

# Glutamine (Gln) Content Assay Kit

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

**Detection instrument:**Spectrophotometer

**Cat No:** NA0149

**Size:** 50T/24S

## Components:

**Extract Solution I:** Liquid 70 mL×1. Storage at 2-8°C.

**Extract Solution II:** Liquid 15 mL×1. Storage at 2-8°C. Chloroform, self prepared.

**Reagent I:** Liquid 3 mL×1. Storage at 2-8°C.

**Reagent II:** Powder×2. Storage at -20°C. Before use, take one and add 0.2 mL distilled water to fully dissolve it, the unused reagent can be stored at 4°C for 4 weeks. Avoid repeated freezing and thawing.

**Reagent III:** Liquid 10 mL×1. Storage at 2-8°C.

**Reagent IV:** Powder×1. Storage at -20°C. Before use, add 40 mL Extract Solution I to fully dissolve it. The unused reagent can be stored at 2-8°C for 4 weeks. Avoid repeated freezing and thawing.

**Reagent V:** Powder×1. Storage at -20°C. Before use, add 2.5 mL Reagent I to fully dissolve it. The unused reagent can be stored at 2-8°C for 4 weeks. Avoid repeated freezing and thawing.

**Reagent VI:** Liquid 8 mL×1. Storage at 2-8°C.

**Standard Solution:** Liquid 1 mL×1. storage at 2-8°C. 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=0.2 mL: 2.8 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 NH<sub>4</sub><sup>+</sup>, under the action of 1-mPMS, WST can react with NADH to produce water-soluble formazan, which has the maximum absorption peak at 450nm, and the glutamine content can be calculated.

## Reagents and Equipment Required but Not Provided:

Spectrophotometer, Desktop Centrifuge, Water Bath/Constant Temperature Incubator, Transferpettor, Mortar/Homogenizer/Cell Ultrasonic Crusher, 1 mL Glass Cuvette, 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  $12000\times g$  for 5 minutes at  $4^{\circ}\text{C}$ , take the supernatant and add  $500\mu\text{L}$  of Extract Solution II, shake vigorously for 5min, centrifuge at  $12000\text{ g } 4^{\circ}\text{C}$  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 200W, working time 3s, interval 7s, The total time is 3min). Centrifuge at  $12000\times g$  for 5 minutes at  $4^{\circ}\text{C}$ , take the supernatant and add  $500\mu\text{L}$  of Extract Solution II, shake vigorously for 5 min, centrifuge at  $12000\text{ g } 4^{\circ}\text{C}$  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 sample and add  $500\mu\text{L}$  of extract II, shake vigorously for 5 min, centrifuge at  $12000\text{ g } 4^{\circ}\text{C}$  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 spectrophotometer 30 minutes, adjust wavelength to 450 nm, set zero with distilled water.

2. Dilution of  $0.4\mu\text{mol/mL}$  standard solution: take  $40\mu\text{L}$  of  $10\mu\text{mol/mL}$  glutamine standard solution, add  $960\mu\text{L}$  distilled water, fully mix. The  $\text{mol/mL}$  standard solution is used after prepared immediately. (In the experiment,  $160\mu\text{L}$  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 ( $\mu\text{L}$ )	Test tube (T)	Control tube (C)	Standard tube (S)	Blank tube (B)
Sample	160	160	-	-
Standard solution	-	-	160	-
Distilled water	-	-	-	160
Reagent II working solution	160	-	160	160
Reagent III	80	240	80	80
Reaction at $37^{\circ}\text{C}$ for 1h				
Reagent IV	640	640	640	640

Reagent V	40	40	40	40
Reagent VI	120	120	120	120

Mix well, place it in 37 °C environment (Light avoidance) for 1h. Centrifuge at 12000×g for 5 minutes at 25 °C, and take 1 mL 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  $\Delta A_T$  is 0.005-0.7.

### III. Calculation:

#### 1) Tissue

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

$$\text{Glutamine content } (\mu\text{mol/mg prot}) = \Delta A_T \times C_S \div \Delta A_S \times V_S \div (C_{pr} \times V_S) = \Delta A_T \times 0.4 \div \Delta A_S \div C_{pr}$$

##### B. Tissue weight

$$\text{Glutamine content } (\mu\text{mol/g quality}) = \Delta A_T \times C_S \div \Delta A_S \times V_S \div W = \Delta A_T \times 0.4 \div \Delta A_S \div W$$

#### 2) Bacteria or cells

$$\text{Glutamine content } (\mu\text{mol}/10^4\text{cell}) = \Delta A_T \times C_S \div \Delta A_S \times V_S \div N = \Delta A_T \times 0.4 \div \Delta A_S \div N$$

#### 3) Serum (plasma) volume

$$\text{Glutamine content } (\mu\text{mol/mL}) = \Delta A_T \times C_S \div \Delta A_S = \Delta A_T \times 0.4 \div \Delta A_S$$

$C_S$ : Concentration of standard solution, 0.4  $\mu\text{mol/mL}$ ;

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

$C_{pr}$ : Supernatant sample protein concentration (mg/mL);

$W$ : Sample weight, g;

$N$ : The total number of bacteria or cells, ten thousand.

### 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  $\Delta A$  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 0.1468 g 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  $A_T=0.535$ ,  $A_C=0.1$ ,  $A_S=0.547$ ,  $A_B=0.067$ ,  $\Delta A_T=0.435$ ,  $\Delta A_S=0.48$ . According to the sample mass:

Glutamine content ( $\mu\text{mol/g}$  quality) =  $\Delta A_T \times 0.4 \div \Delta A_S \div W \times 2 = 4.939 \mu\text{mol/g}$ .

2. Take 0.12g 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  $A_T=0.351$ ,  $A_C=0.136$ ,  $A_S=0.547$ ,  $A_B=0.067$ ,  $\Delta A_T=0.215$ ,  $\Delta A_S=0.48$ . According to the sample mass:

Glutamine content ( $\mu\text{mol/g}$  quality) =  $\Delta A_T \times 0.4 \div \Delta A_S \div W \times 2 = 2.986 \mu\text{mol/g}$ .

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  $A_T=0.402$ ,  $A_C=0.253$ ,  $A_S=0.547$ ,  $A_B=0.067$ ,  $\Delta A_T=0.149$ ,  $\Delta A_S=0.48$ . According to the sample mass:

Glutamine content ( $\mu\text{mol/g}$  quality) =  $\Delta A_T \times 0.4 \div \Delta A_S \div W = 0.124 \mu\text{mol/g}$ .

### 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:

- NA0744/NA0502 glutamic-pyruvic transaminase (GPT) Assay Kit
- NA0743/NA0501 Glutamic-oxalacetic Transaminase (GOT) Assay Kit
- NA0742/NA0500 Amino Acid(AA) Assay Kit
- NA0741/NA0499 Glutamic Acid(Glu) Assay Kit