Caspase 3 Activity Assay Kit

Introduction:

Caspase 3 Activity Assay Kit is a kit detection of Caspase-3 enzyme activity which from cell or tissue lysates or purified Caspase 3 by Spectrophotometric method .

Caspase (Cysteine-requiring Aspartate Protease) is a protease family which play an important role in the process of apoptosis. Caspase-3 is also known as CPP32, Yama or apopain, sometimes by writing caspase-3 or Caspase3, belongs to CED-3 subfamily of caspase family (CED-3, subfamily) is a key enzyme in the process of cell apoptosis. Caspase 3 is the most studied caspase in mammalian cells. Caspase-3 in the normal state is in the form of zymogen exists in cytoplasm and no activity, but in the cell apoptosis, Caspase-3 is activated, activated Caspase-3 is composed of two subunit and two small subunits, cleavage the corresponding cytoplasmic nuclear substrate, eventually leading to cell apoptosis. Caspase 3 can be cut procaspase 3, 6, 7 and 9, and can direct specific shear many caspase substrates, including PARP, ICAD, gelsolin and fodrin etc. These protein which sheared by caspase 3 are important part of the molecular mechanism of apoptosis. In addition, caspase-3 also plays a key role in the nucleus during apoptosis, including chromatin condensation, DNA fragmentation. At the same time, caspase-3 also play a key role in cell blebbing.

The basics of this kit is casepase -3 can catalyze the substrate Ac-LEHD-pNA produce yellow based pNA, pNA have strong absorption in the vicinity of the 405nm, which can be measured by absorbance to detect the activity of caspase 3.

This kit can be detection by enzyme-labeled instrument or capacity of not more than 100 μ l spectrophotometric detection cup. This kit can be used for caspase-9 detection of cultured cells or fresh tissue samples.

Contents:

Cat No.: BD0064-1(20T)	Cat No.: BD0064-2(50T)	Cat No.: BD0064-3(100T)
Lysis buffer,5ml	Lysis buffer,10ml	Lysis buffer,15ml
Ac-DEVD-pNA,200ul	Ac-DEVD-pNA,500ul	Ac-DEVD-pNA,1ml
Detection buffer,5ml	Detection buffer,10ml	Detection buffer,15ml
DTT,100ul	DTT,150ul	DTT,250ul

Procedure:

1 Lysis buffer and detection buffer

According to the sample to prepare lysis buffer and detection buffer, join 10ul DTT to per 1ml buffer.

2 Sample processing

A. Cell samples

1) Collection of 2-5*10^6 cells, 4℃, 500g , centrifugate 2-3 minutes, carefully remove the medium, collection the cells.
2) Use cold PBS washing the cells twice, dry the supernatant as possible each time.
3) Add 100 μl cold lysis buffer, high speed vortex oscillation for 15 seconds.

4) Put the sample on ice for 15 minutes, speed vortex oscillation for 15 seconds every 5 minutes.
5) 4℃, 500g centrifugate 5 minutes.
6) Suction the supernatant to another pre-cooling tube, placed the sample on ice or -80℃ refrigerator for the next step.

B. Tissue sample

1) Shear appropriate tissue sample, add cold lysis buffer (50mg/100 μ l), homogenate the sample to no obvious visible solid tissue by tissue homogenizer (or with liquid nitrogen grinding), put the sample on ice for 15 minutes, suction the supernatant to another pre-cooling tube.
2) 4℃, 500g centrifugate 5 minutes.
3) Suction the supernatant to another pre-cooling tube, placed the sample on ice or -80℃ refrigerator for the next step.

3 Quantitative protein by Bradford method.

4 Detection the activity of Caspase-3

4.1 Suction 10ul supernatant about containing 20-50ug protein to a new tube, join 90ul detection buffer.

4.2 Adding 10ul Ac-LEHD-pNA, reaction for 1-2 hours at 37 ℃, evades the light . Do the next step when yellow color could be detection, if the color change was not obvioued, the time period may be extended, even overnight incubation.

4.3 Determination of A405nm or A400nm.

4.4 Caspase-3 activity was calculated according to the light absorption value of induced apoptosis cell and blank control relative absorption ratio value.

Attention：

1) The laboratory should have microplate which can be measured in A405 or A400 or capacity of not more than 100 μl spectrophotometric detection cup and corresponding spectrophotometer. The priority determination of A405
2) If the color change is not obvious at 37 ℃, may be appropriately extended reaction time.
3) Not suitable for determination of protein concentration by BCA method.
4) If the protein concentration of the samples is low, trying to make the protein concentration in the sample reaches 3mg/ml
5) If the activation of the caspase level of the samples is very low, appropriate regulation the apoptosis time, find a strong point in time detection.
6) Ac-LEHD-pNA stored away from light, use the process to avoid light.
7) Ac-LEHD-pNA solution may precipitate after cryopreservation, please mixing before use.

Storage & Shelf life:

Lysis buffer and detection buffer store at RT, other store at -20℃ and keep in dark place, quality guarantee period is one year.