Glutamic Acid(Glu) Colorimetric Assay Kit

Catalogue No.: K150

Size: 50T/48S

Kit component:

Item	Quantity	Instructions	**Storage**
Standard	1 mL×1 vial	10 μmol/mL Glutamic Acid	2-8℃ for 3 months
Reagent 1	120mL×1 bottle		2-8℃ for 3 months
Reagent 2	5mL×1 bottle		2-8℃ for 3 months
Reagent 3	Powder×1 bottle	Add 55mL reagent 1 before use	-20℃ for 3 months
Reagent 4	Powder×1 bottle	Add 4mL reagent 2 before use	-20℃ for 3 months
Reagent 5	Powder×1 bottle	Add 3.5mL reagent 4 before use	-20℃ for 3 months

Materials Not Supplied:

Ultraviolet spectrophotometer, Benchtop Centrifuge, Adjustable pipette, 1 mL of quartz cuvette, Mortar/homogenizer, Ice, Distilled water.

Principle of the Assay:

Glutamic acid (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 various amino acid synthesis through transamination, and is one of the main amino sources in organisms. In addition, Glu is also the main active ingredient of MSG, commonly used as a food additive and flavor production. Glutamate dehydrogenase (GDH) catalyzes glutamate and NAD to produce α-ketoglutaric acid, NADH and NH4+, which causes the increase of absorbance at 340nm. The content of glutamate is calculated by measuring the change of absorbance at 340nm.

Application:

This kit can be used to measure glutamic acid (Glu) content in serum (plasma), animal tissue, culture cells and cell culture supernatant.

Assay Procedure (For reference):

1. Sample preparation

a. Cells/bacteria according to the number of cells (10^6) : reagent 1 volume (mL) is 5~ 1:1 ratio (it is recommended that 5 million cells/bacteria add 1 mL reagent 1), ice bath ultrasonic crushing cells/bacteria (power 200w, ultrasonication 3 seconds, interval of 10 seconds, total time 3min); At 10000 rpm, centrifuge at 4℃ for 10min, and take the supernatant on ice to be measured.

b. Tissue: According to the mass (g) : reagent 1 volume (mL) is 1:5 ~10 ratio (it is recommended to weigh about 0.1g and add 1mL reagent 1) add reagent 1, after ice bath homogenization, 10000rpm, centrifuge at 4℃ for 10min, and take the supernatant on ice to be measured.

c. Serum and other liquids: Take 0.5mL liquid sample, add 0.5mL reagent 1, fully shake and mix, 10000rpm, centrifuge at 4℃ for 10min, and take the supernatant on ice to be measured.

2. Other preparation work

Before the formal test, 2-3 samples with significant differences should be selected for pre-test.

a. The ultraviolet spectrophotometer is preheated for more than 30min, the wavelength is adjusted to 340nm, and the distilled water is adjusted to zero.

b. Take part of the reagent 3 according to the sample size and preheat it at 37℃ for more than 5min before use.

c. Dilution of the standard: The 10 μmol/mL glutamic acid standard is diluted with distilled water to 0.8, 0.6, 0.4, 0.2, 0.1, 0.05μmol/mL standard, respectively.

3. Add each reagent in turn according to the operation table

Reagent（μL）	Sample tube	Standard tube	Blank tube
Sample	200	-	-
Standard	-	200	-
Distilled water	-	-	200
Reagent 3	800	800	800
Mix fully, measure the OD values A1 at 340nm, and recorded as A1 sample, A1 standard and A1 blank respectively.			
Reagent 5	50	50	50
Mix fully, incubate at 37°C for 30 minutes, then immediately measure the absorbance A2 at 30min10s, recorded as A2 sample, A2 standard and A2 blank respectively. Calculate ΔA standard =A2 standard -A1 standard, ΔA sample =A2 sample -A1 sample. Standard curves and blank tubes only need to be measured 1-2 times.			

4. Calculation

a. Plot the standard curve

The standard curve is established according to the concentration of the standard tube (x, μmol/mL) and the absorbance ΔA standard (y, ΔA standard). According to the standard curve, the ΔA sample(y, ΔA sample) is brought into the formula to calculate the sample concentration (x, μmol/mL).

b. Calculation of glutamic acid (Glu) content

（1）Calculated according to protein concentration: Glu content（μmol/mg prot）= x×V sample÷（Cpr×V sample）×F=x÷Cpr×F

（2）Calculated according to sample weight: Glu content（μmol/g）= x×V sample÷（W÷V extract×V sample）×F =x÷W×F

（3）Calculated according to the number of bacteria or cells: Glu content（μmol/10^6 cell）= x×V sample÷（N÷V extract×V sample）×F = x÷N×F

（4）Calculated according to liquid sample volume: Glu content（μmol/mL）= x×2×F = 2x×F

V extract: the volume of reagent 1 added for pre-treatment, 1mL；

V sample: the volume of the sample added, 0.2mL；

Cpr：the protein concentration of sample, mg/mL；

W：sample weight, g；

N：total number of bacteria or cells, measured in 10^6；

2: dilution times of liquid sample pre-treatment, (0.5mL liquid sample +0.5mL reagent 1) ÷0.5mL liquid sample =2; F: Sample dilution ratio.

Notes:

1. If the measured absorption value exceeds the linear range absorption value, the sample size can be increased or the sample can be diluted before measurement.
2. Sensitivity: 0.023 μmol/mL
3. Range: 0.05-0.8 μmol/mL