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

Catalog No：E-BC-K055-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader（640-660 nm）

Detection range：3.64-100 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
Tel: 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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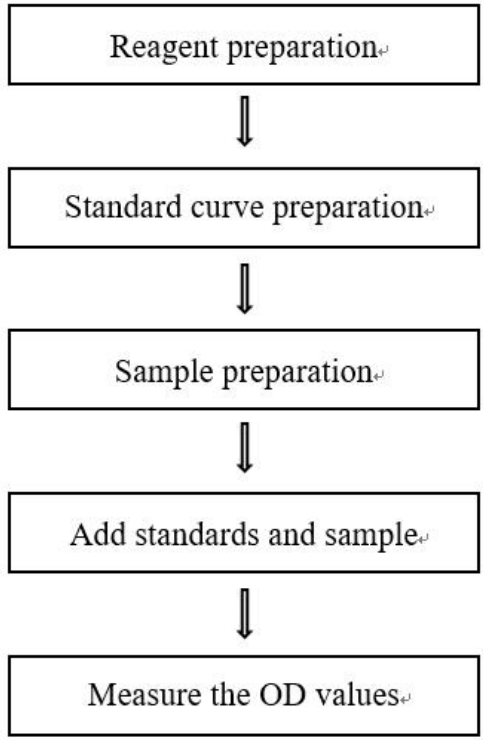
Assay summary



Intended use

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

Detection principle

Copper ions can complex with various amino acids to produce blue-green complex compound, and the depth of color is proportional to the content of total amino acids at a specific wavelength. T-AA content can be calculated with the absorbance at 650 nm.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Powder A	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months
Reagent 2	Acid Reagent	0.4 mL × 1 vial	0.8 mL × 1 vial	2-8°C, 12 months
Reagent 3	Powder B	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months
Reagent 4	Powder C	Powder × 1 vial	Powder × 1 vial	2-8°C, 12 months
Reagent 5	Protein Precipitator	8 mL ×1 vial	15 mL ×1 vial	2-8°C, 12 months
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

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:

Microplate reader (640-660 nm, optimum wavelength: 650 nm), Test tube, Micropipettor, Vortex mixer, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

Size 1(48 T)：

① Equilibrate all reagents to room temperature before use.

② The preparation of powder A working solution：

Dissolve one vial of powder A with 12 mL of double distilled water, stir fully to form a blue turbid liquid, then add 0.35 mL of acid reagent slowly and stir until the turbid liquid turns into light blue transparent liquid. Continue stirring for another 30 minutes. Store at 2-8°C for 1 month.

③ The preparation of powder B working solution：

Dissolve one vial of Powder B with 6 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 1 month.

④ The preparation of 200 mmol/L standard：

Dissolve one vial of powder C with 5 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 1 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, 10, 20, 40, 50, 60, 80, 100 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**10**	**20**	**40**	**50**	**60**	**80**	**100**
200 mmol/L standard(μL)	0	10	20	40	50	60	80	100
Double distilled water (μL)	200	190	180	160	150	140	120	100

Size 2(96 T):

① Equilibrate all reagents to room temperature before use.

② The preparation of powder A working solution:

Dissolve one vial of powder A with 24 mL of double distilled water, stir fully to form a blue turbid liquid, then add 0.7 mL of acid reagent slowly and stir until the turbid liquid turns into light blue transparent liquid. Continue stirring for another 30 minutes. Store at 2-8°C for 1 month.

③ The preparation of powder B working solution:

Dissolve one vial of powder B with 12 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 1 month.

④ The preparation of 200 mmol/L standard:

Dissolve one vial of Powder C with 5 mL of double distilled water. Mix well to dissolve. Store at 2-8°C for 1 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, 10, 20, 40, 50, 60, 80, 100 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**10**	**20**	**40**	**50**	**60**	**80**	**100**
200 mmol/L standard (μL)	0	10	20	40	50	60	80	100
Double distilled water (μL)	200	190	180	160	150	140	120	100

Sample preparation

① Sample preparation

Serum (plasma) and urine: detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for a month.

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 Normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4°C.

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

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-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 plasma	1
Porcine serum	1
10% Rat heart tissue homogenate	1
10% Rat liver tissue homogenate	1
10% Mouse liver 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 powder A working solution, it is necessary to pay attention to whether the powder is completely dissolved.

Operating steps

① Standard tube: Take 30 μL of standard with different concentrations to 1.5 mL EP tubes.
Sample tube: Take 30 μL of sample to 1.5 mL EP tubes.
② Add 120 μL of protein precipitator into each tube.
③ Mix fully with vortex mixer for 5 s and centrifuge at 3500×g for 10 min.
④ Take 100 μL of supernatant from each tube to 1.5 mL EP tubes.
⑤ Add 200 μL of powder A working solution into each tube.
⑥ Mix fully with vortex mixer for 5 s.
⑦ Add 100 μL of powder B working solution into each tube.
⑧ Mix fully with vortex mixer for 3 s, centrifuge at 3500×g for 10 min. Take 300 μL of supernatant to the microplate and measure the OD value of each well at 650 nm with microplate reader.

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).

The sample:

1. Serum (plasma) sample:

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

2. Tissue sample:

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

[Note]

f: Dilution factor of sample before tested.

ΔA_{650}: $OD_{Sample} - OD_{Blank}$.

C_{pr}: Protein concentration of 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)	10.50	42.60	88.10
%CV	4.2	3.8	4.0

Inter-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)	10.50	42.60	88.10
%CV	7.0	6.3	6.2

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 102%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	14.5	45	73.2
Observed Conc. (mmol/L)	14.6	44.6	77.6
recovery rate(%)	101	99	106

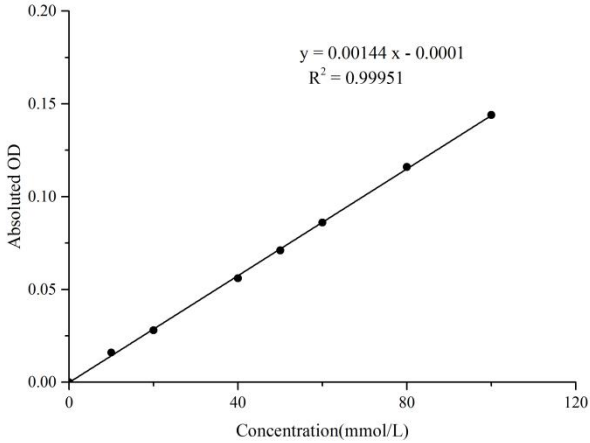
Sensitivity

The analytical sensitivity of the assay is 3.03 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**	**10**	**20**	**40**	**50**	**60**	**80**	**100**
Average OD	0.070	0.086	0.098	0.126	0.141	0.156	0.186	0.214
Absoluted OD	0.000	0.016	0.028	0.056	0.071	0.086	0.116	0.144



Appendix II Example Analysis

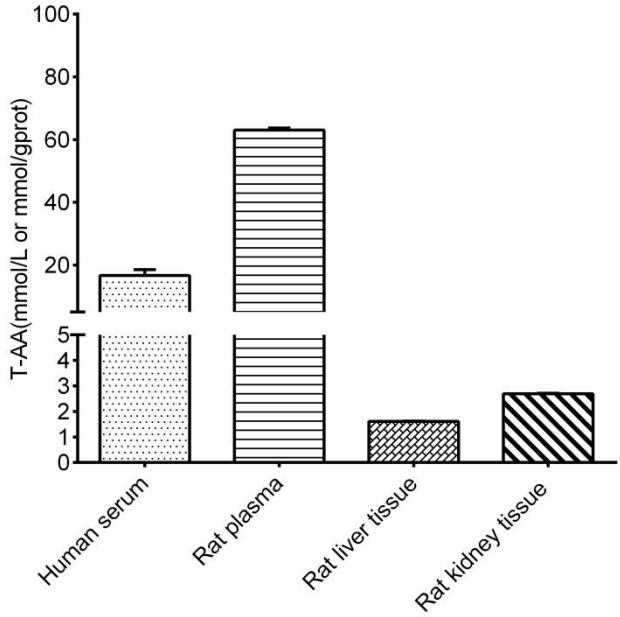
Example analysis：

For human urine, take 30 μL of human urine sample and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 0.0014 x – 0.0001, the average OD value of the sample is 0.134, the average OD value of the blank is 0.070, and the calculation result is:

T-AA content (mmol/L) =（0.134-0.070+0.0001）÷0.0014= 45.79 mmol/L

Detect human serum, rat plasma, 10% rat liver tissue homogenate (the concentration of protein is 9.17 gprot/L) and 10% rat kidney tissue homogenate (the concentration of protein is 7.56 gprot/L) according to the protocol, 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.