Amino Acid (AA) Colorimetric Assay Kit

Cat#: orb1173181 (User Manual)

Micro Amino Acid (AA) Colorimetric Assay Kit
Cat #: KTB1460
Lot #: Refer to product label
Size: 96 T
Detection range: 0.625-40 µmol/mL
Sensitivity: 0.5 µmol/mL
Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant, Bacteria, Urine
Storage: Stored at 4℃ for 12 months

Assay Principle

Animal Liver and kidney are the main organs of amino acid metabolism, so the changes of amino acid in urine can reflect the physiological state of liver and kidney. In addition, amino acids can also reflect burns, typhoid fever and other aspects of the situation. The content of amino acids in plants is important to study the changes of nitrogen metabolism, nitrogen absorption, transportation, assimilation and nutrition of plants under different conditions and in different growth and development stages.

CheKine™ Micro Amino Acid (AA) Assay Kit provides a convenient tool for detection of amino acid. The principle is that the
α-amino of amino acid can react with Ninhydrin hydrate to produce blue-purple substances, which has a maximum absorption peak at 570 nm. The amino acid content of the sample can be calculated by measuring the absorbance at 570 nm.

Materials Supplied and Storage Conditions

Kit components	Size (96 T)	Storage conditions
Extraction Buffer	100 mL	4℃
Assay Buffer	10 mL	4℃
Substrate	1	4℃, protected from light
Standard (10 mg)	1	4℃, protected from light

Materials Required but Not Supplied

·Microplate Reader or Visible Spectrophotometer capable of measuring absorbance at 570 nm
·Metal bath, Refrigerated Centrifuge
·96-well plate or Microglass cuvette, precision Pipettes, disposable Pipette Tips
·Deionized Water
·Homogenizer(for Tissue Samples)
·Ethanol

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Substrate: Add 4 ml 95% ethanol for 96 T to dissolve before use. This solution can be stored at 4℃ for up to 1 week. The solution can also be stored at -20℃ after aliquoting to avoid repeated freezing and thawing.
Standard: Containing 10 mg L-cysteine/vial, add 2.066 mL of deionized water to dissolve before use. The concentration is 40 μmol/mL. This solution can be stored at 4℃ for up to 1 week. The solution can also be stored at -20℃ after aliquoting to avoid repeated freezing and thawing.
Standard curve setting: dilute 40 μmoL/mL Standard with Deionized Water to 40、20、10、5、2.5、1.25、0.625、0 μmoL/mL Standard solution as shown in the table below.

	Volume of Standard	Volume of Deionized Water (μL)	The concentration of Standard
Std.1	200 μL of 40 μmol/mL	0	40 μmol/mL
Std.2	100 μL of Std.1 (40 μmol/mL)	100	20 μmol/mL
Std.3	100 μL of Std.2 (20 μmol/mL)	100	10 μmol/mL
Std.4	100 μL of Std.3 (10 μmol/mL)	100	5 μmol/mL
Std.5	100 μL of Std.4 (5 μmol/mL)	100	2.5 μmol/mL
Std.6	100 μL of Std.5 (2.5 μmol/mL)	100	1.25 μmol/mL
Std.7	100 μL of Std.6 (1.25 μmol/mL)	100	0.625 μmol/mL

Sample Preparation

Note: Fresh samples are recommended. If not assayed immediately, samples can be stored at -80℃ for one month.

1. Animal Tissues: Weigh 0.1 g tissues, add 1 mL Extraction Buffer and homogenize at room temperature. Transfer to 1.5 mL EP tube, cover tightly and place in boiling water for 15 min. And then cooling with running

water, centrifuge at 10,000 rpm for 10 minutes at room temperature. Use supernatant for assay.

2. Plant Tissues: Weigh 0.1 g tissues, add 1 mL Extraction Buffer and mash. Ultrasonic break at room temperature 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Transfer to 1.5 mL EP tube, cover tightly and place in boiling water for 15 min. And then cooling with running water, centrifuge at 10,000 rpm for 10 minutes at room temperature. Use supernatant for assay.

3. Cells or Bacteria: Collect 5 x 10^6 cells or bacteria into the centrifuge tube, wash cells or bacteria with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells or bacteria at room temperature 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Transfer to 1.5 mL EP tube, cover tightly and place in boiling water bath for 15 min. After cooling with running water, centrifuge at 10,000 rpm for 10 minutes at room temperature. Use supernatant for assay.

4. Serum, Plasma, Cell Supernatant, Urine or other Liquid samples: Add 0.5mL samples and 0.5mL Extraction Buffer to 1.5 mL EP tube, cover tightly and place in boiling water bath for 15 min. After cooling with running water, centrifuge at 10,000 rpm for 10 minutes at room temperature. Use supernatant for assay.

Assay Procedure

1. Preheat the microplate reader or Visible Spectrophotometer for more than 30 min, and adjust the wavelength to 570 nm. Visible Spectrophotometer deionized water zero.

Add the following reagents respectively into each EP tubes:

Reagent	Blank tube (μL)	Standard tube (μL)	Test tube (μL)
Deionized Water	10	0	0
Std. with different concentration	0	10	0
Sample	0	0	10
Substrate	20	20	20
Assay Buffer	50	50	50

2. Mix well, then cover tightly and place in boiling water for 5 min. After cooling with running water for 10 seconds, add 120 μL 60% ethanol. Then reverse the EP tube several times and transfer 150 μL of each reaction to a 96-well plate or Microglass cuvette. Then reading the values at 570 nm. The absorbance of Blank tube, Standard tube, Test tube recorded as A_{Blank}, $A_{Standard}$ and A_{Test}, respectively. Finally, calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$; $\Delta A_{Standard}=A_{Standard}-A_{Blank}$. Be sure to finish the measurement within 30 minutes

after color development.

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If A Test is greater than 2.0, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor. The reaction of proline and hydroxyproline with Ninhydrin has no absorption peak at 570 nm, therefore, the determination results at 570 nm do not contain these two amino acids.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the Standard solution as the y-axis and the ΔAStandard as the x-axis, draw the standard curve. Substitute the ΔATest into the equation to obtain the y value (μmoL/mL).

2. Calculate the content of Amino Acid in sample

(1) By sample fresh weight

Amino Acid (μmol/g)=y÷(W÷VExtraction)×n**=y÷W×n**

(2) Calculated by protein concentration Amino Acid (μmol/mg prot)**=y÷Cpr×n**

(3) Calculated by Cells or Bacteria number

Amino Acid (μmol/10^4 cells)=y÷(Cells or Bacteria number÷VExtraction)×n=y÷500×n**=0.002×y×n**

(4) Calculated by liquid volume Amino Acid (μmol/mL)**=y×2×n**

Where: W: sample weight, g; VExtraction: Extraction Buffer volume added, 1 mL; n: dilution factor; Cpr: supernatant protein concentration, mg/mL; 500: Total number of bacteria or cells, 5×10^6; 2: the dilution factor of Extraction the liquid (0.5 mL+0.5 mL)/0.5 mL=2.

Typical Data

Typical standard curve

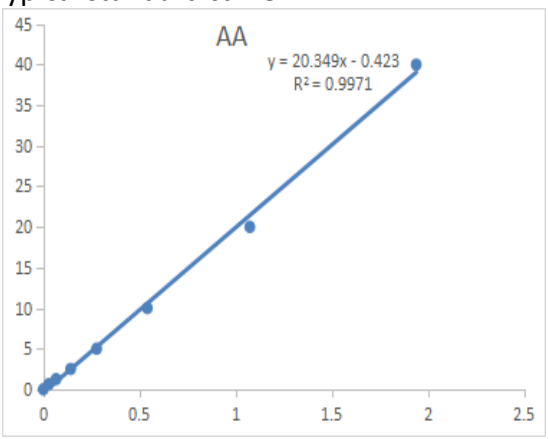

Figure1. Standard Curve of Amino Acid in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Examples

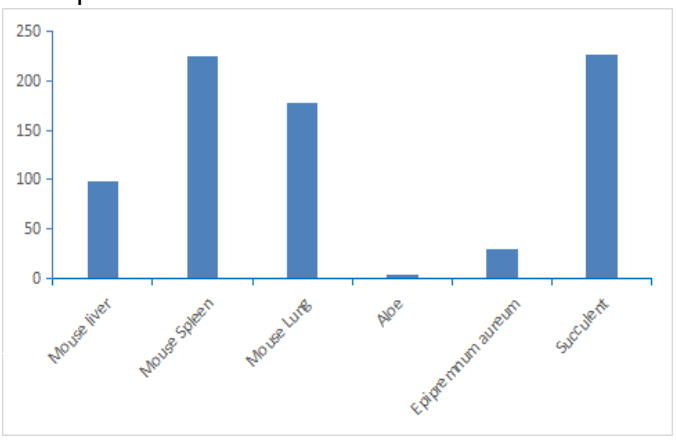

Figure 2. Amino Acid content in Mouse liver, Mouse spleen, Mouse lung, Aloe, Epipremnum aureum and Succulent, respectively. Assays were performed following kit protocol.