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

γ-Glutamate Cysteine Ligase Activity Assay Kit

Catalog No.: BC00156

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	γ-Glutamate Cysteine Ligase Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Bacteria, fungi, tissues, serum, plasma
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (optimal detection wavelength 660 nm)

Product Introduction

GCL is the rate-limiting enzyme in the synthesis of reduced glutathione (GSH), and GSH has a feedback inhibitory effect on GCL. GCL gene expression is regulated by a variety of factors, such as oxidants, antioxidants, growth factors, and inflammatory factors. The level of GCL activity has a significant impact on GSH content and the ratio of reduced to oxidized glutathione (GSH/GSSG).

Principle

In the presence of ATP and Mg^{2+}, GCL catalyzes the synthesis of γ-glutamylcysteine from glutamate and cysteine; at the same time, ATP decomposes to produce inorganic phosphorus. The activity of GCL can be calculated by measuring the rate of increase of inorganic phosphorus.

Components

Components	Size	Storage
Reagent 1	Liquid × 1 bottle	2~8°C
Reagent 2	Powder × 1 bottle	2~8°C
	Add 14mL of distilled water and shake thoroughly to dissolve before use.	

Reagent 3	Powder × 1 tube	2~8℃
	Add 3.5 mL of distilled water and shake well to dissolve before use.	
Reagent 4	Liquid × 1 bottle	RT
Reagent 5	Powder × 1 tube	2~8℃
	Just before use, add 30mL of distilled water and shake thoroughly to dissolve. Slowly add 1.0mL of concentrated sulfuric acid while stirring.	

Storage

The kit has a shelf life of 6 months.

Preparation before the experiment

Sample processing

1. Tissue sample preparation: Homogenize the tissue sample at a ratio of 1:5~10 (tissue mass (g): reagent volume (mL) – it is recommended to weigh approximately 0.1g of tissue and add 1mL of reagent – in an ice bath. Centrifuge at 10000g, 4℃ for 10min, and collect the supernatant on ice for analysis.
2. Bacterial and fungal samples: Disrupt the cells by sonication in an ice bath at a ratio of 500-1000:1 (1 mL of reagent 1 is recommended for 5 million cells). The cells are 10^4 cells per volume of reagent 1. The cells are 300W, 3 seconds of sonication, 7 seconds of interval, and a total of 3 minutes. The cells are then centrifuged at 1000 rpm for 10 minutes at 4°C. The supernatant is collected and placed on ice for testing.
3. Liquids such as serum plasma: sample directly.

Operation process

1. Preheat the spectrophotometer for at least 30 minutes, adjust the wavelength to 660 nm, and zero it with distilled water;

2. Operating steps;

Reagents(μL)	Test tube	Control tube
Test sample	120	--
Reagent 1	240	240
Reagent 2	260	260
Reagent 3	60	60
Mix well and incubate in a water bath at 37°C for 15 minutes.		
Reagent 4	300	300
Test sample		120
Mix well, centrifuge at 10,000 rpm for 10 minutes at room temperature (25°C), and take 500 μL of the supernatant for testing.		
Supernatant	500	500
Reagent 5	500	500
After mixing thoroughly, seal tightly and incubate in a 45°C water bath for 10 minutes. After cooling, zero the blank tube (or use distilled water to zero the tube) and measure the absorbance value of each tube at 660 nm. Calculate: $\Delta A=A_{test}-A_{control.}$		

Result calculation

1. Calculated based on sample protein concentration

Unit definition: At 37°C, one unit (1 U) of GCL enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 μg inorganic phosphorus per minute per milligram of protein.

$$\text{GCL activity (U/mg prot)} = \Delta A \div 0.1427 \times \frac{V_{\text{reaction total}}}{Cpr \times V_{\text{sample}}} \div T = 3.815 \times \Delta A \div Cpr$$

2. Calculated by sample quality

Unit definition: At 37°C, one unit (1 U) of GCL enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 μg inorganic phosphorus per minute per gram of sample.

$$\text{GCL activity (U/g)} = \Delta A \div 0.1427 \times \frac{V_{\text{reaction total}}}{W \times V_{\text{sample}} \div V_{\text{sample total}}} \div T = 3.815 \times \Delta A \div W$$

3. Calculated by cell number

Unit definition: At 37°C, one unit (1 U) of GCL enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 μg inorganic phosphorus per minute per 10^4 cells.

$$\text{GCL activity (U/10}^4\text{cell)} = \Delta A \div 0.1427 \times \frac{V_{\text{reaction total}}}{\text{cell number} \times V_{\text{sample}} \div V_{\text{sample total}}} \div T$$

$$= 3.815 \times \Delta A \div \text{cell number}$$

4. Calculated by sample volume

$$\text{GCL activity (U/mL)} = \Delta A \div 0.1427 \times \frac{V_{\text{reaction total}}}{V_{\text{sample}}} \div T = 3.815 \times \Delta A$$

Annotation

0.1427: coefficients of the regression equation;

$V_{\text{reaction total}}$: Total volume of the reaction system, 980 μL = 0.98 x 10^{-3}L

$V_{\text{sample total}}$: 1 mL of the extracted solution added;

V_{sample} :Volume of supernatant added to the reaction system (mL), 120μL = 0.12mL;

T:Catalytic reaction time, 15 min;

Cpr: Protein concentration in the supernatant (mg/mL), which needs to be determined separately. It is recommended to use our company's BCA protein content assay kit (BC00006).

W: Sample mass, g;

Cell count: 10^4.

Precautions

1. Sample processing and other procedures must be carried out on ice, and enzyme activity must be measured on the same day to avoid affecting its activity. For homogenates, repeated freeze-thaw cycles should be avoided.
2. Once all powder solutions have been prepared, unless otherwise specified, please use them on

the same day.

3. Please wear gloves during the experiment. Reagent 3 contains a highly corrosive substance; please be careful not to splash it on your skin or into your eyes.
4. Absorbance measurements should be completed within 10-40 minutes after the water bath.
5. For sample testing, please take 1-2 samples for preliminary testing first. If the absorbance is found to be too high, it should be diluted with reagent 1 (or physiological saline) by an appropriate factor. Mammalian tissues are generally diluted 3-5 times.
6. During the preparation of reagent 3, a black solid may be generated. Take care not to inhale the black solid when aspirating it, as it will not affect the results.
7. When measuring GCL activity in cells, the cell count should be between 3 million and 5 million. When extracting GCL from cells, reagent 1 (or physiological saline) can be added and then the cells can be ground or sonicated. Cell lysis buffer should not be used to treat the cells.
8. This product is for research purposes only and must not be used for clinical diagnosis. Do not take it.