

## **Amino Acid (AA) Content Assay Kit**

**Note:** Take two or three different samples for prediction before test.

**Detection equipment:** Spectrophotometer

**Cat No:** BC1570

**Size:** 50T/48S

### **Components:**

Reagent I: Liquid 50 mL×1, store at 4°C.

Reagent II: Liquid 50 mL×1, store at 4°C.

Reagent III: Powder×1(brown), store at 4°C and protect from light. Add 4 mL absolute ethyl alcohol before use, then add 56 mL distilled water, mix thoroughly.

Reagent IV: Powder×1, store at 4°C and protect from light. Add 5 mL distilled water before, mix thoroughly.

Standard: Powder×1, 10 mg cysteine, store at 4°C and protect from light. Add 8.26 mL distilled water before use, prepare as 10 μmol/mL cysteine standard solution.

### **Description:**

Animal liver and kidney are the main organs of amino acid metabolism, so the changes of amino acids in urine can reflect the physiological state of liver and kidney. In addition, amino acids can also respond to burns, typhoid and other aspects of the situation. The content of amino acids in plants have a great significance to the study of nitrogen's metabolism, absorption, transport, assimilation and nutritional status under different conditions and at different stages of growth and development.

$\alpha$ -Amino of amino acid can react with hydrated ninhydrin to produce blue purple compound, which has absorption peak at 570 nm, and the content of amino acid is calculated by measuring absorbance of 570 nm.

### **Required but not provided:**

Desk centrifuge, spectrophotometer, water bath, 1 mL glass cuvette, transferpettor, mortar/homogenizer, absolute ethyl alcohol, ice and distilled water.

### **Protocol:**

## I. Sample preparation

1. Tissue: Add 1 mL Reagent I to 0.1 g tissue, fully grind at room temperature, transfer to 1.5 mL centrifuge tube, extract at boiling water for 15 minutes. After cooling, centrifuge at 10000 rpm, 4°C for 10 minutes. Take the supernatant for test.

2. Bacteria or cells: Collect bacteria or cells t centrifuge tube, after centrifuge, discard the supernatant. Add 1 mL reagent I to every 5 million bacteria or cells, ultrasonic smash bacteria or cells (powder 20% , ultrasonic 3s, interval 10s, repeat 30 times). Centrifuge at 10000 rpm, 4°C for 10 minutes. Take the supernatant for test.

## II. Determination protocol

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 570 nm, set the counter to zero with distilled water.

2. Operation table.

Reagent name (μL)	Test tube (A <sub>T</sub> )	Standard tube (A <sub>S</sub> )	Blank tube (A <sub>B</sub> )
Sample	50	-	-
Standard	-	50	-
Distilled water	-	-	50
Reagent II	500	500	500
Reagent III	500	500	500
Reagent IV	50	50	50

Mix thoroughly, incubate at boiling water for 15 minutes, repeatedly overthrow centrifuge several times. Centrifuge at 8000 rpm for 5 minutes, then detect the absorbance of supernatant at 570 nm. Record as A<sub>T</sub>, A<sub>S</sub>, A<sub>B</sub>,  $\Delta A_T = A_T - A_B$ ,  $\Delta A_S = A_S - A_B$ . Detect within 30 minutes after coloration.

## III. Calculation

1. Sample fresh weight.

$$AA (\mu\text{mol/g weight}) = [C_S \times V_S \times (A_T - A_B) \div (A_S - A_B)] \times (V_{RT} \div V_S) \div W = 10 \times (A_T - A_B) \div (A_S - A_B) \div W$$

2. Protein concentration

$$AA (\mu\text{mol/mg prot}) = [C_S \times V_S \times (A_T - A_B) \div (A_S - A_B)] \div (C_{pr} \times V_S) = 10 \times (A_T - A_B) \div (A_S - A_B) \div C_{pr}$$

3. Bacteria or cells amount

$$AA (\mu\text{mol}/10^4 \text{ cell}) = [C_S \times V_S \times (A_T - A_B) \div (A_S - A_B)] \div (500 \times V_S \div V_{ST}) = 0.02 \times (A_T - A_B) \div (A_S - A_B)$$

#### 4. Liquid

$$AA (\mu\text{mol}/\text{mL}) = [C_S \times V_S \times (A_T - A_B) \div (A_S - A_B)] \times 2 = (A_T - A_B) \div (A_S - A_B)$$

$C_S$ : Standard concentration, 10  $\mu\text{mol}/\text{mL}$ ;

$V_S$ : Standard volume, 0.05 mL;

$W$ : Sample weight, g;

$V_S$ : Sample volume, 0.05 mL;

$V_{ST}$ : Sample total volume, 1 mL;

$C_{pr}$ : Supernatant protein concentration, mg/mL;

2: Dilution ratio when extracting liquid,  $(V + V_{ST})/V = 2$ ;

500: Bacteria or cells amount, 5 million.

#### Note:

1. Prepare Reagent III and Reagent IV before use and protect from light.
2. Take 1-2 pre-experiment in order to assure the accuracy, if the absorbance is too high, dilute with distilled water before detecting.
3. The reaction of proline and hydroxyproline with ninhydrin has no absorption peak at 570 nm. Therefore, the determination result at 570 nm does not contain these two amino acids.
4. If the measured value is small, the weight of tissue or cell amount can be increased appropriately, and the proportion of liquid sample and extract can be adjusted (such as changing 0.5 mL liquid + 0.5 mL extract to 0.7 mL liquid + 0.3 mL extract).

#### Technical Specifications:

Minimum Detection Limit: 0.798  $\mu\text{mol}/\text{mL}$

Linear Range: 1-15  $\mu\text{mol}/\text{mL}$

#### Experimental example:

1. Take 0.1g mouse liver to 1ml extract solution, grinding and operate as the procedure after taking the supernatant,  $A_T = 0.592$ ,  $A_S = 0.848$ ,  $A_B = 0.106$ ,  $\Delta A_T = A_T - A_B = 0.592 - 0.106 = 0.486$ ,  $\Delta A_S = A_S - A_B = 0.848 - 0.106 = 0.742$ , calculate content by sample weight:  $AA (\mu\text{mol}/\text{g weight}) = 10 \times \Delta A_T \div \Delta A_S \div W = 10 \times 0.486 \div 0.742 \div 0.1 = 65.5 \mu\text{mol}/\text{g weight}$ .
2. Take 0.1g grass to 1ml extract solution, grinding and operate as the procedure after taking the supernatant,  $A_T = 0.748$ ,  $A_S = 0.848$ ,  $A_B = 0.106$ ,  $\Delta A_T = A_T - A_B = 0.748 - 0.106 = 0.642$ ,  $\Delta A_S = A_S - A_B = 0.848 - 0.106 = 0.742$ , calculate content by sample weight:  $AA (\mu\text{mol}/\text{g weight}) = 10 \times \Delta A_T \div \Delta A_S \div W = 10 \times 0.642 \div 0.742 \div 0.1 = 86.523 \mu\text{mol}/\text{g weight}$ .

**References:**

[1] Lee S W, Lim J M, Bhoo S H, et al. Colorimetric determination of amino acids using genipin from *Gardenia jasminoides*[J]. *Analytica chimica acta*, 2003, 480(2): 267-274.

Kalant H. Colorimetric Ninhydrin Reaction for Measurement of  $\alpha$ -Amino Nitrogen[J]. *Analytical Chemistry*, 1956, 28(2): 265-266.

**Related products:**

BC0290/BC0295 Proline(PRO) Content Assay Kit

BC0180/BC0185 Cysteine(Cys) Content Assay Kit