GENLISA® Glutaminase, GLS Assay

Cat No: KBCA1536

Size: 50T/48S

I. Measurement Significance:

GLS (EC 3.5.1.2) exists in higher animals, some bacteria and plant roots. It catalyzes the hydrolysis of glutamine into glutamate and ammonia and plays an important regulatory role in nitrogen metabolism, especially in regulating free ammonia content and urea metabolism.

II. Principle (Spectrophotometry):

GLS catalyzes the hydrolysis of glutamine into L-glutamate and ammonia. The rate of ammonia increase can be detected using Nessler's reagent to calculate its enzyme activity.

III. Required instruments and supplies:

Benchtop centrifuge, visible spectrophotometer, water bath, 1 ml glass cuvettes, Adjustable pipette, mortar, ice, and distilled water.

IV. Composition and Preparation:

Reagent 1: 60ml×1 bottle, stored at 4ºC.
Reagent 2: 2 bottles of powder, store at 4ºC away from light; add 12.5ml of distilled water to each bottle to dissolve completely before use, prepare before use ;
Reagent 3: 30ml×1 bottle, store at room temperature.
Reagent 4: 10ml×1 bottle, store at room temperature.
Reagent 5: 6ml×1 bottle, store at room temperature.
Reagent 6: 6ml×1 bottle, store at room temperature and away from light.

V. Sample pre-treatment:

1. Tissue: homogenize in an ice bath at a ratio of 1:5-10 (tissue mass (g): reagent volume (ml) (it is recommended to weigh about 0.1g tissue and add 1ml reagent 1), then centrifuge at 8000g, 4ºC for 10min, take the supernatant, and place on ice for testing.
2. Bacteria, fungi, cells: according to the ratio of cell number (104): extract volume (ml) of 500-1000:1 (it is recommended to add 1ml of reagent 1 for 5 million cells), break the cells by ultrasonic wave in an ice bath (power 200w, ultrasonic wave 3s, interval 10s, repeat 30 times), then centrifuge at 8000g, 4ºC for 10min, take the supernatant and place it on ice for testing.
3. Serum/ plasma: direct measurement.

VI. Determination steps:

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 420nm, and zero with distilled water.

2. Sample determination (add the following reagents into the EP tube):

Reagent Name (ul)	Determination Tube	Control Tube
Sample	25	
Distilled water		25
Reagent 1	100	100
Reagent 2	400	400
Mix well and incubate in 37℃ water bath for 1 hour		
Reagent 3		
Mix well, centrifuge at 8000g for 10 min at room temperature, and take the supernatant for testing.		
Supernatant		
Reagent 4		
Reagent 5		
Reagent 6		
Mix well, let stand for 15 minutes, read the absorbance A at 420nm, and calculate $\Delta A = A_{\text{test tube}} - A_{\text{control tube}}$. Only one control tube is needed.		

Note:
1. If reagent VI precipitates, take the supernatant for measurement after it is still.
2. If ΔA is negative, it may be that the enzyme activity is too low. The reaction time can be extended to 2 hours and divided by 2 when calculating.

VII. Calculation of GLS Vitality

Calculation of GLS Vitality:
The standard curve regression equation is y=3.8488x+0.0057, R 2 =0.9983x is the standard concentration (umol/ml), y is the absorbance value A (1) Calculated by protein concentration. One unit of enzyme activity is defined as the amount of glutamine catalyzed to produce 1 nmol of ammonia per minute per mg of protein.

$$\text{GLS Vitality (nmol/min/m gprot)} = \frac{(\Delta A - 0.0057) \times V_{Total} \times 1000}{3.8488 \times V_{Sample} \times Cpr \times T} = \frac{181.8 \times (\Delta A - 0.0057)}{Cpr}$$

Calculated by sample quality:
One unit of enzyme activity is defined as the amount of enzyme activity that catalyzes the conversion of glutamine to produce 1 nmol of ammonia per gram of tissue per minute.

$$\text{GLS Vitality (nmol/min/g Tissue)} = \frac{(\Delta A - 0.0057) \times V_{Total} \times V_{Total\ sample} \times 1000}{3.8488 \times V_{Sample} \times W \times T} = \frac{181.8 \times (\Delta A - 0.0057)}{W}$$

Calculated according to bacterial or cell density:

One unit of enzyme activity is defined as the amount of 10,000 bacteria or cells catalyzing glutamine to produce 1 n mol of ammonia per minute.

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

$$= 0.3637 \times (\Delta A - 0.0057)$$

Serum (plasma) calculation enzyme activity is defined as the amount of glutamine produced by 1ml of serum (plasma) per minute.

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

$$= 181.8 \times (\Delta A - 0.0057)$$

Cpr : supernatant protein content mg/ml;
V_{sample} : sample loading volume, 0.025 ml;
$V_{sample\ total}$: volume of extract added , 1 ml;
V_{total} : total reaction volume, 1.05 ml ; Wsample : sample mass, g ;
T : reaction time, 60 min;
500 : total number of bacteria or cells, 5 million ;
1000 : umol to nmol conversion factor.

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