CheKine™ Micro Amino Acid (AA) Assay Kit

Cat #: KTB1460 Size: 48 T/48 S 96 T/96 S

	Micro Amino Acid (AA) Assay Kit		
REF	**Cat #:** KTB1460	LOT	**Lot #:** Refer to product label
	Detection range: 0.078125-2.5 μmol/mL		**Sensitivity:** 0.078125 μmol/mL
	Applicable samples: Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant, Bacteria, Urine		
	Storage: Stored at 4℃ for 12 months, protected from light		

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 simple, convenient, and rapid method for amino acid, which is suitable for the detection of serum (plasma), animal and plant tissues, cells, cell supernatants, bacteria, and urine. 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		Storage conditions
	48 T	96 T	
Extraction Buffer	70 mL	70 mL×2	4℃
Assay Buffer	7.5 mL	15 mL	4℃
Substrate Cofactor	Powder×2 vials	Powder×4 vials	4℃
Substrate	Powder×1 vial	Powder×1 vial	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 570 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, water bath

- Deionized water, ethanol
- Homogenizer (for tissue samples)

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℃.

Working Substrate Cofactor: Prepared before use. Add 2.5 mL Assay Buffer for each bottle to fully dissolve. Working Substrate Cofactor is freshly prepared.

Working Substrate: Prepared before use. Add 2 mL 95% ethanol for 48 T and 4 mL 95% ethanol for 96 T to fully dissolve. Unused dissolved Substrate can be stored at 4℃ for one week, protected from light.

Note: Working Substrate Cofactor or Working Substrate is toxic and has a irritating property, so personal protection is recommended during use.

Standard: Prepared before use. Add 1.332 mL deionized water to dissolve before use. The concentration is 100 μmoL/mL. Unused dissolved Standard can be stored at 4℃ for 1 month, protected from light.

Standard Curve Setting: Dilute 100 μmol/mL Standard with deionized water to 2.5, 1.25, 0.625, 0.3125, 0.15625,0.078125 μmoL/mL standard solution as shown in the table below.

Num.	Volume of Standard	Volume of Extraction Buffer (μL)	The Concentration of Standard
Std.1	25 μL of 100 μmol/mL	975	2.5 μmol/mL
Std.2	100 μL of Std.1 (2.5 μmol/mL)	100	1.25 μmol/mL
Std.3	100 μL of Std.2 (1.25 μmol/mL)	100	0.625 μmol/mL
Std.4	100 μL of Std.3 (0.625 μmol/mL)	100	0.3125 μmol/mL
Std.5	100 μL of Std.4 (0.3125 μmol/mL)	100	0.15625 μmol/mL
Std.6	100 μL of Std.5 (0.15625 μmol/mL)	100	0.078125 μmol/mL
Blank	0	200	0

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

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 (It is recommended to use screw-cap EP tubes), cover tightly and place in boiling water for 15 min. And then cooling with running water, centrifuge at 10,000 g for 10 min 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 (It is recommended to use screw-cap EP tubes), cover tightly and place in boiling water for 15 min. And then cooling with running water, centrifuge at 10,000 g for 10 min at room temperature. Use supernatant for assay.
3. Cells or Bacteria: Collect 5×10^6 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 (It is recommended to use screw-cap EP tubes), cover tightly and place in boiling water bath for 15 min. After cooling with running water, centrifuge at 10,000 g for 10 min at room temperature. Use supernatant for assay.
4. Serum, Plasma, Cell Supernatant, Urine or other Liquid samples: Add 0.5 mL samples and 0.5 mL Extraction Buffer to 1.5 mL EP tube (It is recommended to use screw-cap EP tubes), cover tightly and place in boiling water bath for 15 min. After cooling with running water, centrifuge at 10,000 g for 10 min at room temperature. Use supernatant for assay.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 570 nm, visible spectrophotometer was returned to zero with deionized water.
2. Add the following reagents respectively into each EP tubes (It is recommended to use screw-cap EP tubes):

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)
Extraction Buffer	10	0	0
Std. with Different Concentration	0	10	0
Sample	0	0	10
Working Sunstrate Cofactor	50	50	50
Working Substrate	20	20	20

3. Mix well, then cover tightly and place in boiling water for 5 min. After cooling with running water for 10 s, 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 min after color development. **Note: (1) In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. (2) If A_{Test} is greater than 2.5 μmol/mL of $\Delta A_{Standard}$, the sample can be appropriately diluted with deionized water, the calculated result multiplied by the dilution factor. If ΔA_{Test} is less than 0.01, increase the sample quantity appropriately. (3) 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. (4) Preparation of Working Reagent (Optional): When dealing with a large number of samples, a working reagent can be prepared by mixing Working Substrate Cofactor：Working Substrate=50 μL:20 μL (total 70 μL, 1T). Note that the Working Reagent is freshly prepared.**

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 $\Delta A_{Standard}$ as the x-axis, draw the standard curve. Substitute the ΔA_{Test} 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÷$V_{Extraction}$)×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)=y÷(cells or bacteria number÷$V_{Extraction}$)×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; $V_{Extraction}$: Extraction Buffer volume added, 1 mL; n: dilution factor; Cpr: supernatant protein concentration, mg/mL; 500: Total number of cells or bacteria, 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

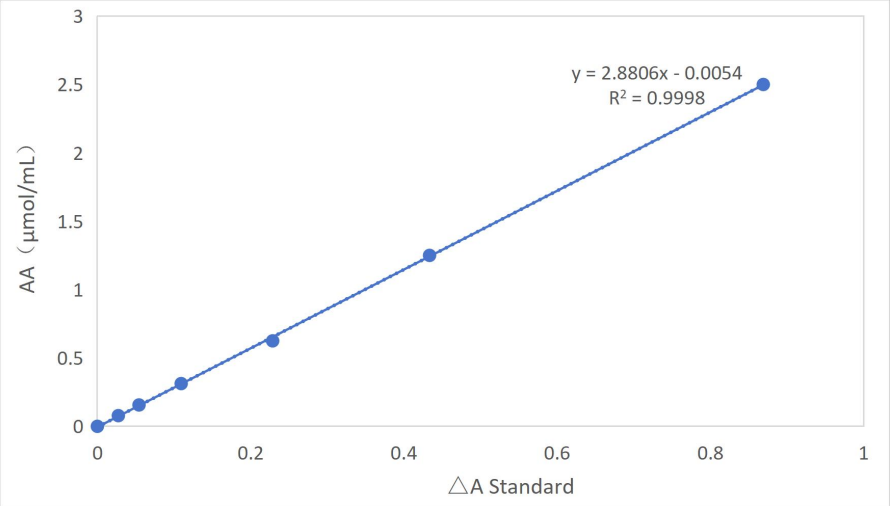

Figure 1. Standard Curve for Amino Acid.

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1430	CheKine™ Micro Proline (PRO) Assay Kit
KTB1440	CheKine™ Micro Glutamate (Glu) Assay Kit
KTB1450	CheKine™ Micro Cysteine (Cys) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.