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CheKine™ Micro Glutamate (Glu) Assay Kit

Cat #: KTB1440 Size: 48 T/48 S 96 T/96 S

	Micro Glutamate (Glu) Assay Kit		
REF	**Cat #:** KTB1440	LOT	**Lot #:** Refer to product label
	Detection range: 100-600 μg/mL		**Sensitivity:** 100 μg/mL
	Applicable samples: Animal and Plant Tissues, Fermented Broth, Cells, Serum (Plasma)		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Glutamate (Glu) is widely present in animals, plants, microorganisms and cultured cells. It is not only one of the 20 amino acids that make up proteins, but also participates in the synthesis of multiple amino acids through transamination. It is one of the main sources of amino acids in organisms. In addition, Glu is also the main effective ingredient of MSG, commonly used in food additives and spice production. CheKine™ Micro Glutamate (Glu) Assay Kit provides a simple method for detecting Glu concentration in a variety of biological samples such as animal and plant tissues, fermented broth, Cells and Serum (Plasma). The kit uses a special extraction solution to extract, and then develops the color with a chromogenic agent. After the color develops, the measurement is performed at 570 nm. It is suitable for determination of glutamate content in fermentation broth or animal and plant tissues.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4℃
Chromogen	Powder×1 vial	Powder×1 vial	4℃, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 570 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Refrigerated centrifuge, water bath, Ice maker
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.
Chromogen Solution: Add 10 mL deionized water to each Chromogen, equilibrate to room temperature before use. Store at 4℃, protected from light. The remaining working solution can be stored at 4℃, protected from light for one week.

Sample Preparation

1. Animal tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and homogenize on ice. Centrifuge at 8,000 g for 10 min at room temperature. Use supernatant for assay, and place it on ice to be tested.
2. Plant tissues: Weigh 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 5 min (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at room temperature. Use supernatant for assay, and place it on ice to be tested.
3. Fermented broth sample: Take 0.1 mL of fermentation broth, add 0.9 mL of Extraction Buffer, then mix well. Centrifuge at 8,000 g for 10 min at room temperature. Use supernatant for assay, and place it on ice to be tested.
4. Cells: Collect 5×10^6 cells into the centrifuge tube, wash cells with cold PBS, discard the supernatant after centrifugation; add 1 mL Extraction Buffer to ultrasonically disrupt the cells 5 min on ice (power 20% or 200 W, ultrasonic 3 s, interval 7 s, repeat 30 times). Centrifuge at 8,000 g for 10 min at room temperature. Use supernatant for assay, and place it on ice to be tested.
5. Serum (Plasma): Take 0.5 mL of Serum (Plasma), add 0.5 mL of Extraction Buffer, then mix well. Centrifuge at 8,000 g for 10 min at room temperature. Use supernatant for assay, and place it on ice to be tested.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 570 nm, visible spectrophotometer was returned to zero with deionized water.
2. Content determination of Glu (The following operations are operated in the EP Tubes):

Reagent	Test Tube (μL)	Blank Tube (μL)
Supernatant	250	0
Extraction Buffer	0	250
Chromogen Solution	50	50

3. Mix well, incubate in boiling water bath for 20 min (cover tightly to prevent water loss), cool to room temperature, add 200 μL into 96-well plate or microglass cuvette, immediately read optical density at 570 nm. The test tube is recorded as A_{Test}, and the blank tube is recorded as A_{Blank}. Finally calculate $\Delta A=A_{Test}-A_{Blank}$.

Note: Blank Tube only needs to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If ΔA_{Test} is less than 0.02, increase the sample quantity appropriately. If the ΔA values are higher than 1.5, dilute the supernatant with Extraction Buffer to an appropriate multiple and repeat this assay. Multiply the results with the dilution factor.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. The regression equation for determination under standard conditions is y=0.0037x-0.5255; x is the glutamic acid content (μg/mL), and y is the absorbance value.

2. Calculation of Glu content

(1) Calculated by volumet of fermentation broth

Glu (μg/mL)=[(ΔA+0.5255)÷0.0037]×10**=2,703×(ΔA+0.5255)**

(2) Calculated by volumet of serum (plasma) liquid volume

Glu (μg/mL)=[(ΔA+0.5255)÷0.0037]×2**=540.6×(ΔA+0.5255)**

(3) Calculated by tissue protein concentration

Glu (μg/mg prot)=[(ΔA+0.5255)÷0.0037×V_{Sample}]÷(V_{Sample}×Cpr)**=270.3×(ΔA+0.5255)÷Cpr**

(4) Calculated by fresh weight of samples

Glu (μg/g fresh weight)=[(ΔA+0.5255)÷0.0037×V_{Sample}]÷(W×V_{Sample}÷$V_{Sample\ Total}$)**=270.3×(ΔA+0.5255) ÷W**

(5) Calculated by number of cells

Glu (μg/10^4)=[(ΔA+0.5255)÷0.0037×V_{Sample}]÷(500×V_{Sample}÷$V_{Sample\ Total}$)**=270.3×(ΔA+0.5255) ÷500**

Where: V_{Sample}: Sample volume added, 0.25 mL; $V_{Sample\ Total}$: Extract Buffer added to samples, 1 mL; $V_{Fermentation\ Broth}$: Fermentation broth volume added, 0.1 mL; Cpr: Sample protein concentration, mg/mL; W: Sample weight, g; 500: Number of cells, 5×10^6; 10: dilution of fermentation broth; 2: dilution of liquid sample.

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1430	CheKine™ Micro Proline (PRO) Assay Kit
KTB1450	CheKine™ Micro Cysteine (Cys) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.