Amino Acid (AA) Content Assay Kit (Ninhydrin, Micro Method)

A1519780

Storage 2-8℃. Protect from light.

Shipping Cold transportation. Please store under the specified storage conditions immediately upon receipt.

Introduction

Animal liver and kidneys are the primary organs for amino acid metabolism; therefore, changes in urinary amino acids most accurately reflect the physiological state of the liver and kidneys. Additionally, amino acid levels can indicate conditions such as burns and typhoid fever. In plants, the amino acid content is significant for studying nitrogen metabolism changes under different conditions and at various growth stages, as well as for understanding nitrogen absorption, transport, assimilation, and nutritional status.

Detection Principle

The α-amino group of amino acids reacts with ninhydrin to produce a blue-purple compound with a characteristic absorption peak at 570 nm. The amino acid content is calculated by measuring the absorbance at 570 nm.

Applicable Samples: Serum (plasma), animal and plant tissues, cells, cell supernatants, bacteria, urine.

Kit Contents

Product number	Component	48T	96T	Storage
A1519780A	Extraction Buffer	70 mL	70 mL×2	2-8℃.
A1519780B	Assay Buffer	7.5 mL	15 mL	2-8℃.
A1519780C	Substrate Cofactor	2 EA	4 EA	2-8℃.
A1519780D	Substrate	1 EA	1 EA	2-8℃. Store in the dark.
A1519780E	Standard	1 EA	1 EA	2-8℃. Store in the dark.

Note:

- Please check the volume of each component before starting the experiment.
- Each component provides an additional 10% beyond the specified amount for standard curve preparation or pilot experiments.

Reagents, consumables and Equipments not provided

- Microplate reader capable of measuring absorbance at 570 nm.
- 96-well microplate.
- PBS, deionized water, anhydrous ethanol.
- Homogenizer (for tissue samples), water bath, incubator, refrigerated centrifuge, adjustable pipettes and tips.

Assay Procedure (For Reference Only)

1. Reagent Preparation

Reagent Name	Preparation Procedure	Notes
Extraction Buffer	Ready to use; equilibrate to room temperature before use.	Store at 4°C.
Assay Buffer	Ready to use; equilibrate to room temperature before use.	Store at 4°C.
Working Substrate Cofactor	Prepare immediately before use. Add 2.5 mL of Assay Buffer to one vial of Substrate Cofactor and dissolve completely.	Use immediately. Toxic and irritant; recommended to wear personal protective equipment during use.
Working Substrate	Prepare immediately before use. For 48T, add 2 mL of 95% ethanol to dissolve completely. For 96T, add 4 mL of 95% ethanol to dissolve completely.	Unused portion can be stored at 4°C protected from light for up to 1 week. Toxic and irritant; recommended to wear personal protective equipment during use.
100 μmol/mL Standard	Prepare immediately before use. Add 1.332 mL of deionized water to dissolve completely to obtain a 100 μmol/mL Glycine Standard.	Unused portion can be stored at 4°C protected from light for up to 1 month.

2. Preparation of Standards

Use the 100 μmol/mL Standard and dilute further according to the table below. Prepare a standard curve for each experiment. Diluted standard solutions are unstable and must be used within 4 hours.

Tube No.	Standard (μL)	Extraction Buffer (μL)	Concentration (μmol/mL)
1	25 μL of 100 μmol/mL Standard	975	2.5
2	100 μL of 2.5 μmol/mL Standard	100	1.25
3	100 μL of 1.25 μmol/mL Standard	100	0.625
4	100 μL of 0.625 μmol/mL Standard	100	0.3125
5	100 μL of 0.3125 μmol/mL Standard	100	0.15625
6	100 μL of 0.15625 μmol/mL Standard	100	0.078125
Blank	0	200	0

3. Sample Preparation

Note: Fresh samples are recommended. If not used immediately, samples can be stored at -80°C for up to 1 month.

3.1 Animal Tissues

Weigh approximately 0.1 g of animal tissue. Add 1 mL of Extraction Buffer and homogenize thoroughly at room temperature.

Transfer to a 1.5 mL screw-cap microcentrifuge tube. Cap tightly (to prevent evaporation) and place in a boiling water bath for 15 min.

Cool under running tap water. Centrifuge at 10,000 g for 10 min at room temperature. Collect the supernatant for analysis.

3.2 Plant Tissues

Weigh approximately 0.1 g of plant tissue. Add 1 mL of Extraction Buffer and macerate.

Sonicate at room temperature for 5 min (power 20% or 200W, 3s pulse on, 7s pulse off, 30 cycles). Transfer to a 1.5 mL screw-cap microcentrifuge tube.

Cap tightly and place in a boiling water bath for 15 min.

Cool under running tap water. Centrifuge at 10,000 g for 10 min at room temperature. Collect the supernatant for analysis.

3.3 Bacteria or Cells

Collect 5×10^6 bacteria or cells. Wash with cold PBS, centrifuge at 800 g for 2 min, and discard the supernatant.

Add 1 mL of Extraction Buffer. Sonicate at room temperature for 5 min (power 20% or 200W, 3s pulse on, 7s pulse off, 30 cycles). Transfer to a 1.5 mL screw-cap microcentrifuge tube.

Cap tightly and place in a boiling water bath for 15 min.

Cool under running tap water. Centrifuge at 10,000 g for 10 min at room temperature. Collect the supernatant for analysis.

3.4 Liquid Samples (Serum/Plasma, Cell Supernatant, Urine)

Pipette 0.5 mL of the liquid sample into a 1.5 mL screw-cap microcentrifuge tube. Add 0.5 mL of Extraction Buffer.

Cap tightly and place in a boiling water bath for 15 min.

Cool under running tap water. Centrifuge at 10,000 g for 10 min at room temperature. Collect the supernatant for analysis.

4. Assay Procedure

4.1 Microplate Reader Preparation

Pre-heat the microplate reader for at least 30 min and set the wavelength to 570 nm.

4.2 Reaction Setup

Prepare the reactions in 1.5 mL screw-cap microcentrifuge tubes as follows:

Reagent	Blank Tube (μL)	Standard Tube (μL)	Test Tube (μL)
Extraction Buffer	10	0	0
Standard (from step 2)	0	10	0
Sample (from step 3)	0	0	10
Working Substrate Cofactor	50	50	50
Working Substrate	20	20	20

4.3 Incubation and Transfer

Mix well. Cap the tubes tightly (to prevent evaporation). Place in a boiling water bath for 5 min. Cool in an ice bath for 30 s. Add 120 μL of 60% ethanol. Invert the tubes several times to mix. Transfer 150 μL from each tube into a well of a 96-well microplate.

4.4 Absorbance Measurement

Measure the absorbance at 570 nm. Record the values as ABlank, AStandard, and ATest. The measurement must be completed within 30 min after color development.

5. Result Calculation

5.1 Data Processing

Calculate $\Delta A_{Test} = A_{Test} - A_{Blank}$

Calculate $\Delta A_{Standard} = A_{Standard} - A_{Blank}$

5.2 Standard Curve

Plot the standard concentration (y-axis, μmol/mL) against the corresponding $\Delta A_{Standard}$ (x-axis) to generate a standard curve. Obtain the linear equation $y = ax + b$. Substitute the ΔA_{Test} value into the equation to obtain the concentration y (μmol/mL) for the sample.

5.3 Calculation of Sample Amino Acid Content

(1) By Sample Mass (Tissue):

Amino Acid Content (μmol/g) = $y \div (W \div V_{extraction}) \times n = y \div W \times n$

(2) By Cell/Bacteria Count:

Amino Acid Content (μmol/10^4 cells) = $y \div (N \div V_{extraction}) \times n = y \div N \times n$

(3) By Liquid Volume (Serum, Urine, etc.):

Amino Acid Content (μmol/mL) = y × 2 × n

(4) By Protein Concentration:

Amino Acid Content (μmol/mg prot) = y ÷ Cpr × n

Parameter Explanation:

y: Amino acid concentration from standard curve (μmol/mL).

W: Sample mass (g).

Vextraction: Volume of Extraction Buffer used for extraction (here, 1 mL).

n: Dilution factor of the sample before addition to the reaction.

N: Number of bacteria or cells, in units of 10^4 (e.g., for 5×10^6 cells, N = 500).

2: Dilution factor for liquid samples: (0.5 mL sample + 0.5 mL buffer) / 0.5 mL sample = 2.

Cpr: Protein concentration of the sample supernatant (mg/mL).

Notes:

Pilot Experiment: Before the formal assay, it is recommended to perform a pilot experiment using 2-3 samples with expected significant differences.

Normalization: For tissue and cell samples, results can be normalized by measuring the protein concentration. BCA Protein Assay Kits are recommended.

Assay Specificity: Proline and hydroxyproline do not produce an absorption peak at 570 nm when reacting with ninhydrin. Therefore, the results measured at 570 nm do not include these two amino acids.

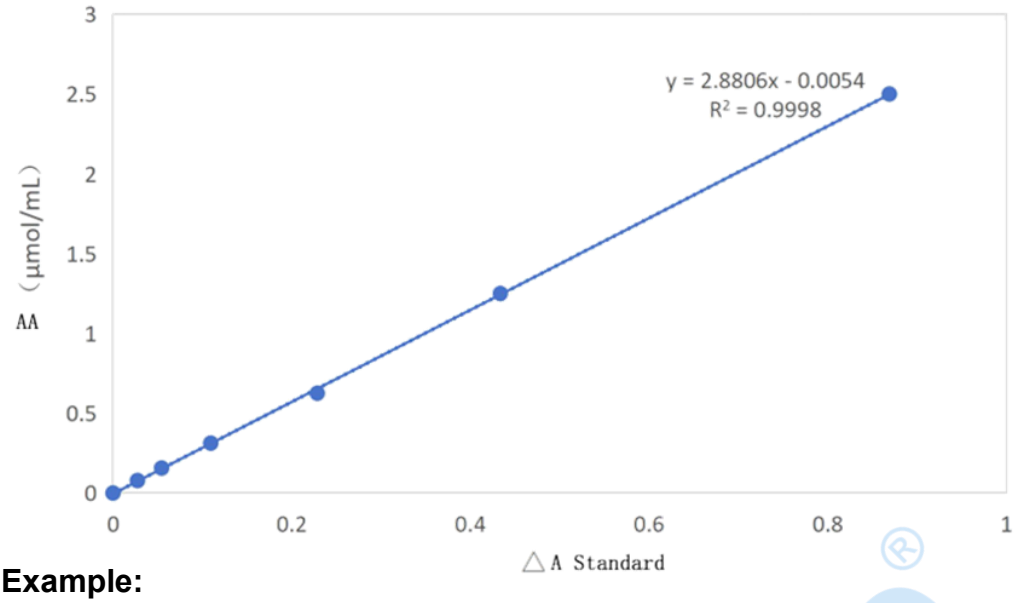
Standard Curve: It is recommended to establish your own standard curve for the most accurate results. If not, the typical standard curve equation provided in the results section can be used as a reference.

Safety: Biochemical detection reagents are generally irritants and have biological toxicity. For your safety and health, please wear appropriate personal protective equipment (lab coat, mask, gloves, head cover) and perform experiments in a fume hood or biosafety cabinet throughout the process.

Intended Use: This product is for scientific research only and is not intended for clinical diagnosis.

Results Display

Typical Standard Curve: y = 2.8806x - 0.0054, R^2 = 0.9998



Example:

0.1 g of Epipremnum aureum leaf was processed according to the procedure and measured in a 96-well plate.

ΔATest = ATest - ABlank = 0.295 - 0.065 = 0.23.

Substituting into the standard curve: y = (2.8806 * 0.23) - 0.0054 = 0.657.

Calculated by sample mass: Amino Acid Content (μmol/g) = y ÷ (W ÷ Vextraction) × n = y ÷ W × n = 0.657 ÷ 0.1 × 1 = 6.57 μmol/g.

Frequently Asked Questions (FAQ)

Q: What should I do if the measured ATest is too high or too low?

A: If ATest is higher than the ΔAStandard of the 2.5 μmol/mL standard (> approx. 2.5 μmol/mL equivalent), dilute the sample further with deionized water, multiply the result by the dilution factor, or reduce the amount of sample used for extraction.

If ATest is < 0.01, consider increasing the sample volume appropriately.