Technical Manual

Caspase 3/7 Activity Assay Kit

- **Catalogue Code: AKES194**
- **Research Use Only**
- **Size: 100 Assays**

1. Introduction

The Assay Genie Caspase 3/7 Activity Assay Kit uses spectrophotometry to detect the Caspase 3/7 activity of cells, tissue lysates or other samples.

2. Kit Components

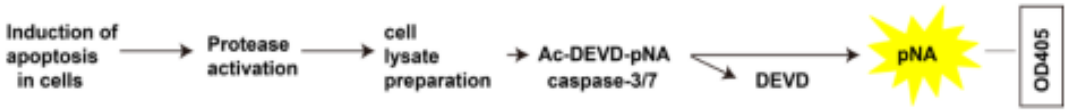
Product	100 Assays	Storage
Cell Lysis Buffer	50 mL	-20°C
2x Reaction Buffer	50 mL	-20°C
Ac-DEVD-pNA (4 mM)	500 µL	-20°C, away from direct sunlight
pNA (10 mM)	1mL	-20°C, away from direct sunlight
Technical Manual	1 copy	-

3. Storage

Store at -20°C for 6 months. It is recommended to aliquot the Ac-DEVD-pNA (4 mM) into smaller quantities and store in the dark. Avoid repeated freezing and thawing.

Detection Principle

This Caspase 3/7 activity assay kit is based on Caspase 3/7 that can catalyze the substrate Ac-DEVD-pNA to generate yellow pNA (p-Nitroaniline). The pNA has a strong absorption near 405 nm, the activity of Caspase 3/7 can be calculated by determining the absorbance at 405 nm.



Detection Sample Types

- ✓ Cell Sample
- ✓ Tissue sample

Materials Not Supplied

1. **Reagents**
   PBS, Protein quantification kit (Bradford method).
2. **Instrument**
   Spectrophotometer/microplate reader, centrifuge, incubator, pipette, mortar or Homogenizer.

Notes

1. All samples need to be detected for protein concentration. Since the cell lysis buffer contain reducing agents, it is not suitable to detect protein concentration with BCA method, the Bradford method is recommended.

2. It is recommended to take 2~3 samples which expected large difference to do pre-experiment before formal experiment. If the absorbance of sample exceeds the measurement range of the standard curve, the sample needs to be diluted or the sample volume needs to be adjusted before measurement.
3. The protein concentration of the sample used for Caspase 3/7 activity detection should be 1~4 mg/mL, otherwise it will affect the accuracy of the experimental results.
4. It is recommended that the number of cells for one sample should not be less than 1×10^6, and the tissue sample should not be less than 50 mg, make sure that the protein concentration is 1~4 mg/mL. Otherwise, it will affect the accuracy of the experiment, resulting in low protein concentration, low activity of Caspase 3/7 in the reaction system, and low OD value.

4. Reagent Preparation

All reagents dissolved after mixing, placed in ice bath for use.

5. Experimental Procedure

Sample Preparation

A. Cell sample

1) Collect the cells and resuspend with PBS, then count the cells, centrifuge at 600×g for 5 min, discard the supernatant and keep the cell pellet. Resuspend the cell pellet with pre-cooled Cell Lysis Buffer according to the ratio of 100 μL Cell Lysis Buffer per 1 million cells, incubate on an ice bath for 30 min (Mix 3~4 times, 10s each time), and then centrifuge the lysed sample at 11000×g for 10~15 min at 4°C. Carefully absorb the supernatant to a new EP tube and place it on ice for use. Meanwhile, determine the protein concentration of supernatant.
   Note: The suspension cells generated after induction of apoptosis should be collected and detected together with the collected adherent cells.

B. Tissue sample

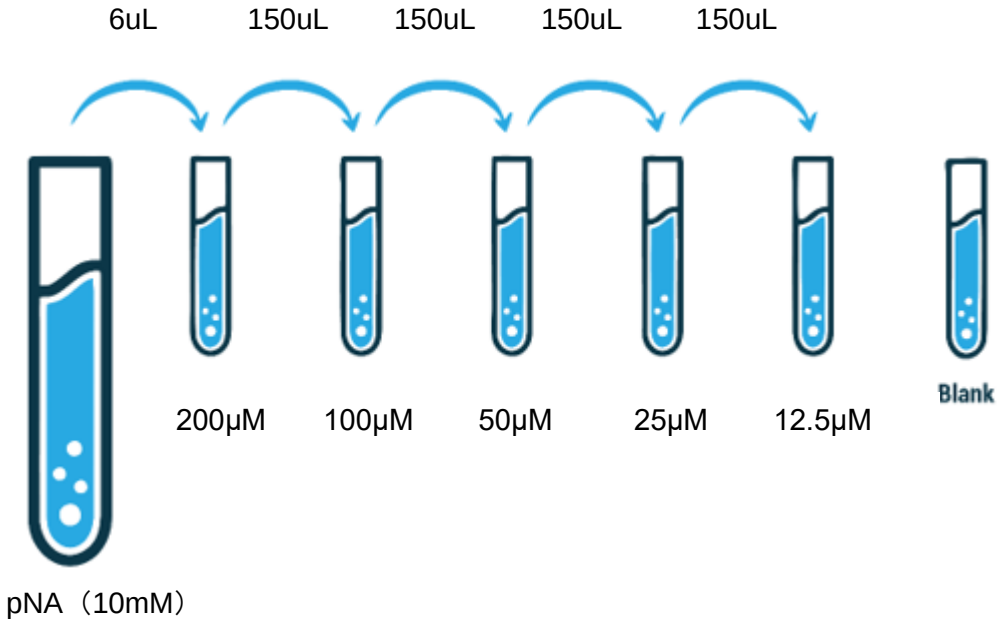
1) A total of 50 mg tissue samples were washed with PBS or normal saline for 1-2 times to remove the residual blood cells in the tissue and cut into pieces with surgical scissors. Add 200 μL of pre-cooled Cell Lysis Buffer and homogenize the sample on ice (If the quality of the tissue is doubled, the pre-cold Cell Lysis Buffer needs to be doubled). Transfer the homogenized samples to a 1.5 mL tube and lyse in an ice bath for 30 min. The lysed samples were centrifuged at 11000×g at 4°C for 10~15 min. Carefully transfer the supernatant to a new EP tube and placed on ice for use. Meanwhile, determine the protein concentration of supernatant.
   Note: The prepared sample should be determined immediately. If it cannot be determined in time, the supernatant after lysis can be stored at -80°C for 2 weeks.

Protocol

1. (Optional) Preparation of pNA standard curve

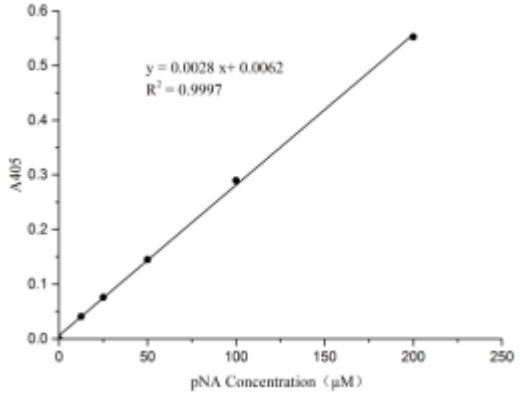
1) Preparation of standard diluent: Take Cell Lysis Buffer and 2×Reaction Buffer according to the ratio of 1:1 to prepare the standard diluent and mix fully.

2) Dilution method: Take 6 EP tubes, add 294 μL of standard diluent to the first tube and 150 μL of standard diluent to the other tubes. Pipette 6 μL of pNA (10 mM) from the first tube and mix to produce a 200 μM pNA working solution. Pipette 150 μL of the solution from this tube into the next one according to this step. The illustration below is for reference. Note: the last tube is regarded as a blank. Don't pipette solution into it from the former tube.



3) Take 100 μL of different concentration standard solutions to the cuvettes or wells of microplate and detect the OD value at 405 nm (In order to ensure the accuracy of experimental results, it is recommended that all samples and standards be assayed in duplicate).

4) Average the duplicate readings for each standard, then subtract the average zero standard OD values. Plot a standard curve with standard concentration on x-axis and absolute OD values on the y-axis. The measured data may be different due to different experimental conditions, detection instruments, etc. and the data in the figure is for reference only.



2. Operation Table:

1) Take 50 μL of lysed sample solution and set the reaction system according to the table below.

	Blank Tube	Experimental Tube
Cell Lysis Buffer	50 μL	0 μL
2×Reaction Buffer	45 μL	45 μL
Sample	0 μL	45 μL
Ac-DEVD-pNA	5 μL	5 μL
Total Volume	100 μL	100 μL

Note: When setting the reaction system, add 2×Reaction Buffer first, then add sample and mix gently. Finally, add Ac-DEVD-pNA and mix it. Pay attention to avoid the production of air bubbles.

2) Incubate at 37°C for 1~2 h, OD405 can be detected when the color change is obvious. If the color change is not obvious, the incubation time can be appropriately extended to 4 h.

Results Calculation

Method 1: Calculate according to the percentage increase of enzyme activity

$$\text{Caspase 3/7 activity (100\%)} = \frac{OD_{sample} - OD_{blank}}{Cpr_{sample}} \div \frac{OD_{negative} - OD_{blank}}{Cpr_{negative}} \times 100\%$$

Note: The negative is a biological control group that does not undergo apoptosis treatment

Method 2: Calculate according to enzyme activity

1) Establish a standard curve: according to the concentration of the standard tube (x, μmol/L), and the absorbance (y, minus the blank tube with a concentration of 0) as the standard equation y = ax+b. The concentration of the sample can be calculated according to the formula based on the OD value of sample. If the sample have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. The actual concentration is the calculated concentration multiplied by dilution factor.

2) Calculate Caspase 3/7 activity:

Definition: One unit is 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.

$$\text{Caspase 3/7 activity (U/mgprot)} = \frac{\Delta A - b}{a} \times \frac{V_1}{V_2 \times Cpr \times T} \times f$$

Note：

Y: ODstandard-ODblack

x: Concentration of standard

a: Slope of the curve

b: Intercept of the curve

△A: ODsample-ODblack

V1: Total volume of the reaction system, 0.1 mL

V2: The sample volume of the addition, 0.05 mL

T: Reaction time, h

Cpr: The protein concentration before the sample was added to the detection system, mgprot / mL

f: The dilution multiple of the sample before adding the detection system

Precautions

1. For maximal assay performance, this reagent should be used within 12 months. Avoid freeze / thaw cycles.

2. This kit is for research use only.

3. It has been reported that the activity of Caspase 3/7 can't be detected in few types of apoptosis, which may be due to the existence of a mechanism independent of the activation of Caspase 3/7. In this case, there will not be a significant change in the activity of Caspase 3/7 by using this kit.

4. Mechanical pNA (4-nitroaniline) is toxic. Avoid direct contact with the human body and inhalation. pNA solidifies at lower temperatures and will stick to the bottom, wall or cap of centrifugal tube. It can be incubated in a water bath at 20~25°C until it is completely dissolved.

5. For your safety and health, please wear a lab coat and disposable gloves before the experiment.

Notes:

Notes:

Notes:

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Contact Details

Email: info@assaygenie.com

Web: www.assayenie.com