Amino Acid (AA) Content Assay Kit

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

Detection equipment: Spectrophotometer/microplate reader

Cat No: NA0500

Size: 100T/96S

Components:

Reagent I: Liquid 100 mL×1, store at 4℃.

Reagent II: Liquid 20 mL×1, store at 4℃.

Reagent III: Powder×1(brown), store at 4℃ and protect from light. Add 1.33 mL absolute ethyl alcohol before use, then add 18.76 mL distilled water, mix thoroughly.

Reagent IV: Powder×1, store at 4℃ and protect from light. Add 2 mL distilled water before, mix thoroughly.

Standard: Powder×1, 10 mg cysteine, store at 4℃ and protect from light. Add 8.26 mL distilled water, 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.

α-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/microplate reader, water bath, micro glass cuvette/96 well flat-bottom plate, 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℃ for 10 minutes. Take the supernatant for test.

2. Bacteria or cells: Collect bacteria or cells to 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℃ for 10 minutes. Take the supernatant for test.

II. Determination protocol

1. Preheat spectrophotometer/microplate reader 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_T)	Standard tube (A_S)	Blank tube (A_B)
Sample	10	-	-
Standard	-	10	-
Distilled water	-	-	10
Reagent II	100	100	100
Reagent III	100	100	100
Reagent IV	10	10	10

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_T, A_S, A_B, $\Delta A_T=A_T-A_B$, $\Delta A_S=A_S-A_B$. Detect within 30 minutes after coloration.

III. Calculation

1. Sample fresh weight.

AA (μmol/g weight)=$(C_S\times V_S\times\Delta A_T\div\Delta A_S)\div(W\div V_{ST}\div V_S)=10\times\Delta A_T\div\Delta A_S\div W$

2. Protein concentration

AA (μmol/mg prot)=$(C_S\times V_S\times\Delta A_T\div\Delta A_S)\div(Cpr\times V_S)=10\times\Delta A_T\div\Delta A_S\div Cpr$

3. Bacteria or cells amount

AA (μmol/10^4 cell) =[C_S×V_S×(A_T-A_B)÷(A_S-A_B)]÷(500×V_S÷V_{ST})=0.02×(A_T-A_B)÷(A_S-A_B)

4. Liquid

AA (μmol/mL) =[C_S×V_S×(A_T-A_B)÷(A_S-A_B)]×2=(A_T-A_B)÷(A_S-A_B)

C_S: Standard concentration, 10 μmol/mL；

V_S: Standard volume, 0.01 mL；

W: Sample weight, g；

V_S: Sample volume, 0.01 mL；

V_{ST}: Sample total volume, 1 mL；

Cpr: 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：0.45 μmol/mL

Linear Range：0.5-18 μmol/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=0.524, A_S=0.458, A_B=0.082, ΔA_T=A_T-A_B=0.524-0.082=0.442, ΔAs=As-A_B= 0.458-0.082=0.376, calculate content by sample weight: AA（μmol/g weight)= 10×ΔA_T÷ΔAs÷W=10×0.442÷0.376÷0.1=117.55 μmol/g weight.
2. Take 0.1g hibiscus to 1ml extract solution, grinding and operate as the procedure after taking the supernatant, A_T=0.674, A_S=0.458, A_B=0.082, ΔA_T=A_T-A_B=0.674-0.082=0.592, ΔAs=As-A_B= 0.458-0.082=0.376, calculate content by sample weight: AA（μmol/g weight)= 10×ΔA_T÷ΔAs÷W=10×

0.592÷0.376÷0.1=157.45 μmol/g weight.

References：

[1] Li Z, Wang R, Gao Y, et al. The Arabidopsis CPSF30‐L gene plays an essential role in nitrate signaling and regulates the nitrate transceptor gene NRT 1.1[J]. New Phytologist, 2017, 216(4): 1205-1222.

[2] Wang C, Zhang W, Li Z, et al. FIP1 plays an important role in nitrate signaling and regulates CIPK8 and CIPK23 expression in Arabidopsis[J]. Frontiers in plant science, 2018, 9: 593.

[3] Wang N, Zhang X, Wang S, et al. Structural characterisation and immunomodulatory activity of polysaccharides from white asparagus skin[J]. Carbohydrate polymers, 2020, 227: 115314.

Related products：

NA0845/NA0603 Proline(PRO) Content Assay Kit

NA0856/NA0614 Cysteine(Cys) Content Assay Kit