(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Total Amino Acid Assay Kit

Catalog No.: BC00176

Size: 50T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Total Amino Acid Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue, serum, plasma, urine
Assay Type	Quantitative
Detection Instrument	Spectrophotometer, Microplate reader (650 nm)

Product Introduction

The liver and kidneys are the major organs for amino acid metabolism. Therefore, changes in urinary amino acids can best reflect the pathological and physiological conditions of the liver and kidneys. In addition, amino acids can also reflect the status of burns, diabetes mellitus, typhoid fever and other diseases.

Principle

Copper ions (Cu^{2+}) can complex with various amino acids to form blue-green complexes. At a specific wavelength, the color intensity is proportional to the content of total amino acids. Therefore, a visible spectrophotometer can be used to measure the absorbance, and the content of total amino acids can be obtained through conversion.

Components

No.	Components	Size	Notes
Reagent 1	Powder	1 vial	Store at 4°C
Reagent 2	Liquid	5 mL	Store at 4°C
Reagent 3	Powder	1 vial	Store at 4°C
Reagent 4	Glycine standard	75.07 mg/vial × 6 vials	Store at 4°C
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Preparation of amino acid reaction solution: Add 160 mL of double-distilled water to Reagent 1, mix thoroughly to form a blue suspension. Then slowly add Reagent 2 dropwise while stirring until the suspension completely turns into a pale blue transparent solution. Store at 4 °C. (The powder is difficult to dissolve; after adding all of Reagent 2, the mixture still needs to be stirred at room temperature for more than 30 minutes to dissolve completely.)

Preparation of amino acid chromogenic agent: Add 80 mL of water to Reagent 3 and mix thoroughly. (Note: Corrosive. Avoid contact with skin during preparation.)

Preparation of 50 μmol/mL glycine standard solution: Dissolve one vial of glycine standard in 20 mL of double-distilled water, mix thoroughly, and prepare freshly before use.

Storage

The kit has a validity period of 6 months.

Preparation

Spectrophotometer with 650 nm wavelength and 1 cm path length cuvette (or microplate reader), bench-top low-speed centrifuge (3500 rpm), pipettes of various volumes, double-distilled water, physiological saline (0.9%) or PBS (0.1 M), vortex mixer, test tubes or centrifuge tubes, protein assay reagent (for tissue and cell samples, available from our company).

Operation process

1. Urine Assay

	Blank tube	Standard tube	Assay tube
Double-distilled water (mL)	1		
50 μmol/mL amino acid standard solution (mL)		1	
Urine (mL)			1
Amino acid reaction solution (mL)	2	2	2
Vortex mixing			
Amino acid chromogenic agent (mL)	1	1	1

Mix well, centrifuge at 3500 rpm for 10 minutes, take the supernatant and measure absorbance at 650 nm with a 1 cm light path, using double-distilled water as zero reference.

2. Serum (Plasma) Assay

	Blank tube	Standard tube	Assay tube
Double-distilled water (mL)	0.3		
50 μmol/mL amino acid standard solution (mL)		0.3	
Serum (plasma) (mL)			0.3
Reagent 5 (mL)	1.2	1.2	1.2
Mix well, centrifuge at 3500 rpm for 10 minutes, then take 1 mL of the supernatant for testing.			
Supernatant (mL)	1	1	1
Amino acid reaction solution (mL)	2	2	2
Vortex mixing			
Amino acid chromogenic agent (mL)	1	1	1
Mix well, centrifuge at 3500 rpm for 10 minutes, take the supernatant and measure absorbance at 650 nm with a 1 cm light path, using double-distilled water as zero reference.			

3. Tissue Assay

Sample pretreatment: Weigh the tissue accurately, add 9 volumes of physiological saline according to the weight-to-volume ratio to prepare a 10% homogenate, centrifuge at 3500 rpm for 10 minutes, and take the supernatant for testing. Meanwhile, determine the protein content of the 10% homogenate supernatant.

	Blank tube	Standard tube	Assay tube
Double-distilled water (mL)	0.3		
50 μmol/mL amino acid standard solution (mL)		0.3	
Homogenate supernatant (mL)			0.3

Reagent 5 (mL)	1.2	1.2	1.2
Mix well, centrifuge at 3500 rpm for 10 minutes, then take 1 mL of the supernatant for testing.			
Supernatant (mL)	1	1	1
Amino acid reaction solution (mL)	2	2	2
Vortex mixing			
Amino acid chromogenic agent (mL)	1	1	1
Mix well, centrifuge at 3500 rpm for 10 minutes, take the supernatant and measure absorbance at 650 nm with a 1 cm light path, using double-distilled water as zero reference.			

Calculation

1. Urine / Serum (Plasma) Assay

$$\text{Total amino acid content (μmol/mL)} = \frac{A_{Assay} - A_{Blank}}{A_{Standard} - A_{Blank}} \times C_{Standard} \times N$$

2. Tissue Assay

$$\text{Total amino acid content (μmol/mg)} = \frac{A_{Assay} - A_{Blank}}{A_{Standard} - A_{Blank}} \times C_{Standard} \div Cpr$$

Annotation:

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

N: Dilution factor of the sample before testing

Cpr: Protein concentration of tissue sample, mg/mL

Precautions

1. When adding Reagent 2, it must be added slowly, and ensure that the suspension is completely dissolved, so that the amino acid reaction solution becomes a light blue transparent solution.
2. Pay attention when preparing Reagent 3. Avoid skin contact as much as possible; this reagent is corrosive to a certain extent.
3. It is recommended to use disposable laboratory test tubes for operation.
4. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.