SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

γ-Glutamylcysteine Ligase(GCL) Activity Assay Kit

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

Detection equipment: Spectrophotometer

Cat No:AK0472

Size:50T/24S

Components:

Reagent I:40 mL×1, store at 4℃.

Reagent II: Powder×1, store at 4℃. Add 14 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 3.5 mL of distilled water when the solution will be used, mix thoroughly.

Reagent IV:16 mL×1,store at 4℃.

Reagent V: Powder×1, store at 4℃. Add 30 mL of distilled water when the solution will be used, mix thoroughly. Then slowly add 1.0 mL of concentrated sulfuric acid(self-prepared) with stir.

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

Description

GCL(Glutamylcysteine ligase) 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, refrigerated centrifuge, water bath,adjustable pipette, 1mL glass cuvette, 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 8000g and 4℃ for 10 minutes, 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 300 w, ultrasonic 3 s, interval 7 s, 3 minutes); Centrifuge at 8000g and 4℃ for 10 minutes, the supernatant is ready for test.

3. Serum: Directly detect.

II. Procedure

1. Preheat Spectrophotometer for 30 min, adjust wavelength to 660 nm, set zero with distilled water.
2. prepare as 0. 1μmol/mL phosphorus standard solution diluted by using distilled water.
3. Add reagents according to the following table.

Reagents (μL)	Contrast tube (A_C)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Reagent I	240	240		
Reagent II	260	260		
Reagent III	60	60		
Sample	-	120		
Mix thoroughly, water bath at 37℃ for 15 minutes.				
Reagent IV	300	300		
Sample	120	-		
Mix thoroughly, incubate at 25℃, Centrifuge at 10000 rpm for 10 minutes;				
Supernatant	500	500	-	-
Phosphorus standard	-	-	500	-
Distilled water	-	-	-	500
Reagent V	500	500	500	500
Mix thoroughly, water bath at 45℃ for 10 minutes, detect the absorbance of 660 nm after cooling, detect as soon as possible. $\Delta A_T=A_T-A_C$, $\Delta A_S=A_S-A_B$.				

III. Calculation

1. Protein concentration

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

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

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℃ each milliliter 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, 0.98 mL;

V_S: Sample volume, 0.12 mL;

Cpr: Supernatant protein concentration, mg/mL;

T: React 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 be used again.

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

Experimental instances:

1. Take 0. 1g of willow leaves, 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 $\Delta A_T = A_T - A_C = 0.923 - 0.635 = 0.288$, $\Delta A_S = A_S - A_B = 0.514 - 0.002 = 0.512$, calculate the enzyme activity according to sample weight:

GCL (U/g weight) $= 0.0544 \times \Delta A_T \div \Delta A_S \div W = 0.306$ U/g weight.

2. Take 0. 1g of spleen, add 1mL of extract solution, fully grinding on ice. Centrifuge at 8000g for 10 minutes at 4℃, take the supernatant, dilute it by 5 times, place it on ice for test according to the measured steps. Calculate $\Delta A_T = A_T - A_C = 0.713 - 0.285 = 0.428$, $\Delta A_S = A_S - A_B = 0.514 - 0.002 = 0.512$, calculate the enzyme activity according to sample weight:

GCL (U/g weight) $= 0.0544 \times \Delta A_T \div \Delta A_S \div W \times 5$ (Dilution Ratio) $= 2.274$ U/g weight

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