Glutamine synthetase（GS） Assay Kit

(Cat/No.:BC110 Size:50T/24S)

1. Measurement significance

Glutamine synthetase (GS) is mainly found in plants and is one of the key enzymes for ammonia assimilation in organisms. It catalyzes the synthesis of glutamine from ammonium ions and glutamate, which not only prevents the toxicity of excessive ammonium ions to organisms, but glutamine is also the main storage and transportation form of ammonia.

2. Determination Principle

GS catalyzes the synthesis of glutamine from ammonium ions and glutamate; glutamine is further converted into γ-glutamyl hydroxamic acid. The complex formed under acidic conditions has a maximum absorption peak at 540nm, and its absorbance value can be measured by a spectrophotometer to determine the activity of GS.

3. Instruments and consumables required

Visible light spectrophotometer, constant temperature water bath, centrifuge, 1mL glass or quartz cuvette, mortar, distilled water, etc.

4. Composition and configuration (The kit is valid for 3 months)

Extract solution：30mL×1 bottle, stored at 4℃.

Reagent 1：12mL × 1 bottle, stored at -20℃. Preheat at 37℃ for 20min before use, mix thoroughly, if there is precipitation, let stand for 10min, and take the supernatant for later use.

Reagent 2：12mL × 1 bottle, stored at -20℃. Preheat at 37℃ for 20min before use, mix thoroughly, if there is precipitation, let stand for 10min, and take the supernatant for later use.

Reagent 3：2 bottles of powder, stored at -20℃. Add 5mL of distilled water to each bottle of powder and dissolve it fully for use.

Reagent 4：15mL×1 bottle, stored at 4℃.

5. Sample pretreatment

1. Tissue：Mix the tissue weight (g) and extract volume (mL) at a ratio of 1:10, homogenize in an ice-water bath, centrifuge at 4000 rpm for 10 min, and place the supernatant on ice for testing.

2. Serum (plasma) : direct sampling and measurement.

3. Cell, bacterial or tissue samples : Collect cells or bacteria in a centrifuge tube, discard the supernatant after centrifugation, mix the cells or bacteria (10^4) and the extract volume (mL) at a ratio of 500:1, and disrupt the cells or bacteria with ultrasound (conditions: ice bath, power 20% or 200W, ultrasound for 3s, interval 10s, repeat 30 times) before testing.

6. Determination steps

	Determination tube	Control tube
Sample to be tested (μL)	175	175
Reagent 1 (μL)	400	
Reagent 2 (μL)		400
Reagent 3 (μL)	175	175
Mix well and incubate at 37℃ (animals) or 25℃ (other species) for 30 min.		
Reagent 4 (μL)	250	250
Mix well, let stand at room temperature for 10 minutes, centrifuge at 4000 rpm for 10 minutes, take the supernatant, adjust the light path to zero with distilled water at 540 nm, measure the OD value of each tube, △A = $A_{determination} - A_{control}$		

Note: Before conducting the formal experiment, it is best to take 1-2 sample supernatants for preliminary testing in order to determine the optimal sample concentration.

7. Calculation Method

The standard curve is: 0.8348x+0.0008, R2 = 0.9999

1. Serum (plasma) calculation method

Unit definition : One unit of enzyme activity is the amount of γ-glutamyl hydroxamic acid produced per mL of serum (plasma) per hour in each mL of reaction system.

Calculation formula :

$$\text{GS activity in serum (plasma) (μmol/h/mL)} = \frac{\Delta A - 0.0008}{0.8348} \times V_{Total} \div V_{Sample} \div T$$

2. Calculation methods for tissues, bacteria, and cells

(1) Calculated according to sample protein concentration :

Unit definition : One unit of enzyme activity is the amount of 1 μmol of γ-glutamyl hydroxamic acid produced per mg of tissue protein per mL of reaction system per hour.

Calculation formula :

$$\text{GS activity in tissues, cells and bacteria (μmol/h/mg prot)} = \frac{\Delta A - 0.0008}{0.8348} \times V_{Total} \div (Cpr \times V_{Sample}) \div T$$

(2) Calculated based on the fresh weight of the sample :

Unit definition: One unit of enzyme activity is the amount of 1 μmol of γ-glutamyl hydroxamic acid produced per gram of tissue in per mL of reaction system per hour.

Calculation formula :

$$\text{GS activity in tissue, cell, and bacteria } (\mu mol/h/g) = \frac{\Delta A - 0.0008}{0.8348} \times V_{Total} \div (W \times V_{Sample} \div V_{Totalsample}) \div T$$

(3) Calculated by bacterial or cell density :

Unit definition : One unit of enzyme activity is when 10,000 cells or bacteria produce 1 μmol of γ -glutamyl hydroxamic acid per hour in each mL of reaction system.

Calculation formula :

$$\text{GS activity in cells or bacteria } (\mu mol/h/10^4 \text{ cell}) = \frac{\Delta A - 0.0008}{0.8348} \times V_{Total} \div (500 \times V_{Sample} \div V_{Totalsample}) \div T$$

V_{total} : total volume of reaction solution, 0.75 mL;

V_{Sample} : sampling volume 0.175mL;

T : reaction time, 0.5h;

Cpr : sample protein concentration, mgprot/mL (prot refers to protein);

W : sample mass, g;

$V_{total\ sample}$: total volume of the extract;

500 : Number of cells, ten thousand.