Glutamine (Gln) Content Assay Kit

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

Detection instrument: Spectrophotometer/Microplate Reader

Cat No: BC5305

Size: 100T/48S

Components:

Extract Solution I: Liquid 80 mL×1. Storage at 2-8℃.

Extract Solution II: Liquid 30 mL×1. Storage at 2-8℃. Chloroform, self prepared.

Reagent I: Liquid 2 mL×1. Storage at 2-8℃.

Reagent II: Powder×1. Storage at -20℃. Before use, take one and add 0.2 mL distilled water to fully dissolve it, the unused reagent can be stored at 4℃ for 4 weeks. Avoid repeated freezing and thawing.

Reagent III: Liquid 5 mL×1. Storage at 2-8℃.

Reagent IV: Powder×1. Storage at -20℃. Before use, add 20 mL Extract Solution I to fully dissolve it. The unused reagent can be stored at 2-8℃ for 4 weeks. Avoid repeated freezing and thawing.

Reagent V: Powder×1. Storage at -20℃. Before use, add 1.5 mL Reagent I to fully dissolve it. The unused reagent can be stored at 2-8℃ for 4 weeks. Avoid repeated freezing and thawing.

Reagent VI: Liquid 4 mL×1. Storage at 2-8℃.

Standard Solution: Liquid 1 mL×1. storage at 2-8℃. Glutamine standard solution with concentration of 10 μmol/mL.

Reagent II Working Solution: An empty brown reagent bottle is provided in the kit. Dilute according to the sample size according to the ratio of Reagent II: distilled water=0.05 mL: 0.7 mL (about 18 samples), The reagent should be prepared just before use, and put it on ice when using.

Product Description:

Glutamine is the amide of glutamic acid and one of the important amino acids that make up proteins. At the same time, glutamine is also the main source of α-ketoglutaric acid in the tricarboxylic acid cycle. Glutamine exists in the organism in two states: free state and bound state. Free glutamine plays an important role in organism metabolism, and its metabolism accounts for more than 60% of free amino acids in cells and blood circulation.

Free glutamine is converted to glutamic acid under the catalysis of glutaminase, Under the catalysis of glutamate dehydrogenase (GDH), glutamate and NAD are produced α- Ketoglutaric acid, NADH and $NH4^+$, under the action of 1-mPMS, WST can react with NADH to produce water-soluble formazan, which has the maximum absorption peak at 450 nm, and the glutamine content can be calculated.

Reagents and Equipment Required but Not Provided:

Microplate Reader/Spectrophotometer, Desktop Centrifuge, Water Bath/Constant Temperature Incubator, Transferpettor, Mortar/Homogenizer/Cell Ultrasonic Crusher, Micro Glass Cuvette/96 Well Flat-bottom Plate, Chloroform and Distilled Water.

Procedure:

I. Sample Extraction:

1. Tissue

According to the proportion of tissue weight (g): the volume of Extract Solution I (mL) is 1:5~10. Suggest add 1 mL of Extract Solution I to 0.1 g of tissue, and fully homogenized on ice bath. Centrifuge at 12000×g for 5 minutes at 4℃, take the supernatant and add 500 µL of Extract Solution II, shake vigorously for 5 min, centrifuge at 12000 g 4 ℃ for 5min, take the upper liquid (in a clear state) and put it on ice for testing (the middle layer turbid substances and the lower layer liquid are not required).

2. Bacteria or cells

Collecting bacteria or cells into the centrifuge tube, discard supernatant after centrifugation. According to the proportion of bacteria or cells (10^4): the volume of Extract Solution I (mL) is 500-1000:1. Suggest add 1 mL of Extract Solution I to 5 million of bacteria or cells. Use ultrasonic to splitting bacteria and cells (placed on ice, ultrasonic power 200 W, working time 3 s, interval 7 s, The total time is 3 min). Centrifuge at 12000×g for 5 minutes at 4 ℃, take the supernatant and add 500 µL of Extract Solution II, shake vigorously for 5 min, centrifuge at 12000 g 4 ℃ for 5 min, take the upper liquid (in a clear state) and put it on ice for testing (the middle layer turbid substances and the lower layer liquid are not required).

3. Serum (plasma) sample

Take the 500 µL of sample and add 500 µL of extract II, shake vigorously for 5 min, centrifuge at 12000 g 4 ℃ for 5 min, take the upper liquid (in a clear state) and put it on ice for testing (the middle layer turbid substances and the lower layer liquid are not required).

Note: If you need to measure the concentration of protein, you need to measure the concentration of protein before adding Extract Solution II.

II. Determination procedure:

1. Preheat the microplate reader/spectrophotometer 30 minutes, adjust wavelength to 450 nm, if you use spectrophotometer, you need to zero the spectrophotometer with distilled water.

2. Dilution of 0.4 µmol/mL standard solution: take 40 µL of 10 µmol/mL glutamine standard solution, add 960 µL distilled water, fully mix. The mol/mL standard solution is used after prepared immediately. (In the experiment, 40 µL is required for each tube. In order to reduce the experimental error, a large volume is prepared.)

3. Add reagents with the following list (reaction in EP tube):

Reagent (µL)	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)
Sample	40	40	-	-
Standard solution	-	-	40	-
Distilled water	-	-	-	40
Reagent II working solution	40	-	40	40
Reagent III	20	60	20	20
Reaction at 37 ℃ for 1 h				

Reagent IV	160	160	160	160
Reagent V	10	10	10	10
Reagent VI	30	30	30	30

Mix well, place it in 37 °C environment (Light avoidance) for 1 h. Centrifuge at 12000×g for 5 minutes at 25 °C, and take 200 μL supernatant to detect the absorbance at 450 nm, record as A_T, A_C, A_S and A_B respectively. $\Delta A_T = A_T - A_C$, $\Delta A_S = A_S - A_B$. The Standard tube and Blank tube only need to be measured 1-2 times. Each Test tube needs to be provided with a Control tube. The range of ΔA_T is 0.005-0.7.

III. Calculation:

1) Tissue

A. protein concentration (The protein concentration needs to be determined by yourself)

Glutamine content (μmol/mg prot)=$\Delta A_T \times C_S \div \Delta A_S \times V_S \div (Cpr \times V_S) = \Delta A_T \times 0.4 \div \Delta A_S \div Cpr$

B. Tissue weight

Glutamine content (μmol/g quality)=$\Delta A_T \times C_S \div \Delta A_S \times V_S \div W = \Delta A_T \times 0.4 \div \Delta A_S \div W$

2) Bacteria or cells

Glutamine content (μmol/10^4cell)=$\Delta A_T \times C_S \div \Delta A_S \times V_S \div N = \Delta A_T \times 0.4 \div \Delta A_S \div N$

3) Serum (plasma) volume

Glutamine content (μmol/mL)=$\Delta A_T \times C_S \div \Delta A_S = \Delta A_T \times 0.4 \div \Delta A_S$

C_S: Concentration of standard solution, 0.4 μmol/mL;

Vs: Sample volume (After adding Extract Solution I), 1 mL;

Cpr: Supernatant sample protein concentration (mg/mL);

W: Sample weight, g;

N: The total number of bacteria or cells, 10^4cell.

Note:

1. If you need to measure the protein concentration, you need to measure the protein concentration before adding extract solution II.

2. If the supernatant to be measured is still turbid after centrifugation, try to increase the centrifugation speed or extend the time, for example, centrifuge at 12000×g for 5 minutes at 4 °C.

3. The measurement range of ΔA is 0.005-0.7. If the measured absorbance value exceeds the linear range, the sample can be diluted with distilled water and then measured again. If the measured absorbance value is less than the linear range, the sample size needs to be increased and then measured again. Pay attention to changing the calculation formula.

Experimental instances：

1. Take 0.1011g of strawberry, add 1 mL of Extract Solution I to tissue, pretreatment according to the

instructions. Dilute the sample twice with distilled water, detect according to the measured steps. Calculate

A_T=0.37, A_C=0.1, A_S=0.466, A_B=0.094, ΔA_T=0.27, ΔA_S=0.372. According to the sample mass:

Glutamine content (μmol/g quality)= $\Delta A_T \times 0.4 \div \Delta A_S \div W \times 2$=5.7433 μmol/g.

2. Take 0.1081g of rabbit muscle, add 1 mL of Extract Solution I to tissue, pretreatment according to the instructions. Dilute the sample twice with distilled water, detect according to the measured steps. Calculate A_T=0.349, A_C=0.147, A_S=0.466, A_B=0.094, ΔA_T=0.202, ΔA_S=0.372. According to the sample mass:

Glutamine content (μmol/g quality)= $\Delta A_T \times 0.4 \div \Delta A_S \div W \times 2$=4.0186 μmol/g.

3. Take 0.5mL of sheep serum, add 1 mL of Extract Solution I to tissue, pretreatment according to the instructions. detect according to the measured steps. Calculate A_T=0.155, A_C=0.118, A_S=0.466, A_B=0.094, ΔA_T=0.037, ΔA_S=0.372. According to the sample mass:

Glutamine content (μmol/mL)= $\Delta A_T \times 0.4 \div \Delta A_S \times 2$=0.0796 μmol/mL.

Recent product citations：

[1] Tsao M, Otter D E. Quantification of glutamine in proteins and peptides using enzymatic hydrolysis and reverse-phase high-performance liquid chromatography[J]. Analytical Biochemistry, 1999, 269(1):143-148.

[2] Seegmiller J E, Schwartz R, Davidson C S. The plasma ammonia and glutamine content in patients with hepatic coma[J]. Journal of Clinical Investigation, 1954, 33(7), 984.

Related products：

BC1550/BC1555   glutamic-pyruvic transaminase（GPT）Assay Kit

BC1560/BC1565   Glutamic-oxalacetic Transaminase (GOT) Assay Kit

BC1570/BC1575   Amino Acid(AA) Assay Kit

BC1580/BC1585   Glutamic Acid(Glu) Assay Kit