

## Glutaminase (GLS) Assay Kit

**Note:** The reagents have been changed, so please be aware of and follow this instruction strictly.

**Operation Equipment:** Spectrophotometer

**Catalog Number:** BC1450

**Size:** 50T/24S

### Components:

Extract solution: 70 mL ×1, storage at 2-8 °C. Dissolve by heating at 40°C or ultrasonication if crystals precipitate.

Reagent I: powder×1, storage at 2-8°C. Add 1 0mL of Extract to dissolve the reagent before use.

Reagent IIA: 1 mL×1, storage at 2-8°C.

Reagent IIB: 4 mL×1, storage at 2-8°C. 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 IIA : Reagent II B = 1:4 before use.

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

Standard: 1 mL ×1, storage at 2-8°C. 10 μmol/mL nitrogen standard solution. Preheat at 37°C before use.

Preparation of 0.3125μmol/mL standard solution: Before use, take 25μL of 10μmol/mL nitrogen standard solution and 775μL of extraction solution to get 0.3125μmol/mL standard solution.

### 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<sup>4</sup>): 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.

2. 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 37°C			-	-
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  $\Delta As = As - Ab$ ,  $\Delta At = At - Ac$ .

**Calculation:**

1. Protein concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 $\mu$ mol of NH<sub>3</sub>-N per hour every milligram of tissue protein.

$$GLS (U/mg \text{ prot}) = \Delta At \div (\Delta As \div Cst) \times Ver \div (Vsa \times Cpr) \div T = 1.5625 \times \Delta At \div \Delta As \div Cpr.$$

2. Fresh weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 $\mu$ mol of NH<sub>3</sub>-N per hour every gram of sample.

$$GLS (U/g) = \Delta At \div (\Delta As \div Cst) \times Ver \div (W \div Ve \times Vsa) \div T = 1.5625 \times \Delta A \div \Delta As \div W$$

3. Number of cells

Unit definition: One unit of enzyme activity is defined as the amount of enzyme catalyzes the production of 1 $\mu$ mol of NH<sub>3</sub>-N per hour every 5 $\times 10^4$  cells.

$$GLS (U/mL) = \Delta At \div (\Delta As \div Cst) \times Ver \div (Vsa \div Ve) \div T = 1.5625 \times \Delta A \div \Delta As$$

Cst: Standard solution concentration, 0.3125  $\mu$ 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:**

1. If 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:**

BC0080/BC0085 Nitrate reductase (NR) Activity Assay kit

BC1450/BC1455 Glutaminase (GLS) Assay Kit

BC1480/BC1485 Nitrite Assay Kit (Water And Soil)