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γ-Glutamylcysteine Ligase(GCL) Activity Assay Kit

Detection equipment: Spectrophotometer/ Microplate reader

Note: Take two or three different samples for prediction before test.

Cat No:AK0471

Size:100T/48S

Components

Reagent I:55 mL×1, store at 4℃.

Reagent II: Powder×1, store at 4℃. Add 6 mL of distilled water when the solution will be used, mix thoroughly. Keep the unused reagents in separate packages at -20 ℃. It is not allowed to freeze and thaw repeatedly.

Reagent III: Powder×1, store at 4℃. Add 1.5 mL of distilled water when the solution will be used, mix thoroughly.

Reagent IV:7mL×1,store at 4℃.

Reagent V: Powder×1, store at 4℃. Add 12 mL of distilled water when the solution will be used, mix thoroughly. Then slowly add 400 μLof concentrated sulfuric acid(self-prepared) with stir.

Standard: 1 mL×1, store at 4℃, prepare as 1 μmol/Lphosphorus standard solution.

Description

GCL(Glutamylcysteineligase) is the rate limiting enzyme of GSH synthesis. GSH has feedback inhibition to GCL. GCL gene express manipulated by many factors, such as oxidants, antioxidants, growth factors and inflammatory factors. The activity of GCL has important influence to GSH content and ratio of GSH/GSSG.

In the presence of ATP and Mg^{2+}, GCL catalyzes the synthesis of γ-glutamyl cysteine from glutamate and cysteine, and Dephosphorylation of ATP produce inorganic phosphorus molecules. The activity of GCL can be calculated by measuring the increasing rate of inorganic phosphorus.

Required but not provided

Spectrophotometer/ Microplate reader, Refrigerated centrifuge, water bath, adjustablepipette, micro glass cuvette/96 well flat-bottomplate, concentrated sulfuric acid and distilled water.

Protocol

I. Sample Extraction:

1. Tissue

The proportion of tissue mass (g): Reagent I (mL) is 1:5 ~ 10 (It is recommended to weigh about 0.1 g of tissue and add 1 mL of Reagent I), carry out ice bath homogenization. Centrifuge at 8000 ×g for 10 minutes at 4℃, the supernatant is ready for test.

2. Bacteria or cells

The proportion of cells(10^4) : Reagent I is 500~1000: 1, It is recommended to 5 million cells and add 1 mL of Reagent I) ultrasonic smash cells (powder 300W, ultrasonic 3s, interval 7s, 3 minutes). Centrifuge at 8000 ×g and 4℃ for 10 minutes, the supernatant is ready for test.

3. Serum: Directly detect.

II. Procedure

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 660 nm, set zero with distilled water.

2. prepare as 0.1 μmol/mL phosphorus standard solution dilutedby using distilled water.

3. Add reagents according to the following table.

Reagents (μL)	Control tube (A_C)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Reagent I	48	48	-	-
Reagent II	52	52	-	-
Reagent III	12	12	-	-
Sample	-	24	-	-
Mix thoroughly, water bath at 37℃ for 15minutes.				
Reagent IV	60	60	-	-
Sample	24	-	-	-
Mix thoroughly, incubate at 25℃, Centrifuge at 10000 rpm for 10 minutes.				
Supernatant	100	100	-	-
Phosphorus standard	-	-	100	-
Distilled water	-	-	-	100
Reagent V	100	100	100	100
Mix thoroughly, 45℃ water bath for 10 minutes, detect the absorbance of 660 nm after cooling, detect as soon as possible.				

III. Calculation

1. Protein concentration

Definition:One unit of enzyme activity is defined as the amount ofenzyme catalyzes the produce of 1 μmol inorganic phosphorus at 37℃every milligram of protein per minute.

$GCL(U/mg\ prot)=[\Delta A_T \div (\Delta A_S \div C_S) \times V_{RT}] \div (Cpr \times V_S) \div T = 0.0544 \times \Delta A_T \div \Delta As \div Cpr$

2. Sample weight

Definition: One unit of enzyme activity is defined as the amount of enzymecatalyzes the produce of 1 μmol inorganic phosphorus at 37℃every gram of sample per minute.

$GCL(U/g)=[\Delta A_T \div (\Delta A_S \div C_S) \times V_{RT}] \div (W \div V_{ST} \times V_S) \div T = 0.0544 \times \Delta A_T \div \Delta A_S \div W$

3. Cells amount

Definition: One unit of enzyme activity is defined as the amount of enzymecatalyzes the produce of 1 μmol inorganic phosphorus at 37℃every 10^4 cells per minute.

$GCL(U/10^4 cell)=[\Delta A_T \div (\Delta A_S \div C_S) \times V_{RT}] \div (\text{cell amount} \times V_S \div V_{ST}) \div T = 0.0544 \times \Delta A_T \div \Delta A_S \div \text{cell amount}$

4. Liquid volume

Definition: One unit of enzyme activity is defined as the amount of enzymecatalyzes the produce of 1 μmol inorganic phosphorus at 37℃every milliliter of liquid per minute.

$GCL(U/mL)= [\Delta A_T \div (\Delta A_S \div C_S) \times V_{RT}] \div V_S \div T = 0.0544 \times \Delta A_T \div \Delta A_S$

V_{RT}: Reaction total volume,0. 196 mL;

V_S :Sample volume, 0.024 mL;

Cpr: Supernatant protein concentration, mg/mL;

T: Reaction time, 15 minutes;

C_S : Concentration of phosphorus standard solution, 0.1 μmol/mL;

V_{ST} : Extraction solution volume, 1 mL;

W: Sample weight, g;

Number of cells: 10^4 as units.

Note:

1. Sample treatment and other processes need to be carried out on ice, detect the enzyme activity within in a day, in order to avoid enzyme activity. If it is homogenate, avoid repeating freeze and thaw.

2. Before sample measurement, take 1-2 samples for pre-experiment. If the absorbance value is greater than 1, use Reagent I (or normal saline) to dilute to an appropriate multiple. Generally, mammalian tissues and blood are diluted by 3-5 times.

3. When GCL activity was measured, the number of cells must be between 3-5 million. When extracting GCL from cells, Reagent I (or normal saline) can be added, and then it can be grinded or treated by ultrasound. Cell lysate cannot be used to treat cells.

4. After preparing Reagent III, store at 4℃, use them within one week.

5. During the preparation of Reagent V, black solid may be produced, which will not affect the results. Pay attention not to inhale the black solid. The solution shall be light yellow after preparation. If it is blue, it is polluted and cannot beused again.

6. Detect the absorbance value within 10-40 minutes after incubating water bath.

Experimental instances：

1. Take 0. 1g of clover, add 1mL of extract solution, fully grinding on ice. Centrifuge at 8000g for 10 minutes at 4℃, take the supernatant, dilute 4 times and place it on ice for test according to the measured steps. Calculate $\Delta A_T = A_T - A_C = 0.784 - 0.415 = 0.369$， $\Delta A_S = A_S - A_B = 0.371 - 0.042 = 0.329$, calculate the enzyme activity according to sample weight:

GCL（U/g weight）$=0.0544 \times \Delta A_T \div \Delta A_S \div W = 0.61$ U/g weight.

2. Take 0. 1g of willow, add 1mL of extract solution, fully grinding on ice. Centrifuge at 8000g for 10 minutes at 4℃, take the supernatant, place it on ice for test according to the measured steps. Calculate $\Delta A_T = A_T - A_C = 0.530 - 0.366 = 0.164$， $\Delta A_S = A_S - A_B = 0.371 - 0.042 = 0.329$, calculate the enzyme activity according to sample weight:

GCL（U/g weight）$=0.0544 \times \Delta A_T \div \Delta A_{标} \div W = 0.271$ U/g weight.

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