

Glutaminase (GLS) Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer

Catalog Number: AK0436

Size:50T/24S

Components:

Extract solution: 70 mL ×1, storage at 4C. Preheat at 37 °C before use.

Reagent I : powder×1, storage at 4C. Add 1 0mL of Extract to dissolve the reagent before use.

Reagent II A: 1 mL×1, storage at 4C.

Reagent II A: 4 mL×1, storage at 4C. Before use, pour Reagent II A into Reagent II B to mix (A:B=1:4 ratio), or prepare according to the volume ratio Reagent II A : Reagent II B = 1:4 before use.

Reagent III: 5 mL×1, storage at room temperature.

Standard: 1 mL ×1, storage at 4C. 10 μmol/mL nitrogen standard solution. Preheat at 37C before use.

Product Description:

GLS (EC3.5. 1.2) is mainly found in higher animals and some bacteria and plant roots, catalyzing the hydrolysis of glutamine into glutamic acid and ammonia, which plays an important role in the regulation of nitrogen metabolism, especially the regulation of free ammonia and urea metabolism.

The kit uses the indophenol blue colorimetric method to determine ammonia produced by glutamine of GLS-catalyzed to indicate activity

Reagents and Equipment Required but Not Provided:

Spectrophotometer, adjustable pipette, mortar/homogenizer, centrifuge, 1 mL glass cuvette, ice and distilled water.

Sample preparation:

1. Tissues: The mass (g): volume of distilled water(mL)= 1:5- 10, suggested 0. 1g of tissues, add 1 mL of Extract solution and fully grind. Centrifuge at 12000g at 4 °C for 15 min, then take supernatant on ice to be tested.
2. Bacteria or cells
Accordance ratio bacteria or cell amount (10^4): volume of Extract solution (mL)=500~1000:1. Suggested 5 million with 1 mL of Extract solution. Use ultrasonic to splitting bacteria or cell (placed on ice, powder: 300W, work time 3s , interval 7s , total time 3 min). Centrifuge at 12000g at 4°C for 15 min. then take supernatant on ice to be tested.

Procedure:

1. Preheat spectrophotometer for 30 min, adjust the wavelength to 630 nm and set the counter to zero with distilled water.

- Dilute the standard solution 32 times with the Extract solution to obtain the standard solution of 0.3125 $\mu\text{mol/mL}$.
- Add reagent to a EP tube:

Reagent name (mL)	Test tube (At)	Control tube (Ac)	Standard tube (As)	Blank tube (Ab)
Sample	80	80	-	-
Extract	-	320	-	400
Reagent I	320	-	-	-
Mix and react for 60 min at 37C			-	-
Standard	-	-	400	-
Reagent II	80	80	80	80
Reagent III	60	60	60	60
Distilled water	460	460	460	460

Mix well, react for 30min at room temperature. Measure the absorbance at 630nm. Recorded as At, Ac, As, Ab. Calculate $\Delta A_s = A_s - A_b$, $\Delta A_t = A_t - A_c$.

Calculation:

- Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of $\text{NH}_3\text{-N}$ per hour every milligram of tissue protein.

$$\text{GLS (U/mg prot)} = \Delta A_t \div (\Delta A_s \div C_{st}) \times V_{er} \div (V_{sa} \times C_{pr}) \div T = 1.5625 \times \Delta A_t \div \Delta A_s \div C_{pr}$$

- Fresh weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of $\text{NH}_3\text{-N}$ per hour every gram of sample.

$$\text{GLS (U/g)} = \Delta A_t \div (\Delta A_s \div C_{st}) \times V_{er} \div (W \div V_e \times V_{sa}) \div T = 1.5625 \times \Delta A_t \div \Delta A_s \div W$$

- Number of cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 μmol of $\text{NH}_3\text{-N}$ per hour every 5×10^4 cells.

$$\text{GLS (U/mL)} = \Delta A_t \div (\Delta A_s \div C_{st}) \times V_{er} \div (V_{sa} \div V_e) \div T = 1.5625 \times \Delta A_t \div \Delta A_s$$

Cst: Standard solution concentration, 0.3125 $\mu\text{mol/mL}$;

Vsa: Supernatant volume added, 0.08 mL;

Ver: Volume of enzymatic reaction, 0.4 mL;

Ve: Volume of add Extract solution, 1 mL;

Cpr: Sample protein concentration, mg/mL;

W: Fresh weight of sample, g;

T: React time, 1 hour.

Note:

- If $\text{OD} > 0.7$, It is recommended to further dilute the supernatant and then measure it. Multiply the dilution ratio in calculation.

2. Reagent II should be used as soon as possible. If discoloration is found, it can no longer be used.

Recent Product citations:

[1] Fu Y, Lei F, Wang J, et al. Maternal Cigarette Smoke Exposure Disturbs Glutamate/GABA Balance in pFRG of Neonatal Rats[J]. Respiratory Physiology & Neurobiology, 2020: 103383.

[2] Liu S, Li N, Lin Q, et al. Glutaminase 1 in mandarin fish *Siniperca chuatsi*: Molecular characterization, expression pattern and function involving in virus replication[J]. Aquaculture, 2020: 734924.

References:

[1] Mahajan R V, Saran S, Kameswaran K, et al. Efficient production of L-asparaginase from *Bacillus licheniformis* with low-glutaminase activity: optimization, scale up and acrylamide degradation studies[J]. Bioresource technology, 2012, 125: 11- 16.

Related Products:

AK0301/AK0300	Nitrate reductase (NR) Activity Assay kit
AK0436/AK0435	Glutaminase (GLS) Assay Kit
AK0432/AK0431	Nitrite Assay Kit (Water And Soil)