Amino Acid (AA) Content Assay Kit

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

Detection equipment: Spectrophotometer/microplate reader

Cat No: BC1575

Size: 100T/96S

Components:

Reagent I: Liquid 110 mL×1, store at 2-8℃.

Reagent II: Liquid 12 mL×1, store at 2-8℃.

Reagent III: Powder×1, store at 2-8℃ and protect from light. Add 0.8 mL absolute ethyl alcohol before use, then add 11.2 mL distilled water, mix thoroughly. Used reagent can be stored at 2-8℃ for 4 weeks.

Reagent IV: Powder×1, store at 2-8℃ and protect from light. Add 1.66 mL distilled water before, mix thoroughly. Used reagent can be stored at 2-8℃ for 4 weeks.

Standard: Liquid 1 mL×1, store at 2-8℃. 10 μmol/mL Glutamic acid standard solution

Preparation of 1.25 μmol/mL Glutamic acid standard solution Take 100 μL of 10 μmol/mL Glutamic acid standard solution, add 700 μL of distilled water and mix well to get 1.25 μmol/mL Glutamic acid 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（≥99%, AR）, ice and distilled water.

Protocol:

I. Sample preparation

1. **Tissue:** according to the ratio of tissue mass (g): reagent I volume (mL) 1: 5~10 (it is recommended to weigh about 0.1g of tissue, add 1mL of reagent I for homogenization in an ice bath, and then placed in a boiling water bath to extract for 15min (sealing film wrapped around the mouth to prevent bursting of the lid), cooled down with tap water and then centrifuged at room

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temperature of 10000rpm for 10min, and then take the supernatant to be measured.

2. **Bacteria or cells:** according to the number of cells (10^6): the volume of reagent I (mL) for the ratio of 5 ~ 10:1 (recommended 5 × 10^6cells to add 1mL of reagent I), ice bath ultrasonic breakage of the cells (power 300w, ultrasound 2 seconds, interval of 3 seconds, the total time of 3min), and then placed in a boiling water bath extraction for 15min (sealing film wrapped around the mouth to prevent the explosion of the lid), tap water cooled 10,000 rpm, 10 minutes of centrifugation at room temperature, and remove the supernatant to be measured.

3. **Serum (plasma) and other liquid samples:** take 0.5mL of liquid and add 0.5mL of reagent I, placed in a boiling water bath extraction for 15 min (sealing film wrapped around the mouth to prevent bursting the lid). After cooling with tap water, centrifuge the sample at 10000rpm for 10min, and take the supernatant for measurement. (If the value is too high or too low, you can adjust the ratio of liquid and reagent I)

II. Determination protocol

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

2. Operation table (1.5mL EP tube)

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

After mixing, cover the bottle tightly (wrap the sealing film to prevent bursting the cap). incubate at boiling water for 15 minutes, repeatedly overthrow centrifuge several times. Centrifuge at 10000 rpm for 10 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. Blank and standard tubes only need to be measured 1-2 times.

III. Calculation

1.Sample fresh weight.

AA (μmol/g weight) $=[C_S\times\Delta A_T\div\Delta A_S]\times V_S\div(V_S\div V_{RT}\times W)\times F=1.25\times\Delta A_T\div\Delta A_S\div W\times F$

2.Protein concentration

AA (μmol/mg prot) $=[C_S\times\Delta A_T\div\Delta A_S]\times V_S\div(Cpr\times V_S)\times F=1.25\times\Delta A_T\div\Delta A_S\div Cpr\times F$

3.Bacteria or cells amount

AA (μmol/10^6 cell) $=[C_S\times\Delta A_T\div\Delta A_S]\times V_S\div(N\times V_S\div V_{ST})\times F=1.25\times\Delta A_T\div\Delta A_S\div N\times F$

4.Liquid

AA (μmol/mL) $=[C_S\times\Delta A_T\div\Delta A_S]\times V_S\times 2\times F=2.5\times\Delta A_T\div\Delta A_S\times F$

C_S: Standard concentration, 1.25 μmol/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, $(V+V_{ST})/V=2$;
N: Bacteria or cells amount, 10^6 cell;
F : dilution factor.

Note:

1. Proline and hydroxyproline react with ninhydrin and have no absorption peak at 570nm, so the amount of these two amino acids is not included in the measurement at 570nm.
2. If ΔA is greater than 1, it is recommended that the sample be diluted with reagent 1 and measured. 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.
3. Since the protein will be denatured during the extraction process, it is necessary to use PBS to extract the protein separately before measurement if the protein concentration is used for calculation.

Experimental example:

1、Take 0.1045g of Acacia leaf tissue and add 1mL of reagent I for sample treatment, take the supernatant and dilute it 2 times according to the measurement steps, use 1mL glass cuvette to measure $\Delta A_T=A_T-A_B=0.540-0.088=0.452$, $\Delta A_S=A_S-A_B=0.596-0.088=0.508$, according to the sample mass calculation content to get

Amino acid content (μmol/g mass) = $1.25 \times \Delta A_T \div \Delta A_S \div W \times F = 10.643$ μmol/g mass.

2. Take 0.1051g of rat liver tissue and add 1mL of reagent I to the sample, take the supernatant and dilute it 6 times and then operate according to the assay procedure, use a 1mL glass cuvette to measure $\Delta A_T= A_T- A_B=0.756-0.088=0.668$, $\Delta A_S=A_S-A_B=0.596-0.088=0.508$, according to the mass of the sample, mass calculation content to get

Amino acid content (μmol/g mass) = $1.25 \times \Delta A_T \div \Delta A_S \div W \times F = 46.918$ μmol/g mass.

3、 Take 0.5mL horse serum and add 0.5mL reagent I for sample treatment, take the supernatant and dilute it 4 times and then operate according to the assay procedure, use 1mL glass cuvette to measure $\Delta A_T= A_T- A_B =0.704-0.088=0.616$, $\Delta A_S=A_S-A_B=0.596-0.088=0.508$, calculate the content according to the sample volume. The content was calculated according to the sample volume:

Amino acid content (μmol/mL) = $2.5 \times \Delta A_T \div \Delta A_S \times F = 6.063$ μmol/mL.

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:

BC0290/BC0295 Proline(PRO) Content Assay Kit

BC0180/BC0185 Cysteine(Cys) Content Assay Kit

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