Technical Manual

Total Amino Acids (T-AA) Colorimetric Assay Kit

- **Catalogue Code: MAES0063**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

3.64-100 mmol/L

Sensitivity:

3.03 mmol/L

Storage:

2-8°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background

Animal liver and kidney are the main organs of amino acid metabolism, so the change of amino acids in urine can best reflect the physiological state of liver and kidney. Amino acid content in plants is of great significance to study changes of nitrogen metabolism, plants on the absorption of nitrogen, transport, assimilation and nutritional status under different conditions and different growth periods.

3. Intended Use

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

4. Detection Principle

Copper ions can complex with various amino acids to produce a 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.

5. Kit Components & Storage

Item	Specification	Storage
Powder A	Lyophilized × 1 vial	2-8°C, 6 months
Acid Reagent	0.8 mL × 1 vial	2-8°C, 6 months
Powder B	lyophilized × 1 vial	2-8°C, 6 months
Powder C	lyophilized × 1 vial	2-8°C, 6 months
Protein Precipitator	15 mL × 1 vial	2-8°C, 6 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipette
- Vortex mixer
- Water bath
- Microplate Reader (640-660 nm, optimum wavelength: 650 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Normal saline (0.9% NaCl)
- PBS (0.01 M, pH 7.4)
- Double distilled water

6. Assay Notes:

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

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **working solution A:** Dissolve a vial of powder A with 24 mL double distilled water, stir fully to form a blue turbid liquid, then add acid reagent slowly and stir until the turbid liquid turns into light blue transparent liquid. Continue stirring for another 30 minutes, and the prepared solution can be stored at 2-8°C for 1 month.

3. Preparation of **working solution B:** Dissolve a vial of powder B with 12 mL double distilled water fully. The prepared solution can be store at 2-8°C for 1 month.

4. Preparation of **200 mmol/L standard:** Dissolve a vial of powder C with 5 mL double distilled water fully. The prepared standard solution can be stored at 2-8°C for 1 month.

8. Sample Preparation

1. Serum/plasma sample:
Detect the sample directly.

2. Tissue sample:
Weigh the tissue accurately. Add PBS (0.01 M，pH 7.4) in a weight (g): volume (mL) ratio of 1: 9, homogenize mechanically in ice water bath to break cells fully. Then centrifuge at 10000 g for 10 min at 4°C and collect the supernatant for measurement. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Sample Notes:

The concentration should be determined before preforming the assay.

If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (3.64-100 mmol/L).

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.

9. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 650 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

10. Operation Steps

The preparation of standard curve

Dilute 200 mmol/L standard 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.

The measurement of samples

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 5s 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 working solution A into each tube of step 4.

6. Mix fully with vortex mixer for 5s.

7. Add 100 μL of working solution B into each tube.

8. Mix fully with vortex mixer for 3s, 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 read

Operation Table

	Standard tube	Sample tube
Standards with different concentrations (μL)	30	
Sample (μL)		30
Protein precipitator (μL)	120	120
Mix fully with vortex mixer for 5 s, centrifuge at 3500 g for 10 min and take 100 μL of supernatant for detection.		
Supernatant (μL)	100	100
Working solution A (μL)	200	200
Mix fully with vortex mixer for 5 s		
Working solution B (μL)	100	100
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.		

11. Calculations

Plot the standard curve by using OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the OD value of sample. The standard curve is: y= ax + b.

Plasma and another liquid sample:

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

Tissue sample:

$$\text{T-AA content (mmol/L)} = (\Delta A_{550} - b) \div a \times f \div C_{pr}$$

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the OD value when the standard concentration is 0)

x: The concentration of Standard

a: The slope of standard curve

b: The intercept of standard curve

ΔA_{650}: $OD_{Sample} - OD_{Blank}$

f: Dilution factor of sample before test

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

12. Performance Characteristics

Detection Range	3.64-100 mmol/L
Sensitivity	3.03 mmol/L
Average inter-assay CV (%)	6.5
Average intra-assay CV (%)	4
Average recovery rate (%)	102

Analysis

For human urine, take 30 μL of human urine sample, and carryout the assay according to the operation table.

The results are as follows:

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

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes.

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