitation, the red signal can easily be overwhelmed by a very strong green signal. Apply appropriate compensation using control samples stained with only one color. Ensuring that the positive control sample (e.g. induction is maximized to fully express caspases) is optimally stained with SR-VAD-FMK will also help to detect both colors simultaneously. However, depending on the strength of the

signal being emitted from the other dye, it may still be difficult to attribute the appropriate fraction of the signal to the sulforhodamine B SR-VAD-FMK probe. For example, fluorescein-conjugated antibodies that detect a cell surface marker present in very high copy number (>100,000) may produce a green signal that is too strong to allow the concurrent detection of the orange-red SR-VAD-FMK reagent bound to intracellular proteases present at a significantly lower level.

Because of its orange-red profile, SR-VAD-FMK can be measured in flow cytometry with the red vital stain, 7-AAD, to detect necrosis concurrently. Careful gating of SR-VAD-FMK and 7-AAD fluorophores (using the FL-2 and FL-3 channels respectively) will distinguish the red 7-AAD live/dead fluorescence signal and the orange-red SR-VAD-FMK caspase-specific signal
within a each sample. This will reveal four populations of cells:

- Live unstained cells do not fluoresce.
- Early apoptotic cells fluoresce orange-red with SR-VAD-FMK
- Late apoptotic cells are dually stained with SR-VAD-FMK and 7-AAD: they fluoresce orange-red (they have active caspases) and red (the cell membrane has permeabilized).
- Necrotic cells fluoresce red with 7-AAD

Follow Staining Protocol for Suspension Cells, Steps 1-11, but omit Hoechst staining in Step 6.

1. Resuspend cells in 300 µl 1X Apoptosis Wash Buffer and place on ice.
2. For single-color analysis, an optimal laser consists of a yellow diode pumped solid state (DPSS) 561 nm laser or a DPSS 532 nm green laser. Measure sulforhodamine B SR-VAD-FMK in the FL-2 channel. If a histogram is generated with the log FL-2 on the X-axis versus the number of cells on the Y-axis, caspase-negative (SR-VAD-FMK-) cells will occur in the lower log fluorescence output decades of the FL-1 (X) axis, whereas caspase-positive (SR-VAD-FMK+) cells will appear as a shoulder on the right side (brighter) or separate peak on the right side of the negative peak histogram. It is possible to observe meaningful results using an argon laser at 488 nm, although it may only detect 10% of the signal in FL-2.

Staining Protocol for Adherent Cells

Adherent cells need to be carefully washed to avoid the loss of any cells that round up and come off the plate surface. Loose cells may be harvested from the plate or slide surface and treated as suspension cells, while those remaining adherent to the surface should be washed as adherent cells. If the adherent cells are trypsinized, the loose cells can be recombined with the trypsinzed pool; alternatively, the loose cells can be recombined with the adherent portion when the analysis is performed. If growing adherent cells in a plate, the entire plate may be gently spun as part of the wash process to sediment any loose floating cells. Avoid trypsinizing cells prior to labeling with a vital dye, like 7-AAD. Cell membranes exposed to trypsin could be transiently permeable to vital dyes for a variable time depending upon the cell line. Cells may be labeled with SR-VAD-FMK before or after trypsinization.

1. Culture cells in T25 flasks and expose to the experimental conditions.
2. Apoptotic cells may detach and begin to float into the media. Save and spin to pellet and include these cells in the analysis.
3. Trypsinize adherent cells; neutralize with trypsin inhibitor present in cell culture media with 20% FBS; pool cells with any pellets created in Step 2; add 2-5 ml media.
4. Centrifuge at 200 x g for 5 minutes.
5. Remove all but ~100 µl supernatant. Resuspend cells in 300-500 µl in cell culture media containing 10-20% FBS.
6. If necessary, count cells and adjust the volume of cell suspension to fi t the experiment. Transfer cells into a 15 ml tube.
7. Add 30X SR-VAD-FMK at 1:30.
8. Incubate 30-60 minutes at 37°C, mixing gently every 10 minutes.
9. Wash by adding 2 ml 1X Apoptosis Wash Buffer.
10. Centrifuge at 200 x g for 5 minutes.
11. Aspirate supernatant and resuspend cells in 2 ml 1X Apoptosis Wash Buffer. Incubate 10 minutes at 37°C to allow any unbound SR-VAD-FMK to diffuse out of the cells.
12. Centrifuge at 200 x g for 5 minutes.
13. Aspirate supernatant and resuspend cells in 1X Apoptosis Wash Buffer. Store cells on ice and protect from light; read

within 4 hours. At this point, cells may be analyzed with a fluorescence microscope, flow cytometer, or plate reader.

a. If analyzing via flow cytometry, cells do not need to be counted. Refer to the Flow Cytometry Analysis section.
b. If analyzing with a fluorescence plate reader, refer to Fluorescence Plat Reader Analysis of Adherent Cells section.

Fluorescence Plate Reader Analysis of Adherent Cells

1. Adherent cells can be grown in a microtiter plate. Use plates with clear bottoms and black walls. Culture cells to approximately 90% confluency.
2. Add 30X SR-VAD-FMK at 1:30.
3. Incubate 30-60 minutes at 37°C; mix gently every 10 minutes.
4. Wash by adding ~400 µl media to each well and incubate 60 minutes at 37°C to allow any unbound SR-VAD-FMK to diffuse out of the cells.
5. Gently centrifuge the entire plate to sediment any loose floating cells.
6. Aspirate the media. Resuspend with fresh media or PBS.
7. Read plates using a bottom-reading instrument.

Staining Protocol for Tissue Sections

1. Prepare frozen tissues according to the experiment.
2. Dry slides and fix with acetone for 1 minute.
3. Rehydrate slides by washing in TBS-Tween (TBSt) or PBS-Tween (PBSt) for 5 minutes.
4. Wash again in TBSt or PBSt for 5 minutes.
5. Block slides for 20 minutes. Use a blocker such as 20% Aquablock in media with 0.2% Tween.
6. Reconstitute each vial of SR-VAD-FMK with 50 µl DMSO to form the stock solution.
7. Dilute the SR-VAD-FMK stock 1:50 in PBS to form the working solution. For example, add 50 µl stock to 2,450 µl PBS (2.5 ml total).
8. Add 50 µl of the working solution and incubate >1 hour protected from light.
9. Wash in TBSt or PBSt for 5 minutes.

10. Wash again in TBSt or PBSt for 5 minutes.
11. Set slides in a dish containing 1X Apoptosis Wash Buffer.
12. If desired, stain nuclei with DAPI (catalog #6244) and apply a coverslip.
13. Store samples at 2-8°C for short-term storage or -20°C for long-term storage. Add the 30X SR-VAD-FMK solution to the medium at a 1:30 ratio. For example, add 10 µl 30X SR-VAD-FMK to 290 – 300 µl medium.

REFERENCES

1. Arends, M.J. and A.H. Wyllie. Apoptosis: Mechanisms and roles in pathology. Int. Rev. Exp. Pathol. 32:223-254 (1991).
2. Ekert, P. G., et al. Caspase inhibitors. Cell Death Differ. 6:1081-1086 (1999).
3. Kumar, S. Mechanisms mediating caspase activation in cell death. Cell Death Differ. 6:1060-1066 (1999).
4. Rotonda, J., et al. The three-dimensional structure of apopain/CPP32, a key mediator of apoptosis. Nature Struct. Biol. 3(7):619-625 (1996).
5. Salvesen, G.S., and V.M. Dixit. Caspases: intracellular signaling by proteolysis. Cell. 91:443-446 (1997).
6. Sleath, P.R., et al. Substrate specificity of the protease that processes human interleukin-1B. J. Biol Chem. 265:14526-14528 (1990).
7. Slee, E. A., et al. Serial Killers: ordering caspase activation events in apoptosis. Cell Death Differ. 6:1067-1074 (1999).
8. Thornberry, N. A., et al. A combinatorial approach defines specificities of members of the caspase family and granzyme B. Functional relationships established for key mediators of apoptosis. J Biol Chem. 272(29): 17907-17911 (1997).
9. Thornberry, N.A. et al. A novel heterodimeric cysteine protease is required for interleukin-1B processing in monocytes. Nature. 356:768-774 (1992).
10. Walker, N. P., et al. Crystal structure of the cysteine protease interleukin-1ß-converting enzyme: a (p20/p10)2 homodimer. Cell. 78:343-352 (1994).
11. Wilson, K. P., et al. Structure and mechanism of interleukin-1 beta converting enzyme. Nature. 370:270-275 (1994).

NOTES

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