Caspase-6 colorimetric activity assay Kit

Cat No: BC3860

Size: 50T

Storage： The reagents are transported at room temperature, stored as required after arrival, and stable within 0.5 year.

Reagent I: 20 mL×1. Storage at 4℃;

Reagent II: 60 mL×1. Storage at 4℃.

Reagent III: 0.55 mL×1. Storage at -20℃, avoid light.

5mM pNA standard: 1 mL×1. Storage at -20℃, avoid light.

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).

Product Description:

Caspase is a family of proteases involved in the process of apoptosis, including more than 10 members. Caspase-6, also known as Mch-2, its precursor protein is cleaved by granzyme B to form activated caspase-6 dimer, which can induce apoptosis. Caspase-6 can cleave PARP, a key regulator of apoptosis, and Lamin A, a key protein on the nuclear membrane. Its recognition site for Lamin A is VEID.

The caspase-6 colorimetric assay is based on the hydrolysis of the peptide substrate Ac-VETD-pNA (N-acetyl-Val-Glu-Ile-Asp-p-nitroanilide) by caspase-6, 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.

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

Procedure:

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 150-200 μL 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（μL）	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℃ 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 $\Delta A_T = A_T - A_B$.			Immediately determine the absorbance at 405nm

III. Activity caculation:

1. Establishment of standard curve

The standard equation is made according to the concentration of standard tube (x, μmol/L) and ΔA_S (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-6 activity = ((experimental treatment group A_T)- A_B) / ((experimental control group A_T)- A_B) × 100%

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

3. Calculated by enzyme activity

One unit is the amount of enzyme that will cleave 1.0 nmol of the colorimetric pNA-substrate per hour at 37℃ under saturated substrate concentrations. we can calculate the caspase activity in the sample.

Caspase-6 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; V_S: volume of added sample, 0.05 mL; T: reaction time, 1 h; Cpr: concentration of sample protein, mg/mL; 10^3: unit conversion coefficient, 1 μmol = 10^3 nmol.

Note:

1. Since Reagent I contains a reducing agent (DTT), it is recommended to dilute the sample 2 times with distilled water and then use the Bradford method to determine the protein concentration to reduce the interference of DTT on the protein concentration determination. It is not recommended to use the BCA method to determine protein concentration.

2. The most common reason for the low Caspase activity value is that the cells have not undergone apoptosis, the amount of cells is too small or observation time is improper. When inducing

apoptosis, it is not that the larger the dose, the longer the time, the higher the Caspase activity. It is recommended to set different doses and time points such as 0, 2, 4, 8, 16, 24 hours to detect the best observation point.

3. When the value of the measured sample is higher than the upper limit of the standard curve, the sample can be diluted with Reagent II and then re-measured.

4. Tightly cover the 96-well plate and seal it with parafilm. Incubate at 37 ºC, the OD405 value when the color turns yellow is about 0.2, which can be measured at this time. The insignificant color change can prolong the reaction or overnight, but when the enzyme activity is strong, too long incubation time will cause the reaction to lose the linear relationship.

References:

[1] Cohen GM. Caspases: the executioners of apoptosis. Biochem J, 1997, 326: 1-16.

[2] Janicke R U, Sprengart M L, Wati M R, et al. Emerging role of caspase-3 in apoptosis[J]. Cell Death and Differentiation, 1999, 6:99-104.

Related products:

BC3810 Caspase-1 activity assay Kit
BC3820 Caspase-2 activity assay Kit
BC3830 Caspase-3 activity assay Kit
BC3840 Caspase-4 activity assay Kit
BC3850 Caspase-5 activity assay Kit
BC3880 Caspase-8 activity assay Kit
BC3890 Caspase-9 activity assay Kit