(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Glutamine Synthetase (GS) Activity Assay Kit

Catalog No.: BC00143

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Glutamine Synthetase (GS) Activity Assay Kit
Detection Method	Colorimetric
Sample Type	Whole blood, serum, plasma
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (optimal detection wavelength 540 nm)

Product Introduction

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

Principle

In the presence of ATP and Mg^{2+}, GS catalyzes the synthesis of glutamine from ammonium ions and glutamate; glutamine is further converted into γ-glutamylhydroxamic acid. The complex formed under acidic conditions has a maximum absorption peak at 540 nm, and its absorbance can be measured with a spectrophotometer to determine the activity of GS.

Components

Components	Size	Storage
Extract	30 mL	2~8℃
Reagent 1	12 mL	-20℃
	Preheat at 37℃ for 20 minutes before use, mix thoroughly. If sediment forms, let stand for 10 minutes, then collect the supernatant for later use.	

Reagent 2	12 mL	-20℃
	Preheat at 37℃ for 20 minutes before use, mix thoroughly. If sediment forms, let stand for 10 minutes, then collect the supernatant for later use.	
Reagent 3	2 vials of powder, 6 mL of diluent	-20℃
	Add 5mL of distilled water to each bottle of powder and dissolve thoroughly before use.	
Reagent 4	15 mL	2~8℃

Storage

The kit has a shelf life of 3 months

Preparation before the experiment

Sample processing

1. Tissue: After mixing 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 take the supernatant and place it on ice for testing.
2. Serum (plasma): direct sampling and testing.
3. Cell, bacterial, or tissue samples: Collect cells or bacteria in centrifuge tubes, discard the supernatant after centrifugation, and mix them at a ratio of 500:1 for the number of cells or bacteria (10^4): extraction liquid volume (mL). Disrupt the cells or bacteria with ultrasound (conditions: ice bath, power 20% or 200W, ultrasound for 3 seconds, interval 10 seconds, repeat 30 times) before analysis.

Operation process

Reagents(μL)	Test tube	Control tube
Sample to be tested	175	175
Reagent 1	400	--
Reagent 2	--	400
Reagent 3	175	175
Mix well and react at 37°C (animals) or 25°C (other species) for 30 min.		
Reagent 4	250	250
Mix well, let stand at room temperature for 10 min, centrifuge at 4000 rpm for 10 min, take the supernatant and measure the OD value of each tube at 540 nm using a 1 cm path length cuvette, zero the instrument with distilled water, and $\Delta A = A_{test} - A_{control}$.		

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

Result calculation

1. Serum (plasma) calculation method :

Unit definition: One enzyme activity unit is defined as the production of 1 μmol of γ-glutamyl isohydroxamic acid per hour per mL of serum (plasma) in each mL of reaction system.

$$\text{GS activity } (\mu\text{mol/h/mL}) = \frac{\triangle A - 0.0008}{0.8348} \times V_{\text{reaction total}} \div V_{\text{sample}} \div T$$

2. Computational methods for tissues, bacteria, and cells

(1) Calculated based on sample protein concentration

Unit definition: One enzyme activity unit is defined as the production of 1 μmol of γ-glutamyl isohydroxamic acid per hour per mg of tissue protein in a mL reaction system.

$$\text{GS activity } (\mu\text{mol/h/mg prot}) = \frac{\triangle A - 0.0008}{0.8348} \times V_{\text{reaction tota}} \div (Cpr \times V_{\text{sample}}) \div T$$

(2) Calculated based on sample fresh weight

Unit definition: One enzyme activity unit is defined as the production of 1 μmol of γ-glutamyl isohydroxamic acid per hour by each g of tissue in each mL of reaction system.

$$\text{GS activity } (\mu mol/h/g) = \frac{\triangle\ A - 0.0008}{0.8348} \times V_{\text{reaction tota}} \div (W \times V_{\text{sample}} \div V_{\text{sample total}}) \div T$$

(3) Calculated by bacterial or cell density

Unit definition: One enzyme activity unit is defined as the production of 1 μmol of γ-glutamyl isohydroxamic acid per hour by 10,000 cells or bacteria in each mL of reaction system.

$$\text{GS activity } (\mu mol/h/10^4 \text{ cell}) = \frac{\triangle\ A - 0.0008}{0.8348} \times V_{\text{reaction tota}} \div (500 \times V_{\text{sample}} \div V_{\text{sample total}}) \div T$$

Annotation

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

V_{sample} : 0.175 mL;

T : Reaction time, 0.5 h;

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

W : Sample mass, g;

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

500 : Cell count, in tens of thousands.

Precautions

This product is for research use only and must not be used for clinical diagnosis. Do not take it.