Glutamic Acid Assay Kit (Microanalysis)

Description

Glutamic acid (Glu) is widely present in animals, plants, microorganisms, and cultured cells. It is not only one of the 20 amino acids that constitute proteins, but also participates in the synthesis of various amino acids through transamination reactions, serving as one of the major amino group donors in living organisms. In addition, Glu is the principal active component of monosodium glutamate (MSG) and is commonly used as a food additive as well as in flavor and fragrance production.

Detection Principle

Extract using the dedicated extraction solution, then develop color with the chromogenic reagent. After color development, measure the absorbance at 570 nm.

Packing

Taking 100T/96S packing for example:

Components	Packing	Storage
CB0085M-ES	110 mL x 1	4 °C
CB0085M-A	1 vial (powder) x 1	Store at 4 °C. Before use, add 5 mL of distilled water and mix thoroughly to dissolve. Any remaining reagent should be stored at 4 °C.
CB0085M-Standard	1 vial (powder) x 1	Store at 4 °C. Before use, add 1 mL of distilled water to dissolve and prepare a standard solution with a concentration of 12.5 μmol/mL.

Instructions

I. **Preparation of Lab Instruments**

Visible spectrophotometer / microplate reader, benchtop centrifuge, micro glass cuvettes / 96-well plate, water bath, adjustable pipettes, mortar and pestle, ice, and double-distilled water.

II. Glutamate Extraction

1. Tissue samples:

Homogenize the tissue on ice according to a tissue weight (g) to extraction buffer CB0085M-ES volume (mL) ratio of 1:5–10 (recommended: weigh approximately 0.1 g of tissue and add 1 mL of CB0085M-ES extraction buffer).

Centrifuge at 8,000 × g for 10 min at 4 °C. Collect the supernatant and keep it on ice for analysis.

2. Serum (plasma) or cell culture medium samples:

Mix the serum (plasma) or cell culture medium with CB0085M-ES extraction buffer at a 1:1 (v/v) ratio (recommended: mix 0.5 mL of serum [plasma] or cell culture medium with 0.5 mL of CB0085M-ES extraction buffer), then homogenize on ice.

Centrifuge at 8,000 × g for 10 min at 4 °C. Collect the supernatant and keep it on ice for analysis.

III. Assay Procedure

1. Preheat the spectrophotometer/microplate reader for at least 30 minutes and set the wavelength to 570 nm.
2. Dilute the 12.5 μmol/mL standard solution with distilled water to prepare a 5 μmol/mL standard working solution.
3. Add the following reagents into EP tubes according to the table below:

	Sample Tube (μL)	Control Tube (μL)	Standard Tube (μL)	Blank Tube (μL)
Sample	250			
Standard Solution			250	
Distilled Water				250
Extraction solution CB0085M-ES		250		
CB0085M-A	50	50	50	50

Mix thoroughly and incubate in a 90 °C water bath for 20 min (ensure the cap is tightly closed to prevent water loss). Cool under running water, then transfer 200 μL to a micro glass cuvette or a 96-well plate.

Measure the absorbance at 570 nm and record the values as A control, A sample, A blank, and A standard.

ΔA sample = A sample - A control

ΔA standard = A standard - A blank

Note: The blank, standard, and control tubes only need to be tested 1–2 times each.

IV. Calculation of Glutamate Content

1. Based on sample protein concentration

Glutamate content (μmol/g protein)=ΔA sample÷ΔA standard×Cstd÷Cpr×F =5×ΔA sample÷ΔA standard÷Cpr×F

2. Based on sample mass

Glutamate content (μmol/g fresh weight)= ΔA sample÷ΔA standard×Cstd÷W×F =5×ΔA sample÷ΔA standard÷W×F

3. Based on liquid volime

Glutamate（μmol /mL）=ΔA sample÷ΔA standard×Cstd×2×F=10×ΔA sample÷ΔA standard×F

Note:

Cstd：concentration of the standard tube, 5 μmol/mL;

W: sample mass, g;

F: sample dilution factor;

Cpr: Protein concentration, mg/mL;

2: dilution factor for liquid sample pretreatment.

Precautions

1. To improve detection sensitivity, the absorbance of the assay tube should be less than 1. If it exceeds 1, the supernatant should be diluted with extraction buffer CB0085M-ES to an appropriate factor before measurement, and the corresponding dilution factor should be multiplied in the calculation.
2. For protein quantification, it is recommended to use BCA Protein Quantification Kit (C0050) produced.
3. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
4. Please wear a lab coat and disposable gloves.