Ver.251201

Caspase-3 Activity Assay Kit

BC158 (20Test)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Caspase is a family of proteases involved in the process of apoptosis, including more than 10 members.

Caspase- 3 is the most important terminal protease in the process of apoptosis, and it is also the most studied caspase; it activates pro-caspase-2,6,7,9, specifically hydrolyzes a variety of key apoptotic proteins, such as PARP, and mediates chromatin condensation, apoptotic body formation and nuclear DNA fragmentation.

The caspase-3 activity assay is based on the hydrolysis of the peptide substrate DEVD-pNA (Asp-Glu-Val- Asp-p-nitroanilide) by caspase-3, resulting in the release of the p-nitroaniline (pNA) moiety. p-Nitroaniline has a high absorbance at 405 nm. The activity of Caspase can be calculated by detecting pNA.

This kit is suitable for mammalian tissue and cell.

Kit components

Reagent	Volume	Storage
Reagent I	20mL	-20℃
Reagent II	30mL	-20℃
Reagent III	250μL	-20℃
Standard	1mL	-20℃

Reagent Preparation

1. **Reagent I:** Subpackage store at -20℃.
2. **Reagent II:** Subpackage store at -20℃.
3. **Standard:** pNA standard solution, 5 mmol/L. The standard solution is cloudy at 4℃, and can be dissolved into a clear state without affecting the use.
4. **Preparation of Standard Diluent:** Take 9 mL of Reagent I and add 1 mL of Reagent II, mix well and wait for use. (it can also be prepared according to the ratio of Reagent I: Reagent II = 9:1)

Reagents and Equipment Required but Not Provided

Spectrophotometer/Microplate reader, 100μL cuvette/ 96-well plate, centrifuge, water bath / incubator, adjustable pipette, mortar / homogenizer, ice and distilled water.

Operation Procedures

I. Sample Preparation

1. Cells

Collect the cells into the centrifuge tube, centrifuge and discard the supernatant; add 100μL Reagent II to the number of cells (about 10^6 cells), shake and resuspend the precipitate, then stand on ice for 15 min, centrifuge 15000g at 4℃ for 10-15 min, take the supernatant and place it on ice for testing. (it can be increased to 150-200μL Reagent II if the cracking is not enough)

2. Tissue

According to the ratio of tissue mass (g): Reagent II volume (mL) of 1:5-10 (it is recommended to weigh about 0.1 g of tissue and add 1 mL of Reagent II), grind it in ice bath or cut it thoroughly, place it on ice for 15 min, centrifuge it at 4℃ for 10-15 min, take the supernatant and place it on ice for testing.

II. Determination procedure:

1. Preheat the spectrophotometer / microplate reader for 30 min, adjust the wavelength to 405 nm, and adjust distilled water to zero.
2. Before use, 5 mmol/L PNA standard solution is diluted to 200, 100, 50, 25, 12.5 and 0 μmol/L standard solution with standard solution diluent.

3. Sample determination (add the following reagents in sequence in 96 well plate/Ep tube)

Reagent name	Test tube (A_T)	Blank tube (A_B)	Standard tube (A_s)
Reagent I	40	40	-
Sample	50	-	-
Reagent II	-	50	-
Reagent III	10	10	-
Standard solution	-	-	100
Mix well, cover 96 well plate tightly and seal with sealing film. Incubate at 37°C for 60-120 minutes. When the color change obvious, the absorbance at 405 nm can be determined. If the color change is not obvious, the incubation time can be extended appropriately, even overnight. Blank tube only need to do 1-2 times. Calculate ΔAT -AT-AB.			Immediately determine the absorbance at 405nm

III. Activity calculation

1. Establishment of standard curve

The standard equation is made according to the concentration of standard tube (x, μmol/L) and ΔAs (y, minus the tube with 0 concentration). The determination of ΔA_T is substituted into the standard equation to obtain x (μmol/L).

2. According to the increase percentage of enzyme activity

Increased percentage of caspase-3 activity = ((experimental treatment group AT)- AB) / ((experimental control group AT)- AB) x 100%

The method is simple and reliable, and can be used to determine the enzyme activity roughly.

3. Calculated by enzyme activity

Caspase activity unit: One unit is the amount of enzyme that will cleave. 1.0 nmol of the colorimetric pNA-substrate per hour at 37°C under saturated substrate concentrations. That is, a unit of enzyme activity is defined as the amount of enzyme that can shear a 1nmol pNA substrate to produce 1nmol free pNA at 37°C for one hour when the substrate is saturated. This allows you to calculate how many caspase enzyme activity units are present in the sample.

Caspase-3 activity (U/mg prot) = $x \times V_R \div (V_S \times Cpr) \div T \times 10^3 = 2x \div Cpr \div T$

V_R: total volume of reaction system, 0.1 mL = 10^{-4}L
Vs: volume of added sample, 0.05 mL
T: reaction time, h
Cpr: concentration of sample protein, mg/mL; 10^3
Unit conversion coefficient, 1 μmol = 10^3 nmol