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Caspase-3 Assay Kit

Catalog No: E-BC-K202
Method: Colorimetric method
Specification: 20T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

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Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Application

This kit can be used to measure the caspase-3 activity in tissue and cell samples.

Detection significance

Caspases (Cysteine-requiring Aspartate protease) is a protease family that plays an important role in the process of apoptosis. Caspase-3 (also known as CPP32, Yama, or apopain) is a member of the CED-3 subfamily of caspases and is one of the critical enzymes of apoptosis. Caspase-3 is the most studied caspase in mammalian cells. Caspase-3 can be used to cut procaspase2, 6, 7, 9, and cut specifically many substrates of caspase directly, including poly(ADP-ribose) polymerase (PARP), the inhibitor of caspase-activated deoxyribonuclease (ICAD), gelsolin and fodrin. Protein shearing mediated by caspase-3 are important component of the molecular mechanism of apoptosis. In addition, caspase-3 also play a key role in the process of nuclear apoptosis including chromatin condensation and DNA fragmentation as well as cell blebbing. Caspase-3 exists in the form of prozyme in the normal state and has no activity. However, during the apoptosis stage, activated caspase-3 is consisted by two large subunits and two small subunits, which cleavage the corresponding substrate of endochylema or cytoplasmic nuclear and eventually lead to apoptosis.

Detection principle

This kit is used to conjugate caspase-3 sequence-specific peptides acetyl-Asp-Glu-Val-Asp p-nitroanilide (Ac-DEVD-pNA) to yellow groups p-nitroaniline (pNA). When the substrate is cut by caspase-3, the yellow group pNA is dissociated. pNA has an absorption peak at 405nm. Measure the OD value at 405 nm and then calculate the caspase-3 activity indirectly.

Kit Components

	Components	**Specifications**	**Storage**
Reagent 1	Lysis Buffer	8 mL ×1 vial	-20℃
Reagent 2	Reaction Buffer	8 mL ×1 vial	-20℃
The Preparation of standard diluent: mix the reagent 1 and reagent 2 at the ratio of 1:9 fully.			
Reagent 3	2 mM Ac- DEVD-pNA	200 μL ×1 vial	-20℃, shading light
Avoid repeated freezing and thawing. It is recommended to aliquot the Reagent 3 into smaller quantities and store at -20℃.			
Reagent 4	10 mM pNA	200 μL ×1 vial	-20℃, shading light

Experiments instruments

High Speed Centrifuge (with low temperature)

Micropipettor

Spectrophotometer (with 100 μL of cuvette) or Microplate Reader

Operation steps

1. Reagent preparation

(1) Mix dissolved lysis buffer fully and put on ice for use.

(2) Mix the dissolved Reaction Buffer fully and put on ice for use.

2. Preparation of standard curve

(1) Dilute **10 mM pNA** with **standard diluent** to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 50, 100, 200 μM.

(2) Measure the OD value of standards with different concentrations at 405 nm with spectrophotometer (100 μL cuvette) or microplate Reader.

3. Preparation of sample

Cells:

(1) Adherent cells should be detached with trypsin and then collected sedimentary cells by centrifugation. (Suspension cell can be collected sediment by centrifugation directly). Centrifuge for 5 min at 600 g, discard supernatant.

(2) Resuspend adherent cells in 1 mL cold PBS, centrifuge for 5 min at 600 g, discard supernatant.

(3) Resuspend cells in Lysis Buffer (add lysis buffer into the cell sediment according to the ratio of Cells number (2×10^6): lysis buffer (μL) =1: 100), then incubate in ice bath for 15 min, and oscillate 3~4 times during incubation.

Tissue:

Add lysis buffer into the cell sediment according to the ratio of weight (mg): lysis buffer (μL) =3~10: 100, then homogenize the sample on ice. Transfer the tissue homogenate to a 1.5 mL centrifuge tube, then incubate in ice bath for 5 min.

Note: Because the activity of caspase-3 is related to the protein content of cell lysis, the protein content can be increased by increasing cell number and tissue quality if OD value is low. (It is recommended that a pre-experiment be carried out before the formal experiment to determine the optimal cell number and tissue quality.)

4. Centrifuge at 16000-20000 for 10~15 min at 4℃.

5. Take the supernatant to a new pre-cool tube, put it on ice for test.

6. Carry out the assay immediately or store the samples at -70℃. Meanwhile, determine the concentration of protein with CBB (Coomassie brilliant blue) method.

7. Detection of Caspase-3 activity

(1) Bring appropriate Ac-DEVD-pNA, put on ice for assay.

(2) Operation table

	Blank tube	Sample tube
Reaction Buffer (μL)	40 μL	40 μL
Sample (μL)		50 μL
Lysis buffer (μL)	50 μL	
2 mM Ac- DEVD-pNA (μL)	10 μL	10 μL

(3) Mix fully and avoid to generate bubble. Incubate at 37℃ for 60-120 min. Measure the OD

value (A405) at 405 nm with spectrophotometer (100 μL cuvette) or microplate Reader when the color changes obviously. Note: the reaction time can be extended or stay overnight if the color doesn't change significantly.

Calculation of results

Plot the standard curve by using OD value of standard and corresponding concentration as y-axis and x-axis, respectively. The concentration of the sample is calculated by the standard curve based on the OD value of sample. Tissue and cell samples need to be divided by protein concentration in the calculation, and finally expressed as mM/g protein. The diluted samples need to be multiplied by dilution times in the calculation.

Definition: a unit Caspase-3 activity is amount of enzyme that will cleave 1.0 nmol of the colorimetric substrate Ac-DEVD-pNA per hour at 37℃ under saturated substrate concentrations.

Notes

1. This kit is for research use only. For your safety and health, please wear lab clothes and gloves.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. If a spectrophotometer is used to determine the OD value, the capacity of the colorimeter must be less than 100 uL.
5. Avoid repeated freeze-thaw cycles with Ac-DEVD-pNA. It is recommended that be aliquoted for optimal storage with shading light.
6. The amount of cells is 3-5 $\times 10^6$ or the fresh tissues is 100 mg, the required quantity of protein is 100-200 μg which meet the requirements of measurement. Because the activity of caspase-3 is related to the protein content of cell lysis, the protein content can be increased by increasing cell number and tissue quality if OD value is low.
7. It has been reported that a few types of apoptosis can not detect the activity of caspase-3, which may be due to the existence of a mechanism independent of the activation of caspase-3, and other signaling pathways in the mechanism of apoptosis need to be considered. In this case, there was no significant change in the activity of caspase-3 by using this kit.
8. When the activity of caspase-3 in the sample is very low, we first confirm whether the phenomenon of apoptosis is obvious or not. If the apoptosis is obvious and we confirm that caspase can be activated, please adjust the time of inducing apoptosis and find a time point which caspase-3 activation is stronger. And then repeat the test.