



## CheKine™ Micro Amino Acid (AA) Assay Kit

Cat #: KTB1460

Size: 96 T/96 S

	<b>Micro Amino Acid (AA) Assay Kit</b>		
<b>REF</b>	<b>Cat #:</b> KTB1460	<b>LOT</b>	<b>Lot #:</b> Refer to product label
	<b>Detection range:</b> 0.625-40 µmol/mL		<b>Sensitivity:</b> 0.5 µmol/mL
	<b>Applicable samples:</b> Serum, Plasma, Animal and Plant Tissues, Cells, Cell Supernatant, Bacteria, Urine		
	<b>Storage:</b> Stored at 4°C 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  $\alpha$ -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°C
Assay Buffer	10 mL	4°C
Substrate	Powder×1 vial	4°C, protected from light
Standard (10 mg)	Powder×1 vial	4°C, 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°C.

**Assay Buffer:** Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

**Working Substrate:** Add 4 mL 95% ethanol to dissolve before use. Unused dissolved Substrate can be stored at 4°C for one week, protected from light. For long-term storage, please store at -20°C after aliquoting, protected from light for 1 month, avoid repeated freezing and thawing.

**Note: Assay Buffer and Substrate is toxic and has a pungent odor, so it is recommended to experiment in a fume hood.**

**Standard:** Add 2.066 mL deionized water to dissolve before use. The concentration is 40 µmol/mL. Unused dissolved Standard can be stored at 4°C for one week, protected from light. For long-term storage, please store at -20°C after aliquoting, protected from light for 1 month, avoid repeated freezing and thawing.

**Standard Curve Setting:** Dilute 40 µmol/mL Standard with deionized water to 20, 10, 5, 2.5, 1.25, 0.625 µmol/mL standard solution as shown in the table below.

Num.	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

**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°C 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 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, cover tightly and place in boiling water for 15 min. And then cooling with running water, centrifuge at 10,000 rpm for 10 min at room temperature. Use supernatant for assay.

3. Cells or Bacteria: Collect  $5 \times 10^6$  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 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, cover tightly and place in boiling water bath for 15 min. After cooling with running water, centrifuge at 10,000 rpm 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:

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
Working Substrate	20	20	20
Assay Buffer	50	50	50

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  $A_{\text{Blank}}$ ,  $A_{\text{Standard}}$  and  $A_{\text{Test}}$  respectively. Finally, calculate  $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Blank}}$ ,  $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$  (Blank tube only needs to make one tube). Be sure to finish the measurement within 30 min after color development.

**Note:** In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If  $A_{\text{Test}}$  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  $\Delta A_{\text{Standard}}$  as the x-axis, draw the standard curve. Substitute the  $\Delta A_{\text{Test}}$  into the equation to obtain the y value (μmol/mL).

2. Calculate the content of Amino Acid in sample

(1) By sample fresh weight

$$\text{Amino Acid } (\mu\text{mol/g}) = y \div (W \div V_{\text{Extraction}}) \times n = \mathbf{y \div W \times n}$$

(2) Calculated by protein concentration

$$\text{Amino Acid } (\mu\text{mol/mg prot}) = \mathbf{y \div Cpr \times n}$$

(3) Calculated by cells or bacteria number

$$\text{Amino Acid } (\mu\text{mol}/10^4) = y \div (\text{cells or bacteria number} \div V_{\text{Extraction}}) \times n = \mathbf{y \div 500 \times n}$$

(4) Calculated by liquid volume

$$\text{Amino Acid } (\mu\text{mol/mL}) = \mathbf{y \times 2 \times n}$$

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

## Typical Data

Typical standard curve

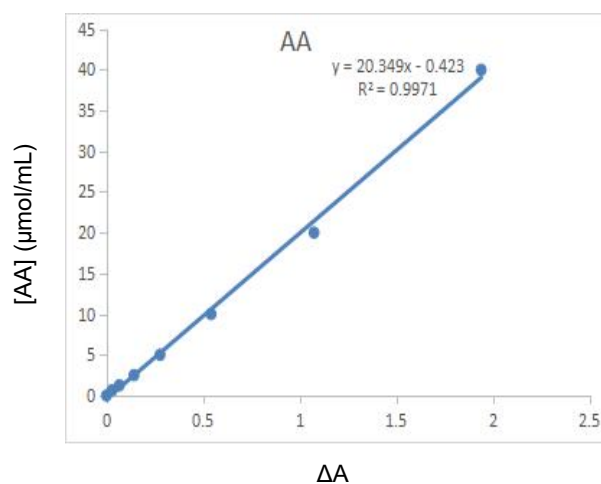


Figure 1. Standard Curve for Amino Acid.

Examples

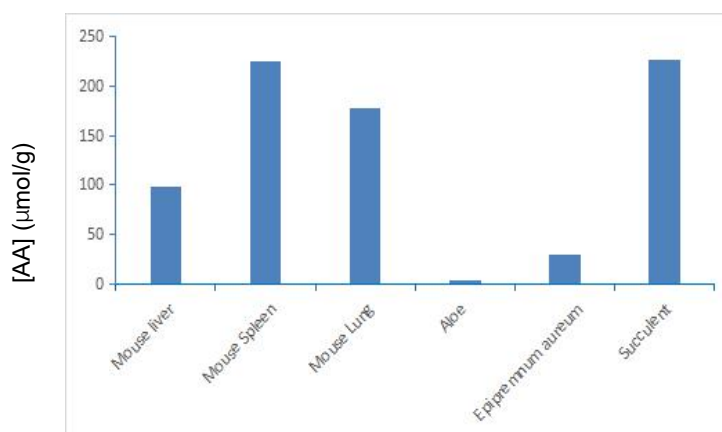


Figure 2. Amino Acid content in Mouse liver, Mouse spleen, Mouse lung, Aloe, Epipremnum aureum and Succulent respectively. Assays were performed following kit protocol.

## 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.