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Glutamic Acid (Glu) Content Assay Kit

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

Detection equipment: Spectrophotometer/microplate reader

Cat No: AK0416

Size: 100T/96S

Components:

Reagent I: Liquid 60 mL×2, store at 4℃ .

Reagent II: Liquid 2 mL ×1, store at 4℃ .

Reagent III: Powder×1, store at -20℃, add 20 mL Reagent I before use.

Reagent IV: Powder×1, store at -20℃, add 1.5 mL Reagent II before use.

Standard: Liquid 0.5 mL×1, store at 4℃ . 10 μmol/mL glutamic acid standard.

Description:

Glu is widely found in animals, plants, microbes and cultured cells. It's not only one of the 20 amino acids that makes up the protein, but also participates in the synthesis of many kinds of amino acids by transaminination It is one of the main amino sources in organism. Besides, Glu is also the main active ingredient of monosodium glutamate, often used as food additive and spice production.

GDH catalyze glutamic acid and NAD to form α-ketoglutaric acid, NADH and NH_4^+, the absorbance of 340 nm was increased and the content of glutamate was calculated by measuring the absorbance of 340 nm.

Required but not provided:

Ultraviolet spectrophotometer/microplate reader, desk centrifuge, transferpettor, water bath, micro quartz cuvette/96 well plate (UV plate), mortar/homogenizer, ice and distilled water.

Protocol:

I. Sample preparation

1. Bacteria or cells: Collect bacteria or cells to centrifuge tube, discard the supernatant after centrifuge. Accordance bacteria or cells : Reagent I=10 million : 1 mL, ultrasonic smash cells (powder 20%, ultrasonic

3s, interval 10s, repeat 30 times)； 10000 rpm centrifuge at 4℃ for 10 min, supernatant is ready for test.

2. Tissue: Add 1 mL extract solution to 0.1 g tissue, homogenate on ice. 10000 rpm centrifuge at room temperature for 10 min, supernatant is ready for test.

II. Detection

1. Preheat spectrophotometer/ microplate reader for 30 min, adjust wavelength to 340 nm, set zero with distilled water.

2. Standard solution: Dilute as 0.625 、0.313 、0.156 、0.078 、0.039 μmol/mL standard solution.

3. Add reagents to centrifuge tube.

A. Standard tube: Add 40 μL standard solution, 160 μL Reagent III and 10 μL Reagent IV to micro quartz cuvette or 96 well plate (UV plate), mix thoroughly, record the absorbance A1 of 20s and absorbance A2 of 5 min20s at 340 nm. ΔAs=A2-A1.

B. Test tube: Add 40 μL sample, 160 μL Reagent III 和 10 μL Reagent IV to micro quartz cuvette or 96 well plate (UV plate), mix thoroughly, record the absorbance A1 of 20s and absorbance A2 of 5 min20s at 340 nm. ΔAt=A2-A1.

III. Calculation

1. Standard curve.

The content of glutamic acid as x-axis, standard tube as ΔAs as y-axis, obtain the equation y=kx+b. Take ΔAt to the equation to acquire x value.

2. Amino acid

A. Protein concentration

Glu (μmol/mg prot)=$x \times V_S \div (Cpr \times V_S) = x \div Cpr$

B. Sample weight

Glu (μmol/g weight)=$x \times V_S \div (W \div V_{ST} \times VS) = x \div W$

3. Bacteria or cells amount

Glu (μmol/10^4 cell) ＝$x \times Vs \div (1000 \div V_{ST} \times V_S) = 0.001x$

V_{ST}: Extract solution volume, 1 mL；

V_S: Sample volume, 0.04 mL；

Cpr: Sample protein concentration, mg/mL；

W: Sample weight, g；

1000: Bacteria or cells amount, 10 million.

Note:

In order to increase detection sensitivity, the absorbance of test tube is less one and ΔA less 0.4, if the value is greater than this, the supernatant should be diluted to the appropriate multiple with Reagent I.

Technical Specifications：

Minimum Detection Limit：0.0037 μmol/mL

Linear Range ：0.0125-0.2 μmol/mL

Recent Product Citations：

[1] Yanan Wang,Chengzhen Liang,Zhigang Meng,et al. Leveraging Atriplex hortensis choline monooxygenase to improve chilling tolerance in cotton. Environmental and Experimental Botany. June 2019;162:364-373.(IF3.712)

References：

[1] Beck R, Malthe-Sørenssen D, Andreassen J P. Polycrystalline growth in precipitation of an aromatic amine derivative and l-glutamic acid[J]. Journal of crystal growth, 2009, 311(2): 320-326.

Related products：

AK0564/AK0563 Proline(PRO) Content Assay Kit

AK0582/AK0581 Cysteine(Cys) Content Assay Kit