Total Amino Acid Assay Kit

(Cat.No: BC118　　80T/78S　Colorimetric method）

1. Composition and Preparation (The kit is valid for 6 months)

Reagent I: Powder × 1 vial, stored at 4℃.

Reagent II: Liquid 5 mL × 1 bottle, stored at 4℃.

Preparation of amino acid reaction solution: Add 160 mL of distilled water to Reagent One and mix thoroughly to form a blue suspension. Then slowly add Reagent Two while stirring to make the suspension completely turn into a light blue transparent solution. Store at 4℃ (The powder is difficult to dissolve, and after adding Reagent Two, it needs to be stirred at room temperature for more than half an hour to fully dissolve. It can be prepared in advance).

Reagent III: Powder × 1 vial, stored at 4℃. Add 80 mL of distilled water to Reagent Three and mix thoroughly to dissolve it. This will be **the Amino Acid color developer**. (Note: It is corrosive. Do not touch the skin when preparing.)

Reagent IV: Glycine standard solution 75.07 mg/vial × 6 vials, stored at 4℃. When using, dissolve one vial of the standard powder in 20 mL of distilled water, mix thoroughly to prepare a **50 μmol/mL glycine standard solution**. Prepare it as needed.

Reagent V: Liquid 100 mL × 1 bottle, stored at 4℃.

2. Determination principle

Copper ions (Cu2+) can form blue-green complexes with various amino acids. The intensity of the color at a certain wavelength is directly proportional to the total content of amino acids. Therefore, the absorbance can be measured using a visible light spectrophotometer, and the total amino acid content can be obtained through conversion.

3. Required instruments and reagents

Adjustable 650nm wavelength spectrophotometer and 1cm cuvette (or microplate reader and 96-well plate), vortex mixer, centrifuge, test tube or centrifuge tube, distilled water, stopwatch, various specifications pipettes.

4. Procedures

(1) Sample pre-treatment:

1. **Tissue sample:** Accurately weigh the tissue sample to approximately 0.1g. Add 0.9mL of physiological saline according to the weight-volume ratio of 0.1g:0.9mL. Homogenize and prepare a 10% homogenate. Centrifuge at 4000 rpm for 10 minutes. Take the supernatant (this supernatant of the homogenate can be used for protein concentration determination) 0.5mL and add 0.5mL of reagent V **(If the sample volume is small, you can reduce the amount of homogenate supernatant and reagent five according to the proportion)**. Vortex mix and then centrifuge at 4000 rpm for 10 minutes. Take the supernatant for the color reaction;
2. **Serum (plasma) and other high-protein liquid samples:** Take 0.2mL of serum (plasma), add 0.8mL of reagent V **(If the sample volume is small, you can reduce the amount of**

homogenate supernatant and reagent V according to the proportion), vortex mix and then centrifuge at 4000 rpm for 10 minutes. Take the supernatant for the color reaction;

3. **Urine and other low-protein liquid samples:** Directly take the sample for the color reaction (if there are solid substances present, centrifuge at 4000 rpm for 10 minutes and then take the supernatant for the color reaction).

(2) Operating Table: (Color Reaction)

	Blank tube	Standard tube	Meansure tube
Distilled water (mL)	0.6		
50 μmol/mL amino acid standard solution (mL)		0.6	
The supernatant of the sample after pre-treatment (mL)			0.6
Amino acid reaction solution (mL)	1.8	1.8	1.8
Vortex mixing			
Amino acid chromogenic reagent (mL)	0.6	0.6	0.6
Vortex mixing, centrifuge at 4000 rpm for 10 minutes. Take the supernatant and measure its absorbance at 650 nm with a 1 cm path length. Zero the spectrophotometer with distilled water. Read the absorbance value A (or take 200 μL of the reaction solution from each tube and add it to a 96-well plate, then read the value at 650 nm with an enzyme reader).			

(3) Calculation formulas:

1. **The tissue samples were calculated based on protein concentration:**

$$\text{Total amino acid content in the tissue (μmol/mgProtein)} = \frac{A_{measure} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \div Cpr \times 2$$

2. **The tissue samples were calculated based on raw weight:**

$$\text{Total amino acid content in the tissue (μmol/gTissue)} = \frac{A_{measure} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \div \frac{W}{V_{totalsamples}} \times 2$$

3. **Calculation of high-protein liquid samples such as serum (plasma):**

$$\text{Total amino acid content in the serum (μmol/mL)} = \frac{A_{measure} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times 5$$

4. **Calculation of urine and other low-protein liquid samples**

$$\text{Total amino acid content in the urine (μmol/mL)} = \frac{A_{measure} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard} \times N$$

$C_{standard}$: Standard solution concentration: 50 μmol/mL;

W: Tissue weight, g;

$V_{total\ samples}$: The total volume of normal saline added during the homogenization of the tissue samples, mL

N: The dilution factor of urine and other low-protein liquid samples before testing;

Note: The constant "2" or "5" represents the dilution factor when adding reagent five to the tissue or serum (plasma) samples. For tissues, it is 1:1 (i.e., 2 times); for serum (plasma), it is 1:4 (i.e., 5 times).

5. **According to the standard curve: (The method for making the calibration curve is attached in Appendix I)**

If performing standard curve calculation, simply replace $\frac{A_{measure} - A_{blank}}{A_{standard} - A_{blank}} \times C_{standard}$

the former formula with the newly obtained standard curve calculation formula, and keep the rest unchanged (the absolute OD value of the sample is equal to $A_{measure}$-A_{blank})

5. Calculation examples

Example 1: After pre-treatment of the 10% mouse liver tissue homogenate supernatant, the detection was carried out according to the operation table. The absorbance of the blank tube was 0.009, that of the standard tube was 0.232, and that of the test tube was 0.035. At the same time, the concentration of 10% homogenate supernatant protein was measured to be 10.25 mg prot/mL. Then the calculation result is:

$$\text{Total amino acid content in the tissue (μmol/mgProtein)} = \frac{0.035 - 0.009}{0.232 - 0.009} \times 50 \div 10.25 \times 2$$
$$= 1.137\ \mu\text{mol/mg Protein}$$

Example 2: After pre-treatment of mouse serum, the test was conducted according to the operation table. The absorbance of the blank tube was measured as 0.009, the absorbance of the standard tube was 0.232, and the absorbance of the test tube was 0.020. Then the calculation result is:

$$\text{Total amino acid content in the serum (μmol/mL)} = \frac{0.020 - 0.009}{0.232 - 0.009} \times 50 \times 5$$
$$= 12.332\ \text{μmol/mL}$$

Example 3: Take the original urine sample and conduct the test according to the operation table. The absorbance of the blank tube is 0.009, the absorbance of the standard tube is 0.232, and the absorbance of the test tube is 0.189. Then the calculation result is:

$$\text{Total amino acid content in the urine (μmol/mL)} = \frac{0.138 - 0.009}{0.232 - 0.009} \times 50 \times 1$$
$$= 28.92\ \text{μmol/mL}$$

6. Advantages

1. Simple and inexpensive. Currently, the methods for detecting amino acids in both domestic and international settings are too complicated and the costs are quite high.
2. Rapid. This reaction can complete the test in just 15 minutes.
3. Good stability. After multiple repeated experiments, the results of this method are quite stable.
4. This method measures the free form of amino acids.

7. Notes:

1. When adding reagent II to the amino acid reaction solution, do it slowly and try to ensure that the suspension is completely dissolved. This will make the amino acid reaction solution turn light blue and transparent.
2. When preparing reagent III, be careful to avoid contact with the skin as it has certain corrosive properties.

Appendix I: Preparation of Standard Curve

I. Procedures:

1. Dissolve a standard sample in 10 mL of distilled water to prepare a 100 μmol/mL standard solution. Then, dilute it with distilled water to obtain standard solutions of 10, 20, 40, 60, and 80 μmol/mL. Mix well and prepare the solutions as needed. The dilution method is as

follows:

Volume of 100 μmol/mL standard solution (mL)	0.1	0.2	0.4	0.6	0.8
Amount of distilled water (mL)	0.9	0.8	0.6	0.4	0.2
Equivalent to the concentration of the standard solution (μmol/mL)	10	20	40	60	80

2. Operations schedule

	Blank tube	Standard tube
Standard amino acid solutions of each concentration (mL)		0.6
Distilled water (mL)	0.6	
Amino acid reaction solution (mL)	1.8	1.8
Vortex mixing		
Amino acid coloration solution (mL)	0.6	0.6
Vortex mixing, centrifuge at 4000 rpm for 10 minutes. Take the supernatant and measure its absorbance at 650 nm with a 1 cm path length. Zero the spectrophotometer with distilled water. Read the absorbance value A (or take 200 μL of the reaction solution from each tube and add it to a 96-well plate, then read the value at 650 nm with an enzyme reader).		

II. Detection results:

Standard solution concentration (mmol/L)	0	10	20	40	60	80	100
Absorbance value (OD value)	0.009	0.045	0.089	0.185	0.269	0.372	0.486
Absolute OD value	0	0.036	0.080	0.176	0.260	0.363	0.477

III. Draw standard curve: