

## $\gamma$ -Glutamylcysteine Ligase (GCL) Activity Assay Kit

**Detection equipment:** Spectrophotometer/Microplate reader

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

**Cat No:**BC1215

**Size:**100T/48S

**Product Composition:** Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Reagent I	Liquid 65 mL×1	2-8°C
Reagent II A	Liquid 5 mL×1	2-8°C
Reagent II B	Powder×1	-20°C
Reagent III	Powder×1	2-8°C
Reagent IV	Liquid 7 mL×1	2-8°C
Reagent V	Powder×1	2-8°C
Reagent VI	Powder×1	2-8°C
Reagent VII	Liquid 5 mL×1	2-8°C
Standard	Liquid 1 mL×1	2-8°C

### Solution Preparation:

- Reagent II B: Add 2 mL of distilled water when the solution will be used, mix thoroughly. Unused reagents can be stored for 4 weeks in separate packages at -20°C. It is not allowed to freeze and thaw repeatedly.
- Preparation of Reagent II: According to the sample number, Reagent II is prepared with the ratio of reagent II A: reagent II B = 260 $\mu$ L: 104 $\mu$ L (total 364 $\mu$ L, 7T).
- Reagent III: Add 5 mL of distilled water when the solution will be used, mix thoroughly. Unused reagents can be stored for 4 weeks at 2-8°C.
- Reagent V: Add 5 mL of distilled water when the solution will be used, mix thoroughly. Unused reagents can be stored for 4 weeks at 2-8°C.
- Reagent VI: Add 5 mL of distilled water when the solution will be used, mix thoroughly. Unused reagents can be stored for 4 weeks at 2-8°C.
- Preparation of working solution: make solution as the volume ratio of Reagent V: Reagent VI: Reagent VII=0.1mL : 0.1mL : 0.1mL(total 0.3mL, 3T), which should be light yellow. It lose efficacy if its colour change. Prepare the reagent when it will be used. It is better to use new beaker, glass rod and glass pipettes, or disposable plastic ware when making reagent to avoid phosphorus pollution.
- Standard: 1 mL×1, store at 2-8°C, 10  $\mu$ mol/mL phosphorus standard solution.
- Preparation of 0.1  $\mu$ mol/mL phosphorus standard solution: 0.1 mL 10  $\mu$ mol/mL standard solution is mixed with 0.9 mL distilled water (1  $\mu$ mol/mL), then 0.1 mL 1 $\mu$ mol/mL standard solution and 0.9 mL

distilled water are mixed to prepare 0.1  $\mu\text{mol/mL}$  phosphorus standard solution.

## Description

GCL (Glutamylcysteinylase) 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  $\text{Mg}^{2+}$ , GCL catalyzes the synthesis of  $\gamma$ -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, adjustable pipette, micro glass cuvette/96 well flat-bottom plate, 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  $\times g$  for 10 minutes at 4°C, 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  $\times g$  and 4°C 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  $\mu\text{mol/mL}$  phosphorus standard solution diluted by using distilled water.

3. Add reagents according to the following table.

Reagents ( $\mu\text{L}$ )	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°C for 15 minutes.				
Reagent IV	60	60	-	-

Sample	24	-	-	-
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Mix thoroughly, incubate at 25°C, Centrifuge at 10000 rpm for 10 minutes.				
Supernatant	100	100	-	-
Phosphorus standard	-	-	100	-
Distilled water	-	-	-	100
Working solution	100	100	100	100
Mix thoroughly, 45°C 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 of enzyme catalyzes the produce of 1 μmol inorganic phosphorus at 37°C every milligram of protein per minute.

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

#### 2. Sample weight

Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the produce of 1 μmol inorganic phosphorus at 37°C 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 enzyme catalyzes the produce of 1 μmol inorganic phosphorus at 37°C every 10<sup>4</sup> cells per minute.

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

#### 4. Liquid volume

Definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the produce of 1 μmol inorganic phosphorus at 37°C 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<sub>RT</sub>: Reaction total volume, 0.196 mL;

V<sub>S</sub>: Sample volume, 0.024 mL;

C<sub>pr</sub>: Supernatant protein concentration, mg/mL;

T: Reaction time, 15 minutes;

C<sub>S</sub>: Concentration of phosphorus standard solution, 0.1 μmol/mL;

V<sub>ST</sub>: Extraction solution volume, 1 mL;

W: Sample weight, g;

Number of cells: 10<sup>4</sup> 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°C, 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 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°C, 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:

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

2. Take 0.1g of willow, add 1mL of extract solution, fully grinding on ice. Centrifuge at 8000g for 10 minutes at 4°C, 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:

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

**Related products:**

BC1170/ BC1175 Reduced Glutathione (GSH) Assay Kit

BC1180/BC1185 Oxidized Glutathione (GSSG) Assay Kit