Elabscience®

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（**FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !**）

Caspase 8 Activity Assay Kit (by Colorimetric)

Catalog No： E-CK-A312

Size： 20 Assays / 50 Assays / 100 Assays

Cat.	Products	20 Assays	50 Assays	100 Assays	Storage
E-CK-A312A	Lysis Buffer	3.0 mL	7.5 mL	15 mL	-20 ℃
E-CK-A312B	2 ×Reaction Buffer	1.0 mL	1.25 mLx2	5.0 mL	-20 ℃
E-CK-A312C	Ac-IETD-pNA	100 μL	250 μL	500 μL	-20 ℃
E-CK-A312D	DTT	50 μL	100 μL	150 μL	-20 ℃
Manual			**One Copy**		

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) Fax: 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Introduction

Elabscience® Caspase 8 Activity Assay Kit can detect the activity of Caspase 8 in cells or tissue lysis.

Detection principle

Caspase (Cysteine-requiring Aspartate Protease) is a protease family that plays an important role in the process of apoptosis. Caspase 8 (also known as FLICE, MACH or mch5) is considered to be a caspase in the upstream of apoptotic signal transduction. Caspase 8 is activated in Fas-receptor and TNFR-1-mediated apoptotic process, forming a dimer composed of P18 and p10, which further activates downstream Caspase 4, Caspase 6, Caspase 9 and Caspase 10. Caspase 8 exists in the form of prozyme in the normal state and has no activity. However, Caspase 8 is activated in the apoptotic stage and participates in the apoptotic process.
This kit is used to conjugate Caspase 8 sequence-specific peptides acetyl-Ile-Glu-Val-Asp p-nitroanilide (Ac-IETD-pNA) to yellow group p-nitroaniline (pNA). When the substrate is cut by Caspase 8, the yellow group pNA is dissociated. pNA has an absorption peak at 405 nm. Measure the OD value at 405 nm and then Caspase 8 activity can be calculated accordingly.

Reagents not included

PBS, Protein Quantitative Kit (Bradford, optional)

Experimental Procedure

1. **Reagent Preparation**
   A. Take out the Lysis Buffer [E-CK-A312A], dissolve fully, mix it and put on ice for use.
   B. Lysis working solution preparation: Take 50 μL Lysis Buffer [E-CK-A312A], add 0.5 μL DTT [E-CK-A312D] to the Lysis Buffer, mix it and put on ice for use.
2. **Sample Preparation**
   A. Suspension Cells
      1) Induce apoptosis of suspension cells with reagents of interest, centrifuge at 2000 rpm for 5 min and discard the supernatant. Add PBS to resuspend gently and count the cells.
      2) Centrifuge at 2000 rpm for 5 min and discard the supernatant. Add 50 μL cold Lysis Buffer working solution to each 2 million cells to resuspend the cells. Incubate in ice bath for 30 min and oscillate 3~4 times during incubation.
   B. Adherent Cells
      1) Adherent cells should be detached with trypsin and then collected sedimentary cells. Collect the cells, centrifuge at 2000 rpm for 5 min and discard the supernatant. Add PBS to resuspend gently and count the cells.
      2) Centrifuge at 2000 rpm for 5 min, discard the supernatant. Add 50 μL cold Lysis Buffer working solution to each 2 million cells to resuspend the cells. Incubate in ice bath for 30 min and oscillate 3~4 times during incubation.

C. Tissue

1) Take 50 mg tissue, cut to small pieces, then add 200 μL cold Lysis Buffer working solution and homogenize the sample on ice.

2) Transfer the tissue homogenate to a 1.5 mL centrifuge tube, then incubate in ice bath for 5 min.

3. Centrifuge at 12,000 rpm for 10~15 min at 4 ℃.
4. Take the supernatant to a new tube, put it on ice for test.
5. Carry out the assay immediately or store the samples at-70 ℃. Meanwhile, you cloud also determine the concentration of protein with Bradford method (It is recommended to use Elabscience® E-BC-K168-S).
6. Caspase 8 Activity Detection

A、 Take Ac-IETD-pNA[E-CK-A312C] and 2 ×Reaction Buffer [E-CK-A312B], dissolve fully and put on ice for use.

B、2 ×Reaction working solution preparation: Add 0.5 μL DTT [E-CK-A312D] to each 50 μL 2 ×Reaction Buffer [E-CK-A312B].

C、Take 45 μL cell lysate or supernatant of tissue homogenate (contain 100~200 μg of protein), if the volume is less than 45 μL, add lysis buffer to 45 μL.

D、 Operation Table

	Blank tube	Sample tube
2 ×Reaction working solution	50 μL	50 μL
Lysis working solution	45 μL	0 μL
Sample	0 μL	45 μL
Ac-IETD-pNA	5 μL	5 μL
Total	100 μL	100 μL

Tips:

1） Add 2 ×Reaction working solution into the tube firstly, then add Sample or Lysis working solution. Mix and avoid bubble formation.

2） Add Ac-IETD-pNA into the tube, Mix it and avoid bubble formation.

E、 Incubate at 37 ℃ for 2~4 h. Measure the OD value (A405) at 405 nm with spectrophotometer (100 μL cuvette) or microplate Reader when the color changes obviously. The reaction time can be extended or stay overnight if the color doesn't change significantly.

F、 Calculate (OD_{Sample}-OD_{Blank}) /($OD_{Negative\ control}$-OD_{Blank}) to determine the activity of Caspase 8.

Definition: One unit Caspase 8 activity is amount of enzyme that will cleave 1.0 nM of the colorimetric substrate Ac-IETD-pNA per hour at 37 ℃ under saturated substrate concentrations.

Storage

Store at -20 ℃. Ac-IETD-pNA[E-CK-A312C] should be stored in dark. Avoid freeze / thaw cycles.

Note

1. For maximal assay performance, this reagent should be used within 12 months. Avoid freeze / thaw cycles.
2. There is reducing agent (DTT) in the Lysis Buffer, It is recommended to Elabscience® Total Protein (TP) Colorimetric Assay Kit (Coomassie Brilliant Blue Method) (It is recommended to use Elabscience® E-BC-K168-S) to measure the protein concentration instead of BCA method.
3. It has been reported that the activity of Caspase 8 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 8, and other signaling pathways in the mechanism of apoptosis need to be considered. In this case, there is no significant change in the activity of Caspase 8 by using this kit.
4. Lysis Buffer in this kit can be used in other Caspase activity assay kits by Elabscience®. And the protein sample can test other Caspase activity.
5. pNA (4-nitroaniline) is toxic. Please be careful when operating, and pay attention to effective protection to avoid direct contact with human body or inhalation. pNA solidifies at lower temperatures and sticks to the bottom, wall or cap of centrifugal tube. It can be incubated in water bath at 20~25 ℃ for a short time until it is completely melted.
6. When the activity of Caspase 8 in the sample is very low. Confirm whether the phenomenon of apoptosis is obvious or not firstly. If the apoptosis is obvious and we confirm that Caspase can be activated, please adjust the time of apoptosis and find a time point which Caspase 8 activation is stronger. And then repeat the test.
7. This kit is for research use only. For your safety and health, please wear lab clothes and gloves. Instructions should be followed strictly, changes of operation may result in unreliable results.