



# Caspase-3 Activity Assay Kit

(Cat/No.:BC158 Size: 20T)

## 1, Product Introduction

This kit is used to detect the activity of Caspase 3 in cell or tissue lysates or the activity of purified Caspase 3 by spectrophotometer or microplate reader.

Caspase (Cysteine-dependent Aspartate Protease) is a family of proteases that play important roles in the process of

apoptosis. Caspase 3, also known as CPP32, Yama, or apopain, sometimes written as caspase -3 or caspase3, belongs to the CED-3 subfamily of the caspase family and is a key enzyme in the process of apoptosis. Caspase 3 is one of the most studied caspases in mammalian cells. Caspase 3 can cleave procaspase 2, 6, 7, and 9, and can directly and specifically cleave many caspase substrates, including PARP (Poly(ADP-ribose) polymerase), ICAD (Inhibitor of caspase-activated

deoxyribonuclease), gelsolin, and fodrin, among others. These protein cleavages mediated by caspase 3 are important components of the molecular mechanism of apoptosis. Additionally, caspase 3 plays a crucial role in nuclear apoptosis processes, including chromatin condensation and DNA fragmentation. Caspase 3 is also essential for cell blebbing.

Under normal conditions, caspase-3 exists in the cytoplasm in the form of zymogen and is inactive. However, during the apoptotic stage of cells, it is activated. The activated caspase-3 consists of two large subunits and two small subunits that cleave the corresponding cytoplasmic and nuclear substrates, ultimately leading to apoptosis.

The Caspase-3 assay kit couples a caspase-3 sequence-specific peptide (Ac-DEVD-pNA (acetyl -Asp-Glu-Val-Asp

p-nitroanilide)) to a yellow group: pNA (p-nitroaniline). When this substrate is cleaved by caspase-3, the yellow group pNA is released. The absorbance value can be measured by a microplate reader or spectrophotometer ( $\lambda = 405\text{nm}$  or  $400\text{nm}$ ) to investigate the activation degree of caspase-3. pNA has strong absorption near  $405\text{nm}$ .

This kit is suitable for detecting Caspase-3 activity in cultured cells and fresh tissues.

## 2. Component

Component	5.0ml (20T)	10.0ml (50T)	15.0ml (100T)	Storage Condition
Cell Lysis Buffer	5.0ml	10.0ml	15.0ml	Store at 4°C
2× Reaction Buffer	1.0ml	2.5ml	5.0ml	Store at 4°C



Ac-DEVD-pNA	100µl	250µl	500µl	Store at -20°C, protect from light
DTT	50µl	100µl	150µl	Store at -20°C

### Instruments and Reagents to be Prepared Independently

Low-temperature high-speed centrifuge, microplate reader or spectrophotometer (100µL cuvette), micropipette, glass homogenizer (for tissue samples), 1.5mL centrifuge tube, PBS, protein quantification reagent, and instrument.

### 3. Storage Conditions

Store at -20°C. Ac-DEVD-pNA needs to be protected from light.

### 4. Precautions

1. Prepare a microplate reader capable of measuring at 405nm or 400nm, or a cuvette with a volume not exceeding 100µl and the corresponding spectrophotometer. Measuring at A405 is preferred; if this is difficult, measurement at 400nm can be used.
2. Try to avoid repeated freeze-thaw cycles of Ac-DEVD-pNA. Please note to aliquot appropriately and store it protected from light.
3. A Bradford protein concentration assay kit is required to measure protein concentration.
4. Literature reports that in some cell types, Caspase 3 activation cannot be detected during apoptosis. There may be mechanisms independent of Caspase-3 activation. In this case, using this kit to detect Caspase-3 activity will show no significant change, and other signaling pathways in the apoptosis mechanism need to be considered.
5. The lysis buffer of this kit is compatible with the lysis buffers of other caspase activity assay kits produced by our company. Therefore, protein samples prepared with this kit's lysis buffer can be used for the detection of other caspase activity assay kits from our company.
6. The number of cells should reach  $3-5 \times 10^6$  or 100mg of fresh tissue to meet the requirement of 100-200µg of protein for measurement. Since the activity of Caspase-3 is related to the protein content of the cell lysate, if the measured OD value is low, the protein amount can be increased by increasing the number of cells or the amount of tissue.
7. The level of activated caspase in the sample is typically low. First, confirm whether the apoptotic phenomenon is evident. If apoptosis is clear and it is confirmed that this caspase can be activated, the time for inducing apoptosis can be appropriately adjusted to find a time point with strong caspase activation. A time-course curve can be made, for example, inducing apoptosis for 0, 2, 4, 8, 16, and 24 hours, or 0, 1, 2, 4, 8, and 16 hours, or 0, 1, 2, 4, 6, and 8 hours, etc. The specific time for inducing apoptosis needs to be determined according to the specific situation.



8. For your safety and health, please wear a lab coat and disposable gloves during operations.

## 5. Operating Procedures

1. Preparation:

- 1) After dissolving the lysis buffer, mix well and place it on ice for later use.
- 2) Preparation of lysis buffer working solution: Add 0.5 $\mu$ l of DTT to every 50 $\mu$ l of lysis buffer before use.
- 3) After dissolving the 2 $\times$  reaction buffer, mix well and place it on ice for later use.

2. Sample Treatment:

1) For suspension cells:

Centrifuge (2000rpm, 5 min) to collect non-apoptosis-induced control samples and apoptosis-induced samples. Carefully aspirate the supernatant, making sure to aspirate as few cells as possible. Wash once with PBS. After completely aspirating the supernatant, add lysis buffer at a ratio of 50 $\mu$ l per 2 million cells, resuspend the precipitate, and lyse on ice for 30 min. Vortex 3-4 times during this period, 10 seconds each time; or perform 2-3 freeze-thaw cycles.

Proceed to step 3 for the experiment.

2) For adherent cells:

Digest adherent cells with trypsin using the conventional method and collect the cells. Wash once with PBS. After completely aspirating the supernatant, add lysis buffer at a ratio of 50 $\mu$ l per 2 million cells, resuspend the precipitate, and lyse on ice for 30 min. Vortex 3-4 times during this period, 10 seconds each time; or perform 2-3 freeze-thaw cycles. Proceed to step 3 for the experiment.

3) For tissue samples:

Place 50mg of solid tissue in a petri dish, cut it into small pieces of about 3mm $\times$ 3mm with surgical scissors, add 50 $\mu$ l of ice-cold lysis buffer working solution, and homogenize with a glass homogenizer on ice. Then transfer the homogenate to a 1.5ml centrifuge tube and lyse on ice for another 5 minutes. Proceed to step 3 for the experiment.

3. Centrifuge at 4 $^{\circ}$ C (12,000rpm, 10-15 min).

4. Carefully aspirate the supernatant (containing lysed proteins) and transfer it to a new tube, placing it on ice for later use.

6. Measure the enzyme activity of caspase 3 immediately or store the sample at -70 $^{\circ}$ C. At the same time, a small amount of the sample can be taken to measure the protein concentration using the Bradford method. If the cells are small, the amount of cells can be appropriately increased.

## 6. Detection of Caspase 3 Enzyme Activity



- 1) Take out an appropriate amount of Ac-DEVD-pNA and 2× reaction buffer and place them on ice for later use.
- 2) Add 0.5µl of DTT to every 50µl of 2× reaction buffer before use.
- 3) Pipette 50µl of cell or tissue lysate supernatant containing 100-200µg of protein. If the volume is less than 50µL, make up to a total volume of 50µl with lysis buffer (the same amount of protein is used for measurement and comparison in each group).
- 4) Set up the reaction system as follows:

	<b>Blank Control</b>	<b>Sample</b>
2× Reaction Buffer	50µl	50µl
Lysis Buffer	50µl	0µl
Sample to be Tested	0µl	50µl
Ac-DEVD-pNA	5µl	5µl
Total Volume	105µl	105µl

- 5) After adding Ac-DEVD-pNA, mix well, and be careful to avoid bubbles during mixing. Incubate at 37°C for 4 hours. Measure A405 when the color change is evident. If the color change is not obvious, the incubation time can be appropriately extended, even overnight.
- 6) Measure the absorbance value with a microplate reader or spectrophotometer (100µl cuvette) at  $\lambda = 405\text{nm}$  or 400nm.
- 7) Determine the activation degree of Caspase-3 in the apoptosis-inducer group by calculating the ratio of OD (induced by apoptosis inducer)/OD (negative control).

7. Refer to the definition of caspase 3 enzyme activity unit by Chemicon: One unit is defined as the amount of enzyme that will cleave 1.0 nmol of the colorimetric substrate Ac-DEVD-pNA per hour at 37°C under saturated substrate concentrations. That is, one enzyme activity unit is defined as the amount of caspase 3 that can cleave 1nmol of Ac-DEVD-pNA to produce 1nmol of pNA at 37°C when the substrate is saturated. In this way, the number of enzyme activity units of caspase 3 in the sample can be calculated. Note: In the detection system of this kit, the initial concentration of the substrate is 0.2mM, and the substrate is saturated at this time. For many samples, the substrate remains saturated within 4 hours of incubation at 37°C. For samples with particularly high caspase 3 enzyme activity, the sample needs to be appropriately diluted with lysis buffer before measurement.