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

Glutaminase (GLS) Assay Kit

Catalog No.: BC00166

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	Glutaminase (GLS) Assay Kit
Detection Method	Colorimetric
Sample Type	Tissues, cells, serum, plasma, bacteria
Assay Type	Quantitative
Detection Instrument	Spectrophotometer (420 nm)

Product Introduction

GLS is present in higher animals, certain bacteria and plant roots. It catalyzes the hydrolysis of glutamine into glutamic acid and ammonia, playing a vital regulatory role in nitrogen metabolism, especially in modulating free ammonia levels and urea metabolism.

Principle

GLS catalyzes the hydrolysis of glutamine into L-glutamate and ammonia. The enzyme activity can be calculated by measuring the increasing rate of ammonia with Nessler's reagent.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	60 mL	Store at 4°C
Reagent 2	Powder	2 vials	Store at 4 °C away from light. Before use, add 12.5 mL distilled water to each bottle and dissolve completely for later use; prepare freshly right before use.
Reagent 3	Liquid	30 mL	Store at room temperature
Reagent 4	Liquid	10 mL	Store at room temperature
Reagent 5	Liquid	6 mL	Store at room temperature
Reagent 6	Liquid	6 mL	Store at room temperature and protect from light.

Storage

The kit has a validity period of 6 months.

Preparation

Benchtop centrifuge, visible spectrophotometer, water bath, 1 mL glass cuvette, adjustable pipette, mortar, ice and distilled water.

- **Sample handling**

1. Tissues: Homogenize tissues on ice at a ratio of tissue mass (g) to Reagent 1 volume (mL) of 1:5-10 (it is recommended to weigh approximately 0.1 g tissue and add 1 mL Reagent 1). Centrifuge the homogenate at 8000 g and 4 °C for 10 min, collect the supernatant, and keep it on ice for subsequent detection.
2. Bacteria, fungi and cells: Lyse cells by sonication on ice at a ratio of cell count (10^4 cells) to extraction buffer volume (mL) of 500-1000:1 (it is recommended to add 1 mL Reagent 1 for 5 million cells). Sonication parameters: power 200 W, sonicate for 3 s, interval 10 s, repeat 30 cycles. After sonication, centrifuge at 8000 g and 4 °C for 10 min, collect the supernatant and place it on ice for testing.
3. Serum/plasma: Direct detection without pretreatment

Operation process

1. Preheat the spectrophotometer for more than 30 min, set the wavelength to 420 nm, and zero the instrument with distilled water.
2. Sample measurement (add the following reagents into EP tubes):

	Assay tube	Control tube
Samples (μL)	25	
Distilled water (μL)		25
Reagent 1 (μL)	100	100
Reagent 2 (μL)	400	400
Mix thoroughly and incubate in a 37 °C water bath for 1 hour.		

Reagent 3 (μL)	525	525
Mix well, centrifuge at 8000 g at room temperature for 10 min; collect the supernatant for detection.		
Supernatant (μL)	650	650
Reagent 4 (μL)	150	150
Reagent 5 (μL)	100	100
Reagent 6 (μL)	100	100
Mix thoroughly and stand for 15 min, then record the absorbance value A at 420 nm. Calculate $\Delta A = A_{sample} - A_{control}$. Only one blank tube needs to be prepared.		

Notes:

① If precipitate forms in Reagent 6, take the supernatant after standing for detection.

② A negative Δ A value may indicate extremely low enzyme activity. Extend the reaction time to 2 hours and divide the result by 2 during calculation.

Calculation

1. Calculation based on protein concentration

Definition of enzyme activity: One unit (U) of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol ammonia from glutamine per milligram of protein per minute.

$$\text{GLS Activity (nmol/min/mg)} = \frac{(\Delta A - 0.0057) \times V_{Total} \times 1000}{3.8488 \times V_{sample} \times Cpr \times T}$$

2. Calculation based on sample mass

Definition of enzyme activity: One unit (U) of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol ammonia from glutamine per gram of tissue per minute.

$$\text{GLS Activity (nmol/min/g)} = \frac{(\Delta A - 0.0057) \times V_{Total} \times V \times 1000}{3.8488 \times V_{sample} \times W \times T}$$

3. Calculation based on cell count

Definition of enzyme activity: One unit (U) of enzyme activity is defined as the quantity of enzyme that catalyzes the production of 1 nmol ammonia from glutamine per 10^4 bacteria or cells per minute.

$$\text{GLS Activity (nmol/min/}10^4) = \frac{(\Delta A - 0.0057) \times V_{Total} \times V \times 1000}{3.8488 \times V_{sample} \times 500 \times T}$$

4. Calculation for Serum (Plasma) Samples

Definition of enzyme activity: One unit (U) of enzyme activity is defined as the amount of enzyme that catalyzes the production of 1 nmol ammonia from glutamine per milliliter of serum (plasma) per minute.

$$\text{GLS Activity (nmol/min/mL)} = \frac{(\Delta A - 0.0057) \times V_{Total} \times 1000}{3.8488 \times V_{sample} \times T}$$

Annotation:

T: Reaction time, 60 min

V_{Total}: Total volume of reaction system, 1.05 mL

V_{sample}: Volume of sample added, 0.025 mL

V: Volume of extraction solution added, 1 mL

W: Sample mass, g

Cpr: Protein concentration of sample, mg/mL

500: Total number of bacteria or cells, 5 million

1000: Conversion factor for converting μmol to nmol

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.