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Glutamine (Gln) Content Assay Kit

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

Detection instrument: Spectrophotometer/Microplate Reader

Cat No: AK0762-100T-48S

Size: 100T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle.

Reagent name	Size	Preservation Condition
Extract Solution I	Liquid 80 mL×1	-
Extract Solution II	Self-Provided Reagent	2-8℃
Reagent I	Liquid 2 mL×1	-20℃
Reagent II	Powder×1	2-8℃
Reagent III	Liquid 5 mL×1	-20℃
Reagent IV	Powder×1	-20℃
Reagent V	Powder×1	2-8℃
Reagent VI	Liquid 4 mL×1	2-8℃
Standard Solution	Liquid 1 mL×1	

Solution Preparation:

1. **Extract Solution II:** Chloroform, self prepared, about 30mL, stored at room temperature; an empty 30mL brown bottle is provided in the kit for dispensing only, please label with the name of the reagent.
2. **Reagent II:** The quality of the reagent is very small and may not be visible to the naked eye, so it can be used directly. Before use, take one and add 0.2 mL distilled water to fully dissolve it, the unused reagent can be stored at -20℃ for 4 weeks. Avoid repeated freezing and thawing. (This reagent is a freeze-dried reagent, and there may be significant differences or even small amounts of reagent observed by naked eye between different bottles. This phenomenon does not affect its use and the actual quality is the same.)
3. **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.
4. **Reagent IV:** Before use, add 20 mL Extract Solution I to fully dissolve it. The unused reagent can be stored at -20℃ for 4 weeks. Avoid repeated freezing and thawing.
5. **Reagent V:** Before use, add 1.5 mL Reagent I to fully dissolve it. The unused reagent can be stored at 2-8℃ for 8 weeks.
6. **Standard Solution:** Glutamine standard solution with concentration of 10 μmol/mL.

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 （>98%，AR）,Ice and Distilled Water.

Operation procedure

I. **Sample preparation** (The sample size to be tested can be adjusted appropriately, and the specific proportion can be referred to the literature.)

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 0.4 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 ℃ environment (Light avoidance) for 1 h. Centrifuge at 12000 g for 5 minutes at 25 ℃, 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) Protein concentration (The protein concentration needs to be determined by yourself)

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

2) Tissue mass

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

3) Bacteria or cells

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

4) 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 mass, 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 ℃.
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 mass)= $\Delta A_T \times 0.4 \div \Delta A_S \div W \times 2$=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 mass)= $\Delta A_T \times 0.4 \div \Delta A_S \div W \times 2$=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. Dilute the sample twice with distilled water,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)= $\Delta A_T \times 0.4 \div \Delta A_S \div W \times 2$=0.0796 μmol/mL.