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

Catalog No：E-BC-K055-S

Specification：50Assays (36 samples)/ 100Assays (86 samples)

Measuring instrument：Spectrophotometer(635-655 nm)

Detection range：4.73-100 mmol/L

Elabscience® Total Amino Acids (T-AA) Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

Email: techsupport@elabscience.com

Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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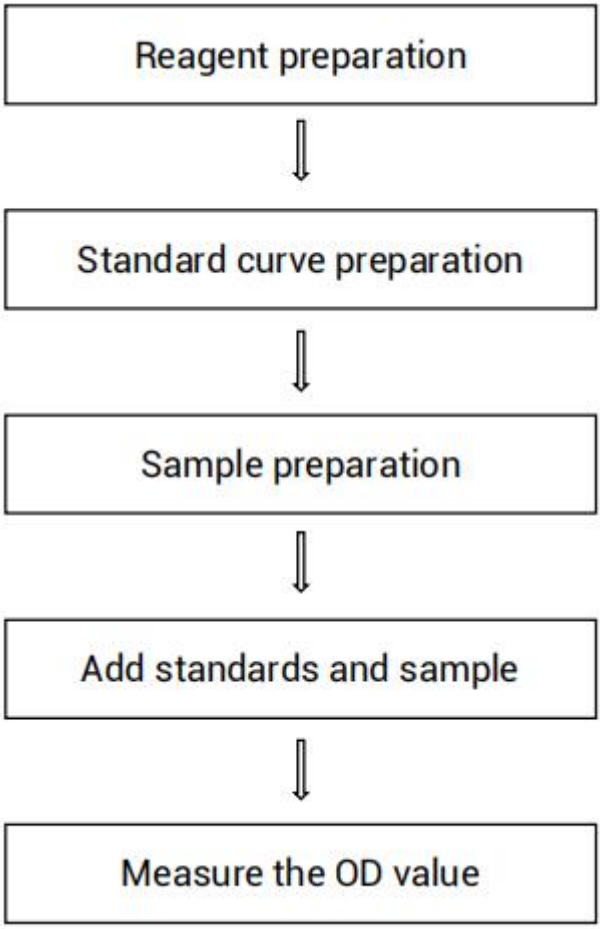
Assay summary



Intended use

This kit can be used to measure the total amino acids (T-AA) content in serum (plasma), urine, animal and plant tissue samples.

Detection principle

Copper ions can form blue-green complexes with various amino acids. The intensity of the color of the complex at 650 nm is directly proportional to the total content of amino acids.

Kit components & storage

Item	Component	Size 1 (50 assays)	Size 2 (100 assays)	Storage
Reagent 1	Powder A	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 months
Reagent 2	Acid Reagent	1 mL × 1 vial	1 mL× 2 vials	2-8℃, 12 months
Reagent 3	Powder B	Powder × 1 vial	Powder × 2 vials	2-8℃, 12 months
Reagent 4	Powder C	Powder × 1 vial	Powder × 1 vial	2-8℃, 12 months
Reagent 5	Protein Precipitator	26 mL × 1 vial	52 mL×1 vial	2-8℃, 12 months

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Spectrophotometer (635-655 nm, optimum wavelength: 650 nm), Vortex mixer, Centrifuge, Magnetic stirrer

Reagents:

PBS(0.01 M，pH 7.4)

Reagent preparation

① Equilibrate all the reagents to 25°C before use.

② The preparation of powder A working solution：
Dissolve one vial of powder A and 45 mL of double distilled water, mix well and stir fully to form a blue turbid solution. Then add 0.875 mL of acid reagent slowly, and stir until the turbid solution turns into light blue transparent solution, continue stirring for 30 min. Store at 2-8°C for a month.

③ The preparation of powder B working solution：
Dissolve one vial of powder B with 22.5 mL of double distilled water, mix well to dissolve. Store at 2-8°C for a month.

④ The preparation of 200 mmol/L standard solution：
Dissolve one vial of powder C with 5 mL of double distilled water, mix well to dissolve. Store at 2-8°C for a month.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 200 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 20, 40, 50, 60, 80, 100 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦
Concentration (mmol/L)	**0**	**20**	**40**	**50**	**60**	**80**	**100**
200 mmol/L (μL)	0	40	80	100	120	160	200
Double distilled water (μL)	400	360	320	300	280	240	200

Sample preparation

① Sample preparation

Serum (Plasma), urine sample: Detect directly.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4℃.

④ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection and detect within 4 h.

⑤ Meanwhile,determine the protein concentration of supernatant (The animal tissue recommends E-BC-K318-M, the plant tissue recommends E-BC-K168-M).

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Human urine	1
Rat serum	1
10% Rat lung tissue homogenate	1
10% Rat spleen tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Epipremnum aureum leaf tissue homogenate	1

Note: The diluent is protein precipitator. For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

When preparing the powder A working solution, it is necessary to pay attention to whether the powder is completely dissolved.

Operating steps

① Standard tube: add 80 μL of standard solution with different concentrations to the tubes.

Sample tube: add 80 μL of sample to the tubes.

② Add 320 μL of protein precipitator into each tube.

③ Mix fully with a vortex mixer for 5 s, centrifuge at 3500 g for 10 min.

④ Take 300 μL of supernatant from each tube to new tubes.

⑤ Add 800 μL of powder A working solution to each tube.

⑥ Mix fully with a vortex mixer for 5 s.

⑦ Add 400 μL of powder B working solution to each tube.

⑧ Mix fully with a vortex mixer for 5 s. Centrifuge at 3500 g for 10 min and take the supernatant. Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 650 nm with a 1 cm optical path cuvette.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard # ①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

1. Serum, plasma and urine samples:

$$\text{T-AA content (mmol/L)} = (\Delta A_{650} - b) \div a \times f$$

2. Tissue samples:

$$\text{T-AA content (mmol/gprot)} = (\Delta A_{650} - b) \div a \times f \div C_{pr}$$

[Note]

ΔA_{650}: $OD_{Sample} - OD_{Blank}$.

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	30.00	60.00	90.00
%CV	3.1	3.6	4.9

Intra-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	30.00	60.00	90.00
%CV	3.5	5.2	6.3

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 100.1%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	20.00	50.00	80.00
Observed Conc. (mmol/L)	19.7	51.6	78.9
Recovery rate (%)	98.5%	103.2%	98.6%

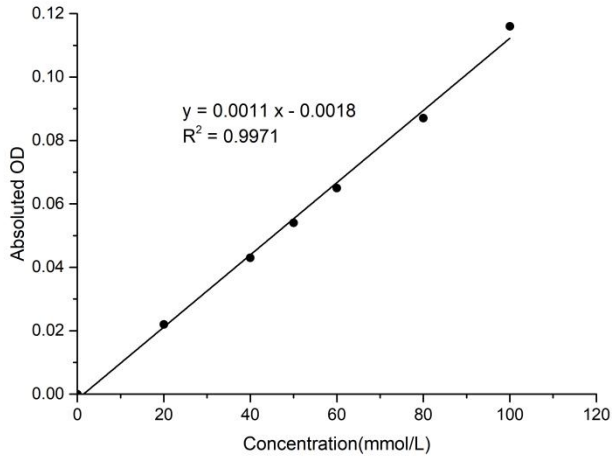
Sensitivity

The analytical sensitivity of the assay is 4.73 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (mmol/L)	0	20	40	50	60	80	100
OD	0.008	0.029	0.051	0.060	0.071	0.091	0.128
	0.008	0.030	0.051	0.063	0.075	0.099	0.119
Average OD	0.008	0.030	0.051	0.062	0.073	0.095	0.124
Absoluted OD	0.000	0.022	0.043	0.054	0.065	0.087	0.116



Appendix Π Example Analysis

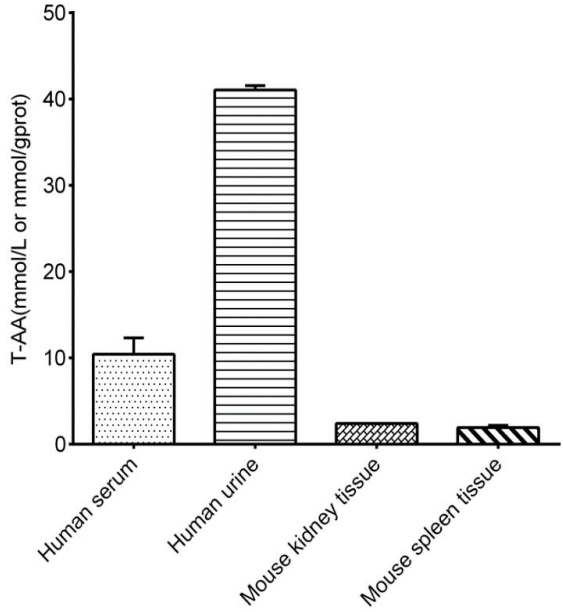
Example analysis：

Take 80 μL of human urine and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0011x - 0.0018, the average OD value of the sample tubes is 0.051, the average OD value of the blank tubes is 0.008 and the calculation result is:

$$\text{T-AA content (mmol/L)} = (0.051 - 0.008 + 0.0018) \div 0.0011 = 40.73 \text{ mmol/L}$$

Detect human serum, human urine, 10% mouse liver tissue homogenate (the concentration of protein is 10.173 gprot/L), 10% mouse spleen tissue homogenate (the concentration of protein is 5.827 gprot/L),the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.