TECHNICAL MANUAL

Total Amino Acids (T-AA) Colorimetric Assay Kit

- **SKU CODE:** MAES0510
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Add protein precipitator
- Mix and centrifuge
- Take supernatant
- Add powder A working solution
- Add powder B working solution
- Measure absorbance at 650 nm

2. 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.

3. 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.

4. Kit components & storage

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

5. 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)

6. Reagent preparation

1. Equilibrate all reagents to 25 °C before use.
2. **Preparation of powder A working solution:** Dissolve one vial of powder A with 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. **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. **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. **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.

7. Sample preparation

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 °C.

4. Centrifuge at 10,000 xg 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.

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only). The diluent is protein precipitator. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

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

8. 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.

9. 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 3,500 xg 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 3,500 xg 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.

10. 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 absolute OD value.

3. Plot the standard curve by using absolute 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:

T-AA content (mmol/L) = (deltaA_{650} - b) ÷ a × f

2. Tissue samples:

T-AA content (mmol/gprot) = (deltaA_{650} - b) ÷ a × f ÷ C_{pr}

[Note]

deltaA_{650}: OD Sample – OD Blank.

f: Dilution factor of sample before test.

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

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Inter-assay Precision

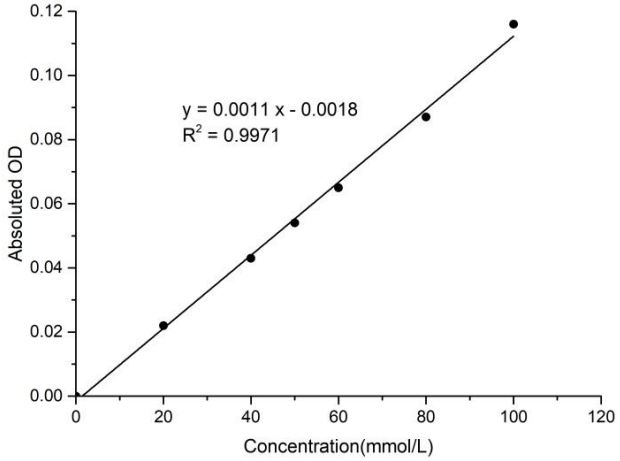
Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

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%.

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.



12. Appendix II Example Analysis

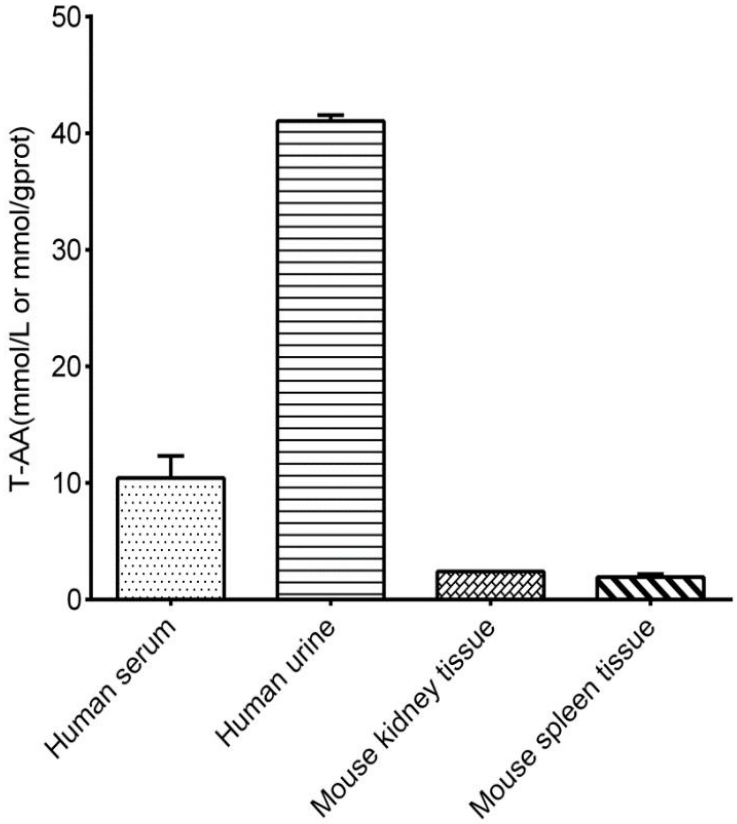
Example analysis:

Take 80 µL of human urine and carry out 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:

T-AA content (mmol/L) = (0.051 - 0.008 + 0.0018) ÷ 0.0011 = 40.73 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:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use

of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.