γ -Glutamyl Cysteine Ligase (GCL) Activity Colorimetric Assay Kit

Cat #: orb1173212 (manual)

Product name: γ -Glutamyl Cysteine Ligase (GCL) Activity Colorimetric Assay Kit

Catalog number: orb1173212

Detection range: 0.0125-0.8 μmol/mL (0.0675-4.32 U/g)

Sensitivity: 0.0125 μmol/mL (0.0675 U/g)

Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Bacteria, Fungus

Storage: Stored at -20℃ for 6 months, protected from light

Assay Principle

γ-Glutamyl Cysteine Ligase (GCL) is the rate-limiting enzyme for GSH synthesis, and GSH has a feedback inhibitory effect on GCL. GCL gene expression is regulated by many factors, such as oxidants, antioxidants, growth factors and inflammatory factors.

The level of GCL activity has an important influence on the GSH content and the GSH/GSSG ratio. Micro γ-Glutamyl Cysteine Ligase (GCL) Activity Assay Kit provides a convenient tool for detection of GCL. The principle is that in the presence of ATP and Mg2+, GCL catalyzes the synthesis of γ -glutamyl cysteine from glutamic acid and cysteine; at the same time, ATP dephosphorylation produces inorganic phosphorus molecules. GCL activity can be calculated by measuring the increase rate of inorganic phosphorus.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	70 mL	70 mL×2	4℃
Reagent I A	2.4 mL	4.8 mL	4℃
Reagent I B	Powder×1 vial	Powder×1 vial	-20℃
Reagent II	Powder×1 vial	Powder×2 vials	4℃
Reagent III	7.5 mL	15 mL	4℃
Reagent IV	Powder×1 vial	Powder×2 vials	4℃, protected from light
Standard	1 mL	2 mL	4℃

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

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Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 660 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, water bath, ice maker, incubator
- Deionized water, concentrated sulfuric acid
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Reagent I A: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent I B: Add 2.4 mL deionized water for 96 T or 1.2 mL deionized water for 48 T to dissolve before use. Fully shake to dissolve. This solution can be stored at -20℃ for 1 month after aliquoting to avoid repeated freezing and thawing.

Working Reagent I: Prepare before use. According to the sample size in accordance with the ratio of Reagent I A: Working Reagent I B=2:1, and used up on the same day.

Working Reagent II: Prepare before use, add 1.53 mL deionized water to each tube, and fully shake to dissolve. This solution can be stored at -20℃ for 1 week after aliquoting to avoid repeated freezing and thawing.

Reagent III: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Working Reagent IV: Add 13.5 mL deionized water for each tube to dissolve before use. After shaking to dissolve, slowly add 450 µL of concentrated sulfuric acid, stirring while adding. This solution can be stored at -20℃ for 1 week after aliquoting to avoid repeated freezing and thawing.

Note: Concentrated sulfuric acid is highly corrosive, please pay attention to safety during operation. During the preparation process, black solids may be produced, which will not affect the results. Be careful not to absorb black solids during operation. Under normal circumstances, the prepared solution should be light yellow. If it is blue, it means that the solution is contaminated and cannot be used.

Setting of standard curve: Dilute the Standard (8 µmol/mL) to 0.8, 0.4, 0.2, 0.1, 0.05, 0.025, 0.0125 µmol/mL standard solution with deionized water, as shown in the following table.

Num.	Volume of Standard	Volume of Deionized Water (µL)	The Concentration of Standard (µmol/mL)
Std.1	100 µL of 8 µmol/mL	900	0.8
Std.2	100 µL of Std.1 (0.8 µmol/mL)	100	0.4
Std.3	100 µL of Std.2 (0.4 µmol/mL)	100	0.2
Std.4	100 µL of Std.3 (0.2 µmol/mL)	100	0.1
Std.5	100 µL of Std.4 (0.1 µmol/mL)	100	0.05
Std.6	100 µL of Std.5 (0.05 µmol/mL)	100	0.025
Std.7	100 µL of Std.6 (0.025 µmol/mL)	100	0.0125

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80 ℃ for one month. Sample processing and other processes need to be carried out on ice, and the enzyme activity must be determined on the same day to avoid affecting its activity. If it is a homogenate, avoid repeated freezing and thawing. When measuring GCL activity in cells, the number of cells must be between 3 million and 5 million. When extracting GCL from cells, Extraction Buffer can be added for grinding or ultrasonic treatment. Cells cannot be treated with cell lysate.

1. Animal or plant tissue samples: Weigh about 0.1 g tissue and add 1 mL Extraction Buffer. Homogenize on ice. Centrifuge at 8,000 g for 10 min at 4°C. Use supernatant for assay, and place it on ice to be tested.
2. Bacteria, fungus, cells: Collect 5×106 bacteria, fungus or cells into the centrifuge tube; add 1 mL Extraction Buffer to ultrasonically disrupt 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at 4℃. Use supernatant for assay, and place it on ice to be tested.
3. Serum (Plasma) and other liquid samples: Tested directly.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 660 nm, visible spectrophotometer was returned to zero with deionized water.
2. Preheat the incubator to 37℃.
3. Add the following reagents respectively into EP tube.

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)	Control Tube (μL)
Sample	0	0	24	0
Extraction Buffer	0	0	48	100
Working Reagent I	0	0	52	0
Working Reagent II	0	0	12	12

Mix well and incubate in 37℃ water bath for 15 min (cover tightly to prevent water loss)

Reagent III	0	0	60	60
Sample	0	0	0	24

Mix well and centrifuge at 10,000 rpm for 10 min at room temperature (about 25℃)

supernatant	0	0	100	100
Different Concentration Std.	0	100	0	0
Deionized Water	100	0	0	0
Working Reagent IV	100	100	100	100

Mix well, incubate in 45℃ water bath for 10 min (cover tightly to prevent water loss), cool to room

temperature. Take 180 μL of the supernatant is added to a 96-well plate or microglass cuvette, then the absorbance value at 660 nm is measured, recorded as A_{Blank}, $A_{Standard}$, A_{Test} and $A_{Control}$. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$. Blank tube only needs to measure one time.

Note: Please complete the measurement of absorbance within10-40 min after water bath. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is greater than 2, the sample can be appropriately diluted with Extraction Buffer (or normal saline), the calculated result multiplied by the dilution factor. If A_{Test} is less than 0.005, the sample size can be increased appropriately increased.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve; Substitute the ΔA_{Test} into the equation to get the y value (μmol/mL).

2. Calculation of GCL activity

(1) Calculated by protein concentration

Active unit definition: One unit defines as the amount of enzyme that catalyzes the production of 1 μmol Inorganic Phosphorus per min per mg of sample at 37℃.

GCL (U/mg prot)=$(y\times V_{Total})\div(Cpr\times V_{Sample})\div T$**=0.54×y÷Cpr**

(2) Calculated by fresh weight of samples

Active unit definition: One unit defines as the amount of enzyme that catalyzes the production of 1 μmol Inorganic Phosphorus per min per g of sample at 37℃.

GCL (U/g fresh weight)=$(y\times V_{Total})\div(W\div V_{Sample\ Total}\times V_{Sample})\div T$**=0.54×y÷W**

(3) Calculated by cells or bacteria number

Active unit definition: One unit defines as the amount of enzyme that catalyzes the production of 1 μmol Inorganic Phosphorus per min per 10,000 cells or bacteria at 37℃.

GCL ($U/10^4$)=$(y\times V_{Total})\div(500\times V_{Sample}\div V_{Sample\ Total})\div T$**=$1.08\times10^{-3}\times y$**

(4) Calculated by serum, plasma and other liquid samples volume

Active unit definition: One unit defines as the amount of enzyme that catalyzes the production of 1 μmol Inorganic Phosphorus per min per mL of sample at 37℃.

GCL (U/mL)=$(y\times V_{Total})\div V_{Sample}\div T$**=0.54×y**

Where: V_{Total}: total reaction volume, 0.196 mL.; Cpr: sample protein concentration, mg/mL.; V_{Sample}: sample volume added, 0.024 mL; T: reaction time, 15 min; W: sample weight, g; $V_{Sample\ Total}$: extract buffer added to samples, 1 mL; 500: Total number of bacteria or cells, 5×10^6.

Typical Data

Typical standard curve:

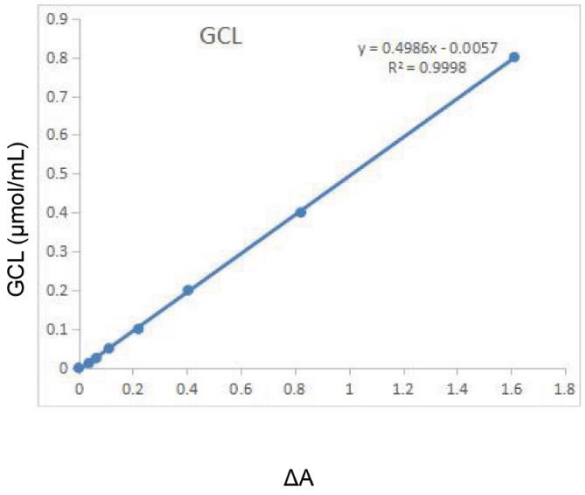

Figure 1. Standard curve for GCL.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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